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Development of multi-epitope driven subunit vaccine in secretory and membrane protein of *Plasmodium falciparum* to convey protection against malaria infection

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ABSTRACT

Malaria infection is the severe health concern for a long time. As per the WHO reports, the malarial infection causes huge mortality all around the world and is incomparable with any other infectious diseases. The absence of effective treatment options and increasing drug resistance to the available therapeutics like artemisinin and other derivatives demand an efficient alternative to overcome this death burden. Here, we performed the literature survey and sorted the *Plasmodium falciparum* secretory and membrane proteins to design multi-epitope subunit vaccine using an adjuvant, B-cell- and T-cell epitopes. Every helper T-lymphocyte (HTL) epitope was IFN-γ positive and IL-4 non-inducer. The physicochemical properties, allergenicity, and antigenicity of designed vaccine were analyzed for the safety concern. Homology modeling and refinement were performed to obtain the functional tertiary structure of vaccine protein followed by its molecular docking with the toll-like receptor-4 (TLR-4) immune receptor. Molecular dynamics simulation was performed to check the interaction and stability of the receptor-ligand complex. Lastly, *in silico* cloning was performed to generate the restriction clone of designed vaccine for the futuristic expression in a microbial expression system. This way, we designed the multi-epitope subunit vaccine to serve the people living in the global endemic zone.

1. Introduction

Malaria is considered as the severe health issue for centuries. It mainly affects the population living in the tropical, subtropical and sub-Saharan Africa region around the globe [1]. As stated by the World Health Organization (WHO) report, half of the world's population is living under the risk of malaria infection and in the year 2015, total 212 million malaria cases and 429,000 deaths were reported, worldwide. Malaria is caused by the intracellular sporozoan parasites belong to the genus *Plasmodium*. Five *Plasmodium* species namely *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* are known to cause the malaria infection among human being. Among these five *Plasmodium* species, *P. falciparum* causes the most lethal form of malaria with highest complications and mortality rates [2]. Transmission of these parasites to humans is caused by the bite of hitherto infected female *Anopheles* mosquitoes [3]. Malaria infection is associated with the severe illness and its symptoms appear usually after 10–15 days of infective mosquitos bite. Primary malaria symptoms include fever, headache, body aches, nausea, vomiting, and chill while severe malaria causes multiple organs disorder in adults and anemia and respiratory distress among children [4].

Even after such severe assault by the malaria parasite, the treatment of uncomplicated malaria only relies upon the artemisinin-based combination therapies (ACTs) consisting of two active molecules with a different mechanism of action. Among this two active constituent, one consists of an artemisinin derivative while another consists of antimalarial drugs like mefloquine, amodiaquine, piperaquine, lumefantrine, or sulfadoxine-pyrimethamine. The treatment of severe malaria depends upon the injectable form of artesunate (2.4 mg/kg body weight) for the time duration of minimum 24 h. It should be continued with the ACTs for consecutive 3 days. Both these treatment options have their respective limitations. As per the WHO reports, parasitic resistance has been reported against almost all known antimalarial drugs that were used against 3 major malarial species namely *P. falciparum*, *P. vivax* and *P. malariae* [5]. The resistance against the highly efficacious artemisinin has also been reported from some parts of the

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South-East Asian region [6]. This resistance is aggravated by cross-resistance, a severe situation in which resistance to one drug deliberates resistance to other drugs which have either the same mode of action or belongs to the same chemical family. Additionally, the combination therapy of artemisinin derivative with mefloquine has been reported to cause acute nausea, anorexia, vomiting, and dizziness in the patient with uncomplicated malaria [7].

A malaria vaccine is a special need of society for the public health concern to attain successful malaria elimination. Unfortunately, to date, there is not a single licensed vaccine against malaria infection for the human use. Recent WHO strategies indicate towards the existence of licensed malaria vaccine for human use by 2030s. That vaccine will immunize against both *P. falciparum* and *P. vivax* and will have at least 75% immunization efficacy against malaria infection [8]. The most advanced vaccine nominee reported till date is RTS, S/AS01 but it is in phase III trial in African countries [9]. Apart from this, there are several vaccine candidates that are either in preclinical trial or different phases of a clinical trial but not licensed for the human use [9].

The complicated life cycle of malaria parasite divides the malaria vaccine into three categories namely pre-erythrocytic malaria vaccines, erythrocytic malaria vaccines and transmission-blocking vaccines. All three vaccines have their own importance in immunization, for example, pre-erythrocytic vaccine targets the *Plasmodium* sporozoite getting entry into the liver cell. While erythrocytic vaccine aims to stop the asexual reproduction of malaria parasite within the red blood cells; the last transmission-blocking vaccines target the sexual reproduction stage of the parasite occurs in the mosquito gut. This study was designed to contribute to the path of pre-erythrocytic and blood-stage malaria vaccine candidate design. As out of nine proteins sorted for the vaccine design, only one protein (CS) is pre-erythrocytic in nature and remaining belongs to the blood stage, the designed vaccine construct will primarily protect against the blood stage parasitic infection but also against pre-erythrocyte parasites. Here, we utilized an immunoinformatics approach to design the subunit vaccine targeting *P. falciparum* membrane and secretory proteins. Subunit vaccine consists of B-cell, cytotoxic T-lymphocyte (CTL) and helper T-lymphocyte (HTL) epitopes along with suitable adjuvant and linkers. Among the physicochemical parameters, thermostability, amphipathic nature, immunogenicity and allergenic nature of vaccine protein was determined. To evaluate the binding affinity of vaccine protein against the TLR-4 immune receptor, the tertiary structure of subunit vaccine was developed followed by molecular docking and molecular dynamics simulation. Lastly, *in silico* cloning was performed to make a restriction clone of optimized codons corresponding vaccine protein into pET28a(+) vector. This study utilizes a combinatorial algorithm to design the subunit vaccine which may have the capability to prevent the pre-erythrocytic and blood-stage malaria parasite. Moreover, this study warrants the experimental validation of subunit vaccine construct to check its immunogenicity and safety behavior.

2. Results and discussion

Malaria is the deadliest parasitic infection covering half of the world's population, but most severely the African region, where highest mortality rate was reported. Increasing drug resistance and the complex life cycle of *P. falciparum* hinders the path of malaria vaccine candidate design. This study was designed to overcome such problems by developing multi-epitope subunit vaccine. Each and every step of this study was designed precisely to obtain an immunogenic and stable subunit vaccine construct which may have the ability to neutralize the futuristic malarial infection (Fig. 1). The different steps of vaccine candidate design and their results are mentioned below ranging from sequence retrieval to *in silico* cloning.

2.1. Sequence procurement and functional domain selection

Surface-associated parasitic proteins play a major role in host-parasite crosstalk which is advantageous for the parasite to coun-

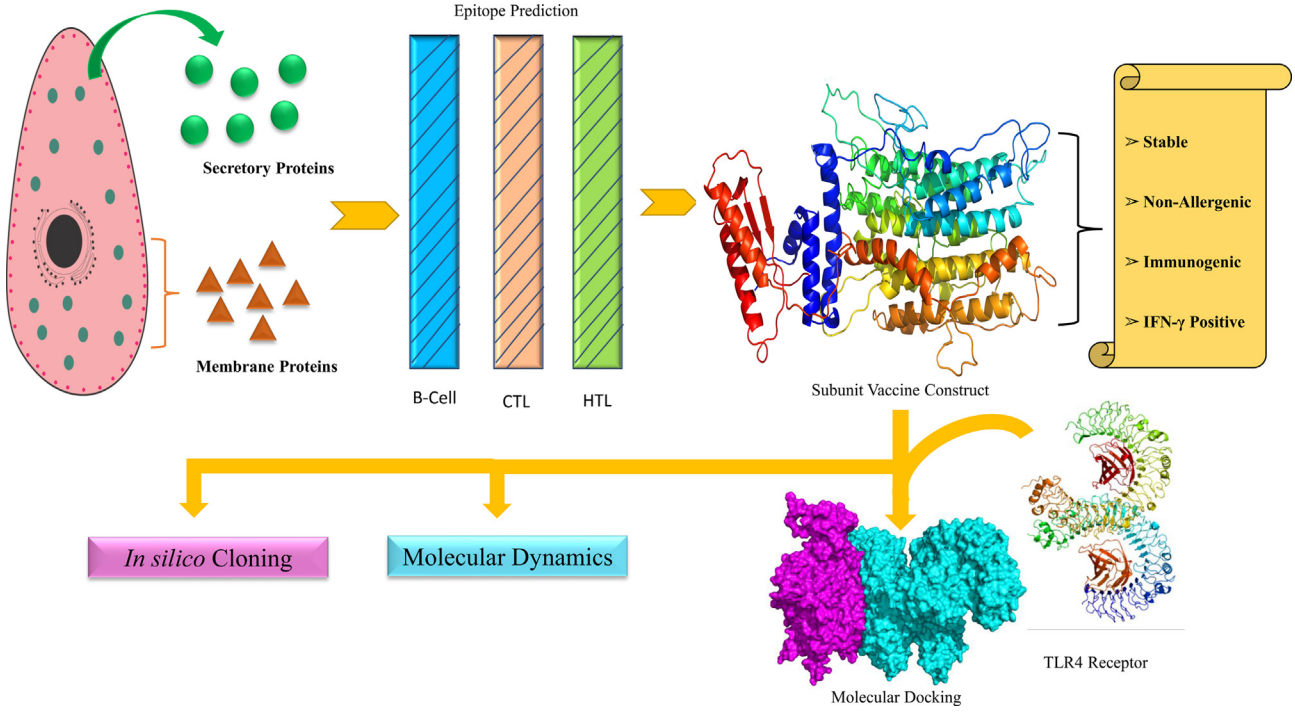

Fig. 1. Schematic representation of multi-epitope subunit vaccine candidate designing using B-cell, CTL and HTL epitopes followed by molecular docking, dynamics simulation and *in silico* cloning.

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