# CHAPTER 1

# INTRODUCTION

## 1.1 Problem Background

Malaria is one of the endemic diseases that are caused by *Plasmodium sp.* and commonly found in developing countries, including Indonesia. In 2018, the World Health Organization (WHO) estimated 228 million cases, with 405 000 deaths occurred worldwide (WHO, 2019). Meanwhile, the Indonesian government estimated about 10 to 12 million cases with 30 000 death each year (The Minister of Health of Indonesia, 2019). From five known human-infecting *Plasmodium* species*, P. falciparum* is the most prevalent malaria infection (WHO, 2019). Numerous efforts have been made to eradicate malaria and its spread throughout the world, such as insect repellent, early diagnostics, prophylaxis, and combination therapy. However, the number of malaria cases is still high and become one of the greatest threats to human health (WHO, 2019).

Artemisinin Combination Treatment (ACT) is the first line of treatment for malaria infection that is commonly used in worldwide (WHO, 2018). The ACT treatment is a combination of an antimalaria drug from the artemisinin group with another antimalaria group. Artemisinin will act as a short-acting drug for rapid parasitemia reduction, while the second antimalaria group serves as a longer-acting drug that eliminates parasite that survives artemisinin. Currently, there are five types of ACT which available in the market, such as artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulfadoxine-pyrimethamine, and dihydroartemisinin-piperaquine (WHO, 2019). The duration of ACT for each malaria patient depends on the cause of the malaria infection experienced by the patient (The Minister of Health of Indonesia, 2018). Unfortunately, the ACT resistance has emerged and lead to the most significant threat in malaria control (Noedl et al., 2008).

The resistance is known to have spread from Cambodia as the first country to report ACT failure to surrounding states (Noedl et al., 2008 and Amato et al., 2018). The previous study conducted by Bakhiet et al. observed the evolution of known drug resistance markers in *P. falciparum* that lead to

ACT failure. The study showed drug resistance genes in Sudan correlate with the spread of the drug deployment pattern (Bakhiet et al., 2018). These findings indicate that the artemisinin is persistent and does not quickly develop at random. The emergence of partial resistance against ACT may lead to the development of complete artemisinin resistance and persistent infection from insufficient treatment. Thus, a new method of treatment should be devised to encounter the spread of ACT resistance.

One of the possible methods is by developing an antimalaria vaccine with a new target candidate. Vaccines are the most promising alternative method for malaria control. Still, their development has been hindered by strain specificity in previously studied antigen, which makes the proposed vaccine is ineffective against all five human-infecting *Plasmodium* (Ramanto and Nurdiansyah, 2020). The decrease in vaccine efficacy towards non-vaccine strain parasite was observed in most leading antimalaria vaccine candidate known as RTS,S/AS01 against *P. falciparum* (Pringle et al., 2018). Previous studies also indicated that genetic diversity in *Plasmodium sp.* might affecting vaccine efficacy (Takala and Plowe, 2009). These findings showed the limitation of strain specificity vaccine toward the efficacy and might affected malaria control. Hence, it is important to develop antimalaria vaccine that works against all human-infecting *Plasmodium.*

In this study, we found several proteins that shared by *Plasmodium* by data mining from PlasmoDB where some of them include in 6-Cysteine (6-Cys) protein family which expressed in different stage throughout the parasitic cycle of *Plasmodium* (Arredondo and Kappe, 2018). Previous evolution studies revealed that three of the 6-Cys family could be used as vaccine target candidate, which are: (i) P41 which has a signal sequence and located on the surface of merozoite; (ii) P48/45 which involved in male or female gamete fusion in the mosquito midgut; (iii) and P230 that also associated with the gamete membrane by binding to P48/45 (Arredondo and Kappe, 2018). Since the 6-Cys protein family members present in all *Plasmodium* species, then the cross-species antimalaria vaccine could be made by using P41, P48/45, and P230.

P41 includes in blood-stage 6-Cys protein, which does not possess glycosylphosphatidylinositol (GPI) moieties that could anchor P41 to the outer leaflet of the plasma membrane (Taechalertpaisarn,

2012). P41 could be a target of infected host immune response because it contains a long spacer region between 6-Cys domains, and human malaria sera could recognize the spacer region (Taechalertpaisarn, 2012). Meanwhile, targeting P48/45 and P230 in the sexual stage and expressed on the surface of gametes could inhibit the successful fusion of gametes in the mosquito gut (Dijk et al., 2010). Since these proteins express in different stages, then there are two types of potential antimalaria vaccine that could be made which are: a merozoite vaccine by using P41 and a gametocyte vaccine by using P48/45 and P230.

Previous study conducted by Ahmed et al. has discovered a low level of polymorphism in both S48/45 domains in P41 from *P. falciparum, vivax, and knowlesi* (Ahmed et al., 2018). In 2018, Srisutham et al. also showed that P48/45 in *P. malariae* has low average pairwise nucleotide diversity and haplotype diversity compared to thrombospondin-related anonymous protein (TRAP) and apical membrane antigen 1 (AMA1) (Srisutham et al., 2018). Another research that was conducted by MacDonald et al. discovers that recombinant P230 domain 1 in P. *falciparum* has a similar secondary structure after heating to denaturation levels and cooling, which indicates P230 has suitable thermal stability for vaccine component (Macdonald et al., 2016). Our study found the unique evolutionary relationship in each of P41, P48/45, and P230 protein between human infecting and non-human infecting *Plasmodium*. Based on the initial structural and domain analysis, we detected signal peptide, coiled-coil, and transmembrane region that could be used as a vaccine target, and unique structural differences were observed in P230 protein (Ramanto and Nurdiansyah, 2020). All of these studies have revealed the characteristic of P41, P48/45, and P230 in *Plasmodium sp.* could be used as the target for the cross-species antimalaria vaccine.

Two different approach known as ancestral and consensus sequence reconstruction could be used to develop cross-species antimalaria vaccine. These approaches will help to produce single antigen that represent characteristic of all human-infecting *Plasmodium* (Joy et al., 2016 and Sterneke et al., 2019). However, the efficacy of vaccine candidate should be validated by assessing the 3-dimensional structure and immunogenicity properties. The study of immunogenicity properties and

protein structure has become a crucial factor in the development of biological drugs, including a vaccine. Immunogenicity in vaccine development aims to induce an optimal immunological reaction that elicited by natural, pathogen-derived peptide epitopes (Mahanty et al., 2015). The Indicator of optimal immunity includes the sustained production of effector lymphocytes. A challenge with this approach is the identification of suitable B and T cell epitopes that could be used as a vaccine candidate. Since the changes in protein structure would affect the protein function, then understanding the 3-dimensional protein structure can assist the identification of suitable epitopes (Frimpong et al., 2018). Epitopes can be designed into accessible forms for easy uptake by immune cells. Furthermore, protein structure analysis provides crucial insight into structural information, which can be exploited to solve some of the challenging problems regarding vaccine development against malaria (Anasir and Poh, 2019). Thus, immunogenicity and 3-dimensional structure analysis could be used to improve the efficacy of antimalaria vaccine from P41, P48/45, and P230 protein in the future.

## 1.2 Problem Formulation

The antimalaria vaccine is the most promising alternative method in malaria control. Numerous ways have been done to find the most effective vaccine candidate, but the development of a vaccine is not an easy task. Some vaccine candidates may have low efficacy and not properly induce host immune system. Furthermore, studied vaccine antigens only designed for specific *Plasmodium* species. P41, P48/45, and P230 as vaccine candidate have shown a great potential to target all human-infecting *Plasmodium*. However, the efficacy of P41, P48/45, and P230 need to be validated.

Immunogenicity and protein structure analysis are the methods that include the use of a computer to analyze and predict the efficacy of vaccine candidates. Immunogenicity analysis reveals the sequence of B-cell, T-cell, and major histocompatibility complex (MHC) binding epitopes in antigenic protein. The best binding epitopes will have a higher score, which means that the epitopes have a higher binding affinity with immune cells. Meanwhile, protein analysis is used to analyze protein activity and stability. Protein analysis can be done by predicting the Post-Translational Modification

(PTM) sites, and hydrophobicity of vaccine candidates. Therefore, these analyses could predict the efficacy of P41, P48/45, and P230 as cross-species antimalaria vaccine.

## 1.3 Research Objectives

- To provide valuable information regarding the structural data and immunogenicity of P41, P48/45, and P230 protein by observing the characteristic differences in tertiary protein structure and immunogenicity properties.
- To identify the suitable antigen that could give a high level of protection against all human infecting *Plasmodium* (*P. falciparum, P. vivax, P. knowlesi, P. ovale, and P. malariae*).
- Utilizing *in silico* approach to support the development of a cross-species antimalaria vaccine using 6-Cys proteins in the blood stage and sexual stage.

## 1.4 Research Scope

This study will be the continuation of the evolutionary studies of *Plasmodium* proteins by taking a deeper analysis in the P41, P48/45, and P230 protein in human infecting *Plasmodium* species. The scope of this study is:

- Data mining of 6-Cys protein sequences from the Genbank and PlamoDB
- Phylogenetic tree and time tree reconstruction
- Ancestral and consensus sequence prediction
- Secondary and tertiary structure prediction of ancestral and consensus protein sequences
- Protein analysis of 6-Cys proteins
- Molecular docking simulation between P12-P41 and P48/45-P230
- Immunogenicity Analysis 6-Cys proteins

## 1.5 Expected Outcome

The research is hoped to get a better understanding of the 6-Cys proteins of *Plasmodium* to gain the edge in creating a vaccine for malaria. The result should serve as the basis for further analysis in this ordeal.