

# ARTEMISININ BASED COMBINATION THERAPIES AND *Pfmdr1* AS A MARKER FOR MONITORING ANTIMALARIAL RESISTANCE IN THAILAND

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**ABSTRACT:** An emergence of antimalarial drug resistant is now becoming a threaten problem of public health worldwide. Artemisinin based therapies (ACT) have been generally recommended for malaria drug policy in most countries. This review stages the problems of drug resistance in malaria parasites and emphasizes the use of molecular makers especially, *Pfmdr1* gene point mutation and *Pfmdr1* gene copy number as a surveillance tool for monitoring ACT resistance.

**Keywords:** artemisinin, antimalarial, resistance, *Pfmdr1*

## THE PROBLEM OF DRUG RESISTANCE

Malaria has been a major problem of public health for several decades in endemic areas of the world's poorest countries. Malaria incidence has increased during the past 20 years and caused more than 300 million acute illnesses resulting in at least one million deaths annually [1]. Ninety percent of deaths were due to malaria occurring in South of Sahara in Africa, particularly among children below five years of age. Malaria is caused by a single cell protozoan parasite of the genus *Plasmodium* spp. With over 100 species, *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae* are naturally transmitted to humans by female *Anopheles* mosquitoes. The *P. falciparum* found to be the most virulent and fatal to human [2].

Although the total number of malaria cases has decreased in some endemic areas, the resistance of *P. falciparum* against many of the routinely used antimalarial drugs has become a serious problem. The resistance means that there is a shift to the right in the dose responses relationship. A parasite resistant strain will be able to survive and/or multiply despite the administration and absorption of the drug. Even though for which the given doses are equal to or higher than those usually recommended but within tolerance of the subject [3]. The emergence of drug resistance is a major obstacle to the provision of prompt and effective treatment and to be effort to control the disease. There are several factors promote the spread of resistance including the proportion of transmissible malaria infections exposed to subtherapeutic concentrations of an antimalarial and the drug concentration profile, the pattern of drug use and the level of immunity in the community [4]. The mechanism of drug resistance have been proposed that, it occur from spontaneous chromosomal point

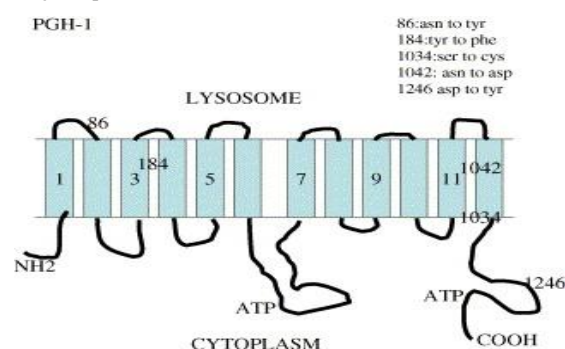
mutations or gene amplifications, which is depend on the drug selection pressure. When the mechanism of resistance occurred, these more resistant parasites have more survival advantage in the presence of antimalarial drugs. This is determined by the intrinsic frequency with which point mutation occur and the degree of resistance conferred by the change. Antimalarial resistance results in prolonged illness, hospitalization and leading to death as well as a circle of an increase in treatment failure consequently the increasing of gametocyte carriage and thus increased malaria transmission.

The conventional methods *in vivo* and *in vitro* drug susceptibility test are still the gold standard for the surveillance of drug efficacy but these techniques need expertise for interpretation and time consuming. Therefore the alternative reliable molecular markers of antimalarial resistance could also play an important role in the surveillance of drug efficacy. For example, screening for *pfcr* K76T mutation which is strongly associated with chloroquine resistance *in vitro* and chloroquine treatment failure in clinical settings [5, 6]. Survey of single-nucleotide polymorphism in *P. falciparum* dihydropteroate synthase (DHPS) and dihydrofolate reductase genes (DHFR) are similarly to monitoring resistance to sulfadoxine/pyrimethamine [7-9]. The progression of chloroquine and sulfadoxine/pyrimethamine resistance leaves few alternative treatment regimens for malaria patients. Currently, antimalarial regimen are base on ACTs or Artemisinin base Combination Therapy which usually include an artemisinin derivative such as artesunate, artemether or dihydroartemisinin as a fast-acting component and mefloquine, lumefantrine, a quinoline, or an antifolate as a long acting drug [10]. Several studies have been shown that *Pfmdr1* and *Pfap6* can be used for ATCs monitoring markers [11]. In this review focus only on the used of *Pfmdr1* gene as molecular marker because the

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**Table 1** Common artemisinin based combination therapies (Modified from Lin et al. [10])

| Artemisinin component | Partner drug              | Elimination half-life of partner drug | Areas where ACT is commonly deployed           |
|-----------------------|---------------------------|---------------------------------------|------------------------------------------------|
| Artesunate            | Mefloquine                | 2-3 weeks                             | Southeast Asia, South America                  |
| Artemether            | Lumefantrine              | 3-4 days                              | Africa, South Asia, Middle East, South America |
| Artesunate            | Amodiaquine               | ~ 10 days                             | West Africa                                    |
| Artesunate            | Sulfadoxine-pyrimethamine | 3-7 days                              | South Asia, Middle East, South America         |
| Dihydroartemisinin    | Piperaquine               | 4-5 weeks                             | Southeast Asia, China, Africa                  |

**Figure 1** The Pgh1 protein of *P. falciparum*. Polymorphic amino acid are indicated [13]

mutations and amplification in *Pfmdr1* proven to associate with antimalarial drug resistance [12].

### ***Pfmdr1* GENE AND GENERAL CHARACTERIZATION**

The *P. falciparum* multidrug resistance gene (*Pfmdr1*) has been reported to associated with the alteration of susceptibility to variety of current antimalarial drugs. This gene located on chromosome 5 encodes 12 transmembrane-domain proteins, also call Pgh1 (P-glycoprotein homologue 1) (Figure 1). This protein localized in digestive vacuole, where is the site of action of chloroquine and other quinoline-base antimalarial drugs [13]. However, conclusive evidence of a direct association between *Pfmdr1* and resistance to these antimalarials has remained controversy, and a single genetic cross has suggested that Pgh1 is not involved in resistance to chloroquine and mefloquine [14, 15]. Nevertheless, Reeds and colleagues provide direct proof that mutations in Pgh1 can confer resistance to mefloquine, quinine and halofantrine. The same mutations influence parasite resistance towards chloroquine in a strain-specific manner and the level of sensitivity to the structurally unrelated compound, artemisinin. This has important implications for the development and efficacy of future antimalarial agents [16]. Furthermore, the amplification of *Pfmdr1* gene has been reported to associated with malaria multidrug resistant. Mefloquine resistant culture-adapted clone lines identified increases in *Pfmdr1* copy number [17]. There have been several studies

using *Pfmdr1* copy number as a molecular tool for monitoring antimalarial drug efficacy and found that this could be an attractive alternative way for antimalarial resistant surveillance [18, 19].

### ***Pfmdr1* GENE AND ANTIMALARIAL RESISTANCE IN THAILAND**

Antimalarial drug resistance has been spread in Thailand over 50 years. At present, the situation of drug resistance is more seriously. The evidences both *in vitro* and *in vivo* of *P. falciparum* which is the most resistant strain has been confirmed. There are some studies reported that the falciparum malaria in Thailand already resistant to chloroquine, sulfadoxine-pyrimethamine, mefloquine and quinine [20]. The declining of mefloquine susceptibility leading to the addition of artesunate in the first-line drug regimen in Trat, Chantaburi, Sa Kaeo which are located in Thai-Cambodian border, and Tak in Thai-Myanmar border. The resistance of *P. falciparum* is the main factor contributing to the obstruction of malaria control programme in Thailand.

In response to the emergence of multidrug resistance, artemisinin-based combination treatment regimens have been introduced Southeast Asia over the past decade [21]. At the beginning these regimens are highly effective and seem to promote protection against the emergence and spread of resistance. The early therapeutic response is due to rapid killing by artesunate and the remaining parasites are still present after 3 days that will be eliminated by the longer lasting partner drug. The common artemisinin based therapies are shown in Table 1. Artesunate-mefloquine combination was the first used at Thailand's border since 1994 for an attempt to overcome with the high level of mefloquine resistant parasites. Unfortunately, the efficacy of ACT at one study site in the Thai-Myanmar border decreased 50% within 2 years of implementation and the incidence continued to fall thereafter. This drop in incidence went beyond the effectiveness of successful treatment that is believed due to a consequence from a reduction in gametocyte carriage after treatment, making patients less infective to mosquitoes. Currently, artesunate-mefloquine is used in Cambodia, Thailand and Myanmar and in some countries in

South America. Its safety in African children and in pregnancy is under investigation. However, the long elimination half-life of mefloquine, approximately 2 to 3 weeks, makes it unsuitable for hyperendemic settings in Africa, where subtherapeutic drug levels for up to 3 to 5 weeks following treatment in the face of high rates of reinfection can quickly lead to drug resistant [10]. In Thailand, *Pfmdr1* copy number was found to be the best predictor of treatment failure after 3 days of ACT treatment, mefloquine given with artesunate for 3 days (MSA3), with a sensitivity of 77% and specificity of 65% [22]. Genetic analysis also suggested that it was the mefloquine component in the small proportion of patients who received MSA3 and presented with late more than 7 days recrudescence infection.

### ***Pfmdr1* AS A MARKER FOR MONITORING ACTS RESISTANCE IN THAILAND**

Molecular markers for monitoring antimalarial resistance are urgently needed to guide the malaria treatment policy in the era of artemisinin based combination therapies. Molecular markers for resistance are based on genetic changes that confer malaria parasite resistance to antimalarial drugs. At present, the validation process of these markers had been slow and their application as surveillance of drug resistance has been limited.

The development of mammalian multidrug resistance gene (*mdr*), both gene amplification and gene mutation of multidrug resistant transporter gene has been shown to occur. Similarly to the *Pfmdr1* gene in *P. falciparum*, there are two ways in which gene polymorphisms may lead to drug resistance both through gene amplification and mutation [23-25]. Amplification of *Pfmdr1* was found to occur through multiple and independent places [26]. Inheritance of point mutations, is likely to be more efficient means of transmitting a resistance through a population. Mutations in the *Pfmdr1* gene were identified by sequencing a series of laboratory strains from different geographical areas [25]. Polymorphism was observed at five position codons 86, 184, 1034, 1042 and 1246. When field isolates are analyzed, both mutation and amplification are found to be wild spread in numerous geographical areas [27].

In Thailand, there are several studies pointed that the *Pfmdr1* mutation and amplification are associated with drug resistance in *P. falciparum*. Mungthin and colleagues investigated pattern of *Pfmdr1* of *P. falciparum* collected from several endemic areas in Thailand during 1988 to 2003 and found the prominent mutation pattern at codons 86, 184, 1034, 1042 and 1246 were N/SND with prevalence increasing from 40% to 95% during the 10 years period. This study suggested that the role of *Pfmdr1* in modulating the susceptibility to chloroquine, quinine and mefloquine. The

frequencies of the S1034C and N1042D mutations and *Pfmdr1* copy number were significantly different in isolates collected during the two periods [28]. In a cohort study of malaria patients receiving mefloquine treatment on the Thai-Myanmar border there was no significant association between either *Pfmdr1* point mutations and *in vitro* drug susceptibility and mefloquine resistant *in vivo*. However the increasing of *Pfmdr1* gene copy number was significantly associated with recrudescence. From this study confirmed that the use of *Pfmdr1* gene copy number can be a useful surveillance tool for mefloquine resistant falciparum malaria in Thailand [29]. Pickard and colleagues also emphasized that *Pfmdr1* copy number ( $\geq 3$  copy number) couple with *Pfmdr1* point mutations enhanced parasites more resistant to mefloquine, quinine, artemisinin, and artesunate and more sensitive to chloroquine [30]. Furthermore, the study enrolled in 2004 at Pailin, Thai-Cambodian border found that the parasites with  $\geq 3$  of *Pfmdr1* copy number before treatment was significantly associated with recrudescence. This finding revealed that artemisinin based treatment selected for increased *Pfmdr1* copy number because isolates from recurrent episodes had higher copy numbers than the paired enrollment samples [31].

### **CONCLUSION**

The development of antimalarial resistance has been a serious problem. The ACT combinations have now been adopted in all countries as malaria drug policy. The emergence of artemisinin and artemisinin derivatives resistance is widely concerned. More studies about all the factors surrounding this problem are urgent needed for better understanding. The rational ACT used should be emphasized. Additionally, the potential molecular marker surveillance tool should be implied to prolong the therapeutic lives of ACT. The findings from several studies have pointed out that *Pfmdr1* mutations and *Pfmdr1* copy number could be used as a monitoring tool for ACT efficacy both *in vivo* and *in vitro* studies.

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