CHAPTER I
INTRODUCTION

Malaria remains one of the most important public health problems worldwide including Thailand despite considerable effort during the last century to eradicate or control the disease. Four species of malarial parasite cause infection in humans, but it is *Plasmodium falciparum* with which creates most problems including its prevalence, virulence and drug resistance (Bull World Health Organ, 1980). Over 40% of the global populations are at risk, with 300-500 million clinical cases and 1-2 million deaths annually (WHO). In Thailand, although annual reported malaria cases have continued to decrease over the past two decades and have disappeared from most of the major cities, people in rural areas, especially in villages on the Thai-Myanmar and Thai-Cambodian borders and forested mountainous areas, remain at great risk. The incidence rate was reported as 0.39 *per* 1,000 cases in 2003 (Malaria unit, Department of Disease Control, Ministry of Public health of Thailand, 2003).

Chemotherapy and chemoprophylaxis has been the mainstay in malaria control. However, the emergence and spreading of resistant of *P. falciparum* to almost all available antimalarials has being reported from all endemic areas. Resistance to chloroquine, a 4-aminoquinoline, was firstly reported in Thailand in 1962 and since then, has spreaded rapidly all over the country (Harinasuta *et al*., 1962). Multidrug resistant *P. falciparum,* including resistance to structurally related antimalarial aminoquinolines, *i.e,* quinine and mefloquine, is currently highly prevalent especially along border areas (Looareesuwan *et al.,* 1992). There has been as yet, clinical evidence for resistance of *P.falciparum* malaria to artemisinin and derivatives (Price *et al.*, 1996). Resistance of the parasite to these drugs arises *via* selection of mutations, and is decisive in determining the lifetime of antimalarial agents. The mechanisms of action and mechanisms of resistance to most drugs currently in use nevertheless, are essentially unknown or debated.

Reasons for the development and spreading of drug resistance involve the interaction of characteristics of the drug itself, human host factors, parasite

characteristics, vector environment factors, and the pattern drug use or drug pressure of policy of each country (Wernsdorfer *et al.*, 1991 and Wongsrichanalai *et al*., 2001).

Drug pressure has been identified as one of the key factors contributing to the emergence of resistance to antimalarial drugs. Selection of resistant strains can occur when a drug is misused or used extensively as monotherapy. The development of resistance is a function of the half-life of a drug. Antimalarials with long half-lives, *e.g.*, mefloquine (half-life of 14-20 days) and chloroquine (half-life of 1 month) are likely to exert undesirable drug pressure for a long time once their concentrations drop below critical threshold and may select resistant parasites. However, a short half-life (in hours) does not a guarantee of persisting drug effectiveness. The parasite-killing capacity of a drug depends largely on the proportion of sensitive parasites within the parasite biomass. Resistance selection is higher in drugs with lower kill-rates due to the high proportion of parasites that survive at given drug concentrations that could be selected (White *et al*., 1989). In Thailand, due to the rapid spreading of multidrug resistance falciparum malaria, antimalarial treatment policy has been changed from time to time. Chloroquine was introduced for clinical use during the period 1965-1974 and followed thereafter, by Fansidar® (sulfadoxine/pyrimethamine), quinine and mefloquine during the period 1974-1980, 1980-1986 and 1986-1995, respectively. At present, combination therapy of mefloquine and artemisinin derivatives is being used as first-line treatment for uncomplicated falciparum malaria all over the country (Ministry of Public Health of Thailand). Different degree of selective drug pressure would be expected and influence the pattern of drug resistance at molecular level and as a consequence, clinical efficacy of antimalarials. Thorough understanding of the mechanisms of such resistance and developing new treatments, including new drugs, novel compounds or modifications of existing drugs are urgently required to cope with the situation.

Molecular methods that detect genetic markers of drug resistance in parasites are potentially powerful tools to detect and track drug resistant malaria. Furthermore, molecular data can also be used to guide development of novel antimalarial compounds to bypass drug resistance mechanisms. Chloroquine resistance has been linked to mutations in two genes, *pfmdr1* and *pfcrt*, that encode the digestive vacuole transmembrane proteins Pgh1 and PfCRT, respectively (Reed *et al.*, 2000). Transfection studies with *pfmdr1* suggest that mutation in Pgh1 may modulate the chlorquine resistance phenotype *in vitro*; however, *in vivo* studies have shown an inconsistent association between mutation in Pgh1 and chloroquine resistance (Van *et al.*, 1994). More recently, a series of point mutations in the *pfcrt* gene have been associated with chloroquine resistance (Durand *et al.*, 2001) (One mutation at position 76(K76T) was present in all *in vitro* resistant parasites and has been proposed as a molecular marker for surveillance of chloroquine-resistant falciparum malaria (Djimde *et al.*, 2001). There is some evidence to support the role of *pfmdr1* mutations in accounting for reduced susceptibility to quinine. In a Brazilian study of *pfmdr1* mutations (Asn86, Cys1034, Asp1042, Tyr1246), chloroquine resistant strains were found to have low susceptibility to quinine and a Gambian study (Zalis *et al.*, 1998), Tyr86 was weakly associated with decreased quinine sensitivity. In addition, a molecular study has also suggested that the *pfmdr1* Tyr86 variant may also be associated with increased sensitivity to artemisinin (Pickard *et al.*, 2003). For mefloquine on the other hand, copy number and polymorphisms of the *pfmdr1* gene have been investigated as molecular markers of mefloquine resistance but the evidence on increased *pfmdr1* copy number as a molecular marker for mefloquine resistance remains conflicting (Price *et al.*, 2004). Two studies in Thailand suggested that a higher copy number confers mefloquine resistance but other studies from Thailand, as well as Brazil and Africa did not confirm that finding (Nelson *et al.*, 2005, Basco *et al*., 1995).

In the present study, the influence of antimalarial drug pressure resulting from clinical usage of different antimalarial drugs, *i.e*., chloroquine, quinine, mefloquine and artesunate on the molecular markers *pfmdr1* and *pfcrt* genes in *P. falciaprum* strains

collected during the periods 1988-1989, 1991-1993, and 2003 were investigated. In addition, association between *in vitro* susceptibility patterns of all isolates to each antimalarial and patterns of these genetic markers were investigated.