CHAPTER I

INTRODUCTION

Malaria is the name of a tropical diseases caused by microscopic unicellular organism called protozoa. They are larger than bacteria and capable of free movement. The specific malaria parasites all belong to the genus Plasmodium . There are 4 plasmodia species that infect man and each causes a different form of malaria. Plasmodium falciparum causes Falciparum malaria or Malignant tertian malaria, Plasmodium vivax causes Vivax malaria or Benign tertian malaria, Plasmodium ovale causes Ovale malaria or Ovale tertian malaria ,and Plasmodium malariae causes Quartan malaria or Benign quartan malaria.

Although all 4 species can be associated with significant morbidity, P. falciparum is the most dangerous species because it causes the most serious illness and acute mortality especially in children in endemic areas of Africa. It is highly effective and widely distributed in tropical and subtropical areas. Tourists ,immigrants, and the imported cases of malaria are increasingly seen in non-endemic areas. P. vivax is the most widely distributed species , being prevalent throughout the tropics and in many temperate regions. Like

infection with P. ovale , which is found chiefly in the tropical Africa , Vivax malaria is characterized by relapses-reappearance of symptoms after a latent period of up to 5 years. P. malariae is widely distributed but much less common than P. vivax or P. falciparum.

Therefore malaria remains the most important of the tropical diseases, and is widespreads not only throughout the tropics, but also to some extent in many temperate regions. Two billion people are considered at risk and 267 million are infected. Clinical cases are estimated at 107 million per year and mortality at 1-2 million per year. Eightythree percent of the total number of cases reported annually to WHO are concentrated in 9 countries :- Afghanistan, Brazil, China, India, Mexico, The Philipines, Sri Lunka, Vietnam, and Thailand.

In Thailand the number of cases reported went down by about 20% ; however incidence has recently increased markedly along the border provinces of Trat (bordering Kampuchian) and Tak (bordering Mayanmar). Forty percent of all cases originated from Trat province, fifteen percent from Tak, Yala, and Chantaburi provinces (WHO, 1991). Treatment and control have become more difficult with the spread of drug-resistant strains of P. falciparum , and insecticide-resistant strains of the mosquito vectors.

Malaria parasites are inoculated in human beings by the bite of infected female mosquitoes of the genus Anopheles. The

parasites multiply many times , first in the liver and then in the red blood cells. The mosquitoes become infected by feeding on the blood of infected persons, and the parasites then undergo another phase of reproduction in these infected vectors. Malaria symptoms begin as a flu-like illness eight days or more after the infected mosquito bite. Typical cycles of fever, shaking chills and drenching sweats may then develop. The frequency depends on the malaria species, coinciding with cycles of parasite multiplication and the erythrocyte destruction. Falciparum malaria may not always show this cyclic pattern and can be fatal if untreated or treated with insufficiently effective drugs ; death may be due to parasitized red cells in the blood vessels supplying the brain-cerebral malaria- or damage to other vital organs.

In countries where malaria is a major cause of morbidity and mortality, like Thailand, control activities aim to stop preventable deaths and to minimize suffering from the disease. Prompt diagnosis and adequate treatment of cases using basic health services are fundamental to this strategy and require good diagnostic facilities to detect the type and severity of the infection rapidly (WHO, 1991).In order to achieve this, there is a need to improve both basic biological and clinical knowledge. Research includes studies of parasite biology to understand why the succeptibility of falciparum malaria parasites to the drug currently used for treatment of malaria

has decreased during recent years, and thus thwarted the control of the disease.

In recent years a number of biochemical techniques have been developed to examine genetic variation of falciparum malaria parasites, which can now be detected in the DNA structure of different P. falciparum isolates. Pulsed field gradient gel electrophoresis(PFGE) is proving to be useful for strain characterization. It allows the fractionation of DNA molecules of 30 - 3,000 kilobases. This range encompasses the sizes of intact chromosomal DNA molecules from eukaryotes such as yeast,and many protozoa including Plasmodium spp..

Analysis of the chromosomes of P. falciparum by classical cytological techniques has been hampered by the failure of chromosomes to condense into discrete entities during metaphase and by the complexity of the life cycle. By means of PFGE, the P. falciparum genome can be separated (probably) into 14 chromosomes, ranging in size from 800 to 3,500 kb.

This analysis also reveals surprising size polymorphisms among equivalent chromosomes of different isolates. It is clear that chromosomal polymorphisms could arise both at mitosis and meiosis as will be shown in Chapter II. While the majority of studies have been performed on isolates adapted to in vitro culture, chromosomal polymorphisms have also been observed in natural infections .

Possible mechanisms underlying these polymorphisms could

involve deletions of repetitive DNA or of coding sequences. It has also been proposed that chromosome length polymorphism is the consequence of homologous recombination between subtelomeric repeat units.

Another possible explanation of chromosome size polymorphisms of the parasites is drug resistance as mentioned aboved. PFGE, can be used to examine the relationship between Plasmodium chromosomes and various types of drug resistance.So far, resistance to pyrimethamine in P. falciparum has been reported to be ascribed to 3 mechanisms. First is by amplification of the DHFR-TS gene; second is by structural changes in the DHFR-TS enzyme , and the third is by the point mutations.

The scope of the thesis

This thesis was set out to determine whether P. falciparum chromosomes which were separated by PFGE showed any polymorphisms among parasite clones, and if so, whether chromosome 4 - sized variation was correlated with the property of pyrimethamine resistance of each clone. Such comparison might give insight into the mechanism of pyrimethamine resistance development. In addition, dot blot analysis using DHFR and β-tubulin genes as probes was done to test whether the mechanism of pyrimethamine resistance of P. falciparum is due to DHFR-TS gene amplification.