Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(13)60181-1

Polymorphic patterns of *pfcrt* and *pfmdr1* in *Plasmodium falciparum* isolates along the Thai-Myanmar border

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PEER REVIEW

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Comments

This study is very interesting and applicable that established prevalence of *pfcrt* and *pfmdr1* polymorphims in provinces along Thai-Myanmar border after chloroquine withdrawal and estimately 15 years of artesunate and mefloquine usage. Application of well known drug resistance molecular markers; *pfcrt* and *pfmdr1* could be applied for surveillance of chloroquine, artesunate and mefloquine resistance progression among the four provinces. Furthermore, the degree of resistance estimated from this study is advantage to classify requirement of intensive monitoring in each location.
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ABSTRACT

Objective: To investigate the distribution and patterns of *pfcrt* and *pfmdr1* polymorphisms in *Plasmodium falciparum* (*P. falciparum*) isolates collected from the malaria endemic area of Thailand along Thai-Myanmar border.

Methods: Dried blood spot samples were collected from 172 falciparum malaria patients prior received treatment. The samples were extracted using chelex to obtain parasite DNA. PCR-RFLP was employed to detect *pfcrt* mutation at codons 76, 220, 271, 326, 356 and 371, and the *pfmdr1* mutation at codon 86. *Pfmdr1* gene copy number was determined by SYBR Green I real-time PCR.

Results: Mutant alleles of *pfcrt* and wild type allele of *pfmdr1* were found in almost all samples. *Pfmdr1* gene copy number in isolates collected from all areas ranged from 1.0 to 5.0 copies and proportion of isolates carrying>1 gene copies was 38.1%. The distribution and patterns of *pfcrt* and *pfmdr1* mutations were similar in *P. falciparum* isolates from all areas. However, significant differences in both number of *pfmdr1* copies and prevalence of isolates carrying>1 gene copies were observed among isolates collected from different areas. The median *pfmdr1* copy number in *P. falciparum* collected from Kanchanaburi and Mae Hongson were 2.5 and 2.0, respectively and more than half of the isolates carried>1 gene copies.

Conclusions: The observation of *pfmdr1* wild type and increasing of gene copy number may suggest declining of artesunate-mefloquine treatment efficacy in *P. falciparum* isolates in this border area.

KEYWORDS

Plasmodium falciparum, Multidrug resistance, *Pfcrt*, *Pfmdr1*, Gene mutation, Gene copy number

1. Introduction

Malaria is one of the major infectious diseases that causes a number of deaths in tropical and subtropical countries. In Thailand, the mortality rate had raised to 36 per 100 000 population in 1958, but continuously declined after the launch of malaria control program[1,2]. Most of the affected populations are those who reside in/near forests and hilly areas along the international borders. The highest incidence has been reported from areas bordering

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Foundation Project: Supported by the National Research University Project (NRU) of Thailand (Grant No. 10/2555) and Thammasat University (Grant for student No. 33/2555).

Article history:
Received 28 Sep 2013
Received in revised form 10 Oct, 2nd revised form 15 Oct, 3rd revised form 21 Oct 2013
Accepted 20 Nov 2013
Available online 28 Dec 2013

Thai-Myanmar, followed by Thai-Malaysia, Thai-Cambodia and Thai-Laos PDR. A serious problem that limits the effectiveness of malaria control program of the country is the emergence and spread of multidrug resistance *Plasmodium falciparum* (*P. falciparum*)[3,4]. To deal with the situation, the artemisinin-based combination therapy, a three-day artesunate-mefloquine combination is currently being used as first-line treatment of multidrug resistance *P. falciparum* according to recommendation of World Health Organization[4].

The gold standard for monitoring antimalarial drug efficacy mainly relies on *in vivo* investigation with supplemented information of *in vitro* parasite susceptibility. In recent years, attempt has been made to apply valid molecular markers of antimalarial drug resistance to predict treatment outcome following treatment with an antimalarial drug regimen[5]. The two candidate malarial parasite genes, *P. falciparum* chloroquine resistant transporter (*pfcrt*) and *P. falciparum* multidrug resistance 1 (*pfmdr1*) that express the transport proteins on the plasma membrane of the parasite's food vacuole *pfcrt* and *pfmdr1*, respectively, have been confirmed to link with resistance of the parasite to antimalarial drugs[6]. Mutation of *pfcrt* associated with chloroquine resistance in *P. falciparum* and distinct genotype polymorphisms depends on its origination. Most of *P. falciparum* isolates collected from Thailand carry *pfcrt* mutations at codons K76T, A220S, Q271E, N326S and R371I[7]. The mutation at codon 86 of *pfmdr1* (86Y) related with chlolroquine resistance, while *pfmdr1* wild type at the same codon (N86) including increased *pfmdr1* gene copy number linked to resistance of the parasite to mefloquine and artesunate[8-10]. The aim of the present study was to investigate the distribution and patterns of *pfcrt* and *pfmdr1* polymorphisms in *P. falciparum* isolates collected from the malaria endemic area of Thailand along Thai-Myanmar border.

2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 172 *P. falciparum*-infected dried blood spot samples were collected prior to treatment, from patients with acute uncomplicated *P. falciparum* malaria during 2009-2010 from the four malaria endemic areas along Thai-Myanmar border of Thailand, *i.e.*, Mae Hongson (MH, 41 samples), Tak (TK, 82 samples), Kanchanaburi (KN, 6 samples) and Ranong (RN, 43 samples) provinces (Figure 1). Genomic DNA was extracted from each sample using chelex resin according to the previously described method[11].

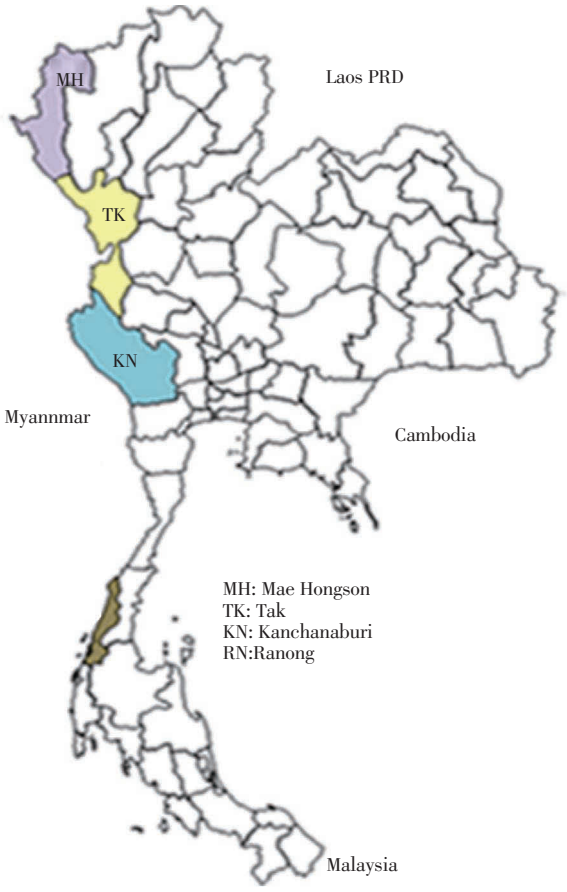

Figure 1. Map of Thailand presenting the four malaria endemic areas along the Thai-Myanmar border.

2.2. Determination of pfcrt and pfmdr1 single nucleotide polymorphisms

PCR-RFLP was employed to detect *pfcrt* mutation at codons 76, 220, 271, 326, 356 and 371[12] and *pfmdr1* mutation at codon 86[13]. DNA of *P. falciparum* laboratory clones G112 and K1 served as control for chloroquine sensitive and chloroquine-resistant genotype, respectively.

2.3. Determination of pfmdr1 gene amplification

Pfmdr1 gene copy number in all samples was investigated by SYBR Green I real-time PCR[14]. DNA of 3D7 (1 *pfmdr1* copy number) and Dd2 (4 *pfmdr1* copy number) *P. falciparum* laboratory clones provided by Professor Dr. Steven A. Ward (School of Tropical Medicine, Liverpool, UK) were used as the internal control. The copy number was determined by relative quantification between *pfmdr1* (target gene) and *pf*β-*actin* (reference gene, an endogenous house-keeping gene which carries only a single copy) that was calculated using the comparative C_t method (also known as the $2^{-\Delta\Delta Ct}$ method).

2.4. Statistical analysis

Qualitative variables were summarized as proportions and