IN VITRO SENSITIVITY ASSAY TO ANTIFOLATES AND GENETIC ANALYSIS OF *Plasmodium vivax* ISOLATES FROM THAILAND

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ABSTRACT

The objective of the present study was to investigate the association between *Pvdhfr* and *Pvdhps* genotype and *in vitro* sensitivity of *Plasmodium vivax* to the antifolates pyrimethamine, WR99210, chlorcycloguanil, sulfadoxine and dapsone. A total of 32 patient isolates collected from Mae Sot, Tak Province were assessed from Mae Sot Thailand. Two *in vitro* systems for assessment of sensitivity of *P. vivax* isolates were applied: a sensitivity assay based on schizont maturation inhibition, utilizing short term culture of parasites, and a yeast expression system. The geometric mean of IC_{50} (the concentration of antimalarial drug that inhibiting schizogony by 50%) and standard deviation values based on schizont maturation assay for pyrimethamine, chlorcycloguanil, WR99210, sulfadoxine and dapsone were 85 (88), 784 (662), 95 (87), 2424 (2784), and 1625 (1801) nM, respectively. Sequencing revealed 5 different *Pvdhfr* alleles and four *Pvdhps* alleles. Twenty six of the 32 isolates carried quadruple mutant alleles of *Pvdhfr* (F57L/S58R/T61M/S117T or F57I/S58R/T61M/S117T). In addition, there were four triple mutants (S58R/T61M/S117T, K49C/S58R/S117N) and two double mutant isolates (S58R/S117N). All isolates carried *Pvdhps* 585V. Twenty four isolates carried double

mutant *Pvdhps* (A383G/A553G), six carried an additional mutation at residue 382 (S382A,C/A383G/A553G) and two carried a single mutation at residue 383 (A383G). A trend of increasing geometric mean IC_{50} values was observed with increasing number of *Pvdhfr* mutations from double to quadruple. The sensitivity of four *Pvdhfr* mutant alleles in the yeast expression system showed that all mutant alleles exhibited increased resistance to pyrimethamine and chlorcycloguanil by more than 50 folds when compared with wild-type allele. In contrast, all mutant alleles particularly the S58R/S117N allele were relatively sensitive to WR99210 (relative resistance 1.3-83), Results suggest that quadruple mutant alleles are linked to decreased sensitivity to pyrimethamine but retained sensitivity to WR99210, suggesting that this compound should be a potential candidate drug for treatment of sulfadoxine/pyrimethamine resistant *P. vivax* malaria in Thailand.

We also examined the prevalence of point mutations in *Pvdhps* and *Pvdhfr* in 160 *P. vivax* isolates collected from different endemic areas along the international borders of Thailand by direct sequencing of both strands of the purified PCR products. The distribution patterns of the *Pvdhfr* and *Pvdhps* genotypes were investigated. Results show that the majority of the isolates harbored quadruple mutation in *Pvdhfr* and double mutation in *Pvdhps*, especially those isolates collected from areas along the Thai-Myanmar border, whereas majority of the isolates obtained from areas along the Thai-Cambodian and Thai-Malaysian borders carried double mutation in *Pvdhfr* and single mutation in *Pvdhps.* Novel mutations that have not been previously identified at codon 512 in *Pvdhps* (K512M, K512E, K512T) was also found.

KEY WORDS: *Plasmodium vivax*, ANTIFOLATE, SENSITIVITY ASSAY, DHFR, DHPS

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