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The association between genetic polymorphisms of heme oxygenase-1, tumor necrosis factor-α (TNF-α), and malaria severity in different ethnic group of patients

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Abstract

Malaria is one of the most important public health problems in several countries in the world. The knowledge on pathogenesis of severe malaria particularly cerebral malaria remains controversial and debatable. Heme oxygenase (HO) enzyme has been proposed as one of the factors that may play significant role in pathogenesis including susceptibility and severity of malaria infection. TNF-α is a cytokine produced primarily by monocytes and macrophages in many inflammatory diseases. The polymorphisms of the TNF promoter have been reported to be associated with susceptibility to severe malaria. In this study, we analyzed $(GT)_n$ repeat polymorphism in the promoter region of the inducible HO-1 and six mutations of TNF-α from malaria patients (Thais, Burmeses and Karens), to determine the association between genetic polymorphisms of HO-1, TNF-α and severity of malaria infection. Our result indicated that the genotype of $(GT)_n$ repeat between ethnic group of patients was significantly different.

Keywords**:** heme oxygenase, malaria, *Plasmodium falciparum*, disease severity

Introduction

Malaria remains health problem in Thailand. Cerebral malaria (CM) is one of the major pathological complications of *Plasmodium falciparum* infection in humans manifesting as coma that can lead to death. The pathogenesis of CM remains controversial but major factors involved, *i.e.*,cytokines and adhesion molecules are well documented (1). Malaria is problematic along international borders of Thailand where there is significant population movement. The highest cases of malaria in 2009 were reported from Mae Sot District, Tak Province. Heme oxygenase (HO) enzyme has been proposed as one of the factors that may play significant role in pathogenesis including susceptibility and severity of malaria infection (2, 3). The two isiforms, HO-1 and HO-2, are microsomal enzymes that play important role in heme catabolism to produce biliverdin/bilirubin, carbonmonoxide (CO) and iron (2, 4,). The polymorphism of human HO-1 gene promoter may contribute to the fine tuning of the transcription. Long $(GT)_n$ alleles have been found associated with susceptibility to several diseases, while they may be linked to resistance to CM (3). TNF-α is a cytokine produced in many inflammatory diseases. Polymorphism in TNF promoters have been reported to be associated with susceptibility to severe malaria, and TNFP-D allele has been shown significantly associated with cerebral malaria in Karen and Burmese populations (5). The objective of this study was to investigate the association between the genetic polymorphisms of HO-1, tumor necrosis factor-α (TNF-α) and severity of malaria infection in three ethnic

groups of patients. This information would hopefully be exploited for development of antimalarial drug targets that interrupt the progress of malaria pathogenesis.

Methods

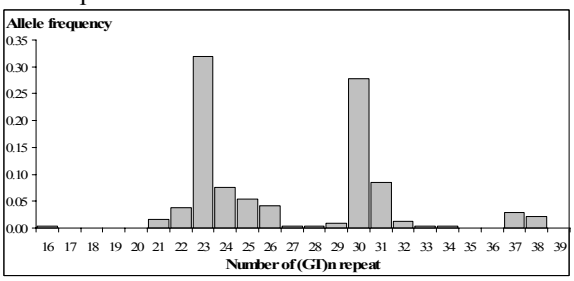
Blood samples were collected from 488 malaria patients (mean age 28 years, parasitemia 77-1,840,000/µl) who were present at Mae Sot General Hospital, Mae Sot, Tak Province, Thailand. The study protocol was approved by the Ethics Committee of the Ministry of Public Health, Thailand. All patients gave informed consents for study participation prior to the study enrolment. Severity of malaria pathology was classified based on the type and parasitemia as non-severe (uncomplicated falciparum malaria and vivax malaria) and severe malaria (falciparum malaria with hyperparasitemia). Microsatellite polymorphisms were used for analysis of HO-1 and direct sequencing was used for analysis of TNF-α. The association of malaria severity, polymorphisms of HO-1 and TNF-α genotypes in different ethnic groups was analyzed using chi-square test at a statistical significance level (α) of 0.05.

Results

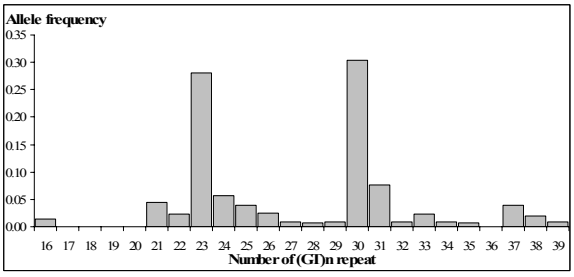
The polymorphism of HO-1 in three ethnic groups of malaria patients

The number of $(GT)_n$ repeats of the HO-1 gene varied between 16 and 39 in all patients (Figure 1). The allele frequencies of $(GT)_n$ allele were similar in the three ethnic groups. With regards to the frequencies of the genotypes (S/S, S/M, S/L, M/M, M/L, L/L) of $(GT)_n$ repeats (Table 1), significantly lower frequency of S/L but higher frequency of MM genotype was found in Thai patients compared with Burmese patients. Burmease patients however carried S/S and M/L genotypes at significantly lower frequencies than Karen patients. No association between disease severity and HO-1 genotype including L and non-L allele frequencies were observed.

a. Thai patients



b. Burmese patients



c. Karen patients

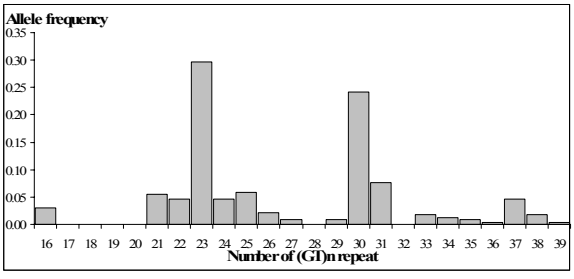


Figure 1. Frequency distribution of $(GT)_n$ repeats in three ethnic groups of patients

	Ethnic		
	Thai	Burmese	Karen
n	119	240	118
Alleles, *n* (%)			
S	132 (55.5%)	236 (49.2%)	133 (56.4%)
M	93 (39.1%)	205 (42.7%)	81 (34.3%)
L	13 (5.5%)	39 (8.1%)	22 (9.3%)
Genotypes, *n*			
S/S	39 (32.8%)	57 (23.8%)[a]	40 (33.9%)
S/M	50 (42.0%)	97 (40.4%)	45 (38.1%)
S/L	4 (3.4%)[b]	25 (10.4%)	8 (6.8%)
M/M	18 (15.1%)	49 (20.4%)[c]	12 (10.2%)
M/L	7 (5.9%)	10 (4.2%)[d]	12 (10.2%)
L/L	1 (0.8%)	2 (0.8%)	1 (0.8%)

Table 1. Distribution of HO-1 promoter genotypes and allele frequencies of the malaria patients

[a] $p = 0.042$, 95%CI: 0.3746-0.9849 for Burmese vs Karen
[b] $p = 0.021$, 95%CI: 0.1016-0.8804 for Thai vs Burmese
[c] $p = 0.015$, 95%CI: 1.1545-4.4483 for Burmese vs Karen
[d] $p = 0.026$, 95%CI: 0.109-0.9169 for Burmese vs Karen

The polymorphism of TNF-α alleles in three ethnic groups of malaria patients

The patterns of SNPs in TNF-α alleles in the three ethnic groups were observed based on five (5) and three (6) point mutations as shown in Table 2 and 3. For the five SNPs of biallelic polymorphism sites (-1031, -863, -857, -308 and -238), 6 types of TNF promoter (TNFP) alleles were found, *i.e.,* TNFP-A, TNFP-B, TNFP-C, TNFP-D, TNFP-E and TNFP-F. For the pattern of three SNPs of biallelic polymorphism sites (-1031, -863, -857), 11 types of TNFP alleles were observed, *i.e.,* TNFP1, TNFP-2, TNFP-3, TNFP-4, TNFP-5, TNFP-6, TNFP-7, TNFP-8, TNFP-9, TNFP-10 and TNFP-11. There was no significant difference in the frequencies of each mutation among all the three ethnic groups. In addition, lack of association between disease severity and SNP in TNF-α alleles was found.

Table 2. Five TNF- α alleles detected in Thai, Burmese and Karen patients with malaria diseases

TNF type	Polymorphic sites of the promoter region of TNF-α gene					Ethnic		
	-238	-308	-857	-863	-1031	Thai (%)	Burmese (%)	Karen (%)
TNFP-A	G	G	C	C	T	49.4	50.3	51.0
TNFP-B	G	G	C	A	C	37.0	33.8	36.5
TNFP-C	R	G	C	M	C	1.3	4.7	3.1
TNFP-D	G	G	Y	M	Y	6.5	4.1	3.1
TNFP-E	G	R	C	M	Y	5.2	5.6	4.2
TNFP-F	G	G	C	A	T	0.6	1.6	2.1

Table 3. Eleven TNF- α alleles detected in Thai, Burmese and Karen patients with malaria diseases

TNF type	Polymorphic sites of the promoter region of TNF-α gene			Ethnic		
	-857	-863	-1031	Thai (%)	Burmese (%)	Karen (%)
TNFP-1	C	C	T	28.6	31.1	37.5
TNFP-2	C	A	C	11.7	9.9	16.7
TNFP-3	C	M	C	1.3	3.7	4.2
TNFP-4	Y	M	Y	3.9	3.1	2.1
TNFP-5	C	M	Y	44.2	42.2	31.3
TNFP-6	C	A	T	0.0	0.6	0.0
TNFP-7	C	C	Y	0.0	3.7	2.1
TNFP-8	Y	C	T	7.8	4.3	2.1
TNFP-9	C	A	Y	1.3	0.6	2.1
TNFP-10	Y	M	T	0.0	0.0	2.1
TNFP-11	Y	C	Y	1.3	0.6	0.0

Discussion

The $(GT)_n$ repeat polymorphism in the HO-1 gene promoter has been demonstrated to link with different diseases in various studies with different ethnic backgrounds (7, 8, 9). In malaria, the association between the microsatellite polymorphism of HO-1 gene promoter and malaria disease was previously reported in Burmese and Karen patients, where a significant association between short $(GT)_n$ alleles (S/S) and high incidence of cerebral malaria was found (10). This observation could be confounded by the influence of ethnics. Nevertheless, in this study, the different in HO-1 genotype among the three ethnic groups of malaria patients was observed with S/S, S/L, M/M and M/L, but not with S/M, and L/L genotypes. Individuals with HO-1 genotypes with S allele would be expected to have increased HO-1 transcription and enzyme activity, with the activity in descending order as follow: S/S > S/M > S/L > M/M > M/L > L/L. Unfortunately, the activity of HO-1 could not directly be determined to support this conclusion.

TNF-α is one of candidate genes to determine the susceptibility for severity of malaria. In Thailand, the level of TNF-α and severe malaria have been reported. Three single nucleotide polymorphisms (SNPs) of the tumor necrosis factor alpha (TNF) promoter -1031, -863, and -857 have been studied and the frequency of TNF U04 allele was found to be significantly higher in patients with cerebral malaria than that with mild malaria (6). The biallelic polymorphic sites at nucleotides -238, -308, -857, -863, and -1031, and seven alleles have been identified in patients from Burma who lived near Thai-Burmese border (5) and TNF promoter (TNFP)-D allele was significantly associated with cerebral malaria in Karen and Burmese patients.

Conclusion

The findings support the hypothesis for the contribution of ethnicity and genetic polymorphism of HO-1 to the severity of malaria infection. The knowledge could be exploited for developing of drugs that might interrupt the progression of severity of disease pathogenesis.

Acknowledgments

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