

The genetic polymorphism of merozoite surface protein-1 in *Plasmodium falciparum* isolates from Aceh province, Indonesia

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Abstract. An estimated of 3.3 million Indonesian population were infected with malaria. However, extensive genetic polymorphism of the field isolates msp-1 of *P. falciparum* represents a major obstacle for the development of malaria treatment. The aim of this study was to investigate the genetic diversity of msp-1 genotype in field isolates of *P. falciparum* collected in Aceh Province. A total of 90 patients with malaria (+) were selected from eleven district hospitals in Aceh from 2013-2015. Data were collected by anamnesis, complete physical examination and laboratory tests for msp-1. All protocols to diagnose malaria followed the WHO 2010 guideline. All samples were stored in Eijkman Biology Molecular Institute, Jakarta. Among 90 samples, 57.7% were male, and 42.3% were female with the most cases found between 21-30 years old. From the allele typing analysis of *P. falciparum* from Aceh; K1, MAD20, and RO33 allele types were identified. MAD20 type was the highest allele found in this study (57.9%). It was found in single and mixed infection. A moderate level of the mixed allele was also observed.

1. Introduction

An estimated of 3.3 million Indonesian population were infected with malaria including 1.2 million in the risk areas which *Plasmodium (P) falciparum* is dominant with an Annual Parasite Incidence (API) of 1.0/1,000 population.[1-3] Unfortunately, the manifestation of malaria varies. However, extensive genetic polymorphism of the field isolates of *P. falciparum* represents a major obstacle for the development of clinical manifestation and malaria treatment. In this study, genetic of msp-1 among *P. falciparum* field isolates from Aceh Province was analyzed. Malaria is the most significant of the parasitic diseases, affecting 198 million people worldwide.[4-7] Parasite virulence contributes directly to the clinical outcome, and parasite diversity influences the speed at which strain-specific immunity develops in the host population.[8,9] The aim of this study was to investigate the genetic diversity of msp-1 genotype in field isolates of *P. falciparum* collected from Aceh Province.



2. Methods

This study has been approved by Gadjah Mada University ethical committee reference no: KE/FK/173/EC. A cross-sectional study with 90 participants was enrolled. Samples were selected from positive *P. falciparum* tested microscopically and above 18 years of age from eleven district hospitals in Aceh Province which were collected from October 2013-February 2015. Malaria case was an individual who had positive *P. falciparum* from microscopic examination and nPCR. Data was collected by anamnesis, complete physical examination and laboratory tests (microscopic and nPCR for MSP-2 allele). [11-13] All samples were stored in Eijkman Biology Molecular Institute, Jakarta. All protocol of assignment and malaria treatment followed the manufacturer manuals and WHO 2010 guideline. Malaria was diagnosed by using finger-prick blood samples which were collected with Whatman 3 M filter paper (GE Healthcare, Buckinghamshire, UK) and stained with 20% Giemsa for 20 min [14-15] for species identification. [16-19] *Plasmodium* species were identified by using double assignment microscopic test followed by nPCR with five sets of primers (20 mM), nested-1 using primers r-PLU-5 and r-PLU-6 (25 µL total PCR reaction) and nested-2 using primers with PCR condition from Snounow *et al.* (1993). [20-23] Bands were visualized by ultraviolet illumination with a DNA ladder of 100 bp from Vivantis, Selangor, and Malaysia. DNA was extracted from peripheral blood collected in ethylenediaminetetra acetic acid (EDTA) tubes using the *Chelex-100* kit according to the manufacturer's instructions. The polymorphic region of block 2 of MSP-1 was amplified by nested PCR using the protocol described by Ntoumi *et al.*

Table 1. The primer nested PCR sequences for identifications of *P. falciparum* genus and species.

Name	Primers	Nucleotide sequences	Amplifications (bp)
Nested-1 (<i>P. Genus</i>)	r-PLU-5	F-5: CTT –GTT-GTT-GCC-TTA-AAC-TTC-3	1.2 kb
	r-PLU-6	R-5: TTA-AAA-TTG-TTG-CAG-TTA-AAA-CG-3	
Nested-2 (<i>P.falciparum</i>)	r-FAL-1	F-5: TTA-AAC-TGG-TTT-GGG-AAA-ACC-AAA-TAT-ATT-3	205 bp
	r-FAL-2	R-5 ACA-CAA-TGA-ACT-CAA-TCA-TGA-CTA-CCC-GTC-3	
msp-1	K1	F-5:AAA-TGA-AGA-AGA-AAT-TAC-TAC-AAA-AGG-TGC-3	176 bp
		R-5: GCT-TGC-ATC-AGC-TGG-AGG-GCT-TGC-ACC-AGA-3	
msp-1	MAD20	F-5: AAA-TGA-AGG-AAC-AAG-TGG-AAC-AGC-TGT-TG-3	201 bp
		R-5:ATC-TGA-AGG-ATT-TGT-ACG-TCT-TGA-ATT-ACC-3	
msp-1	RO33	F-5:TAA-AGG-ATG-GAG-CAA-ATA-CTC-AGT-TGT-TG-3	215 bp
		F-5: CAT-CTG-AAG-GAT-TTG-CAG-CAC-CTG-GAG-ATC-3	

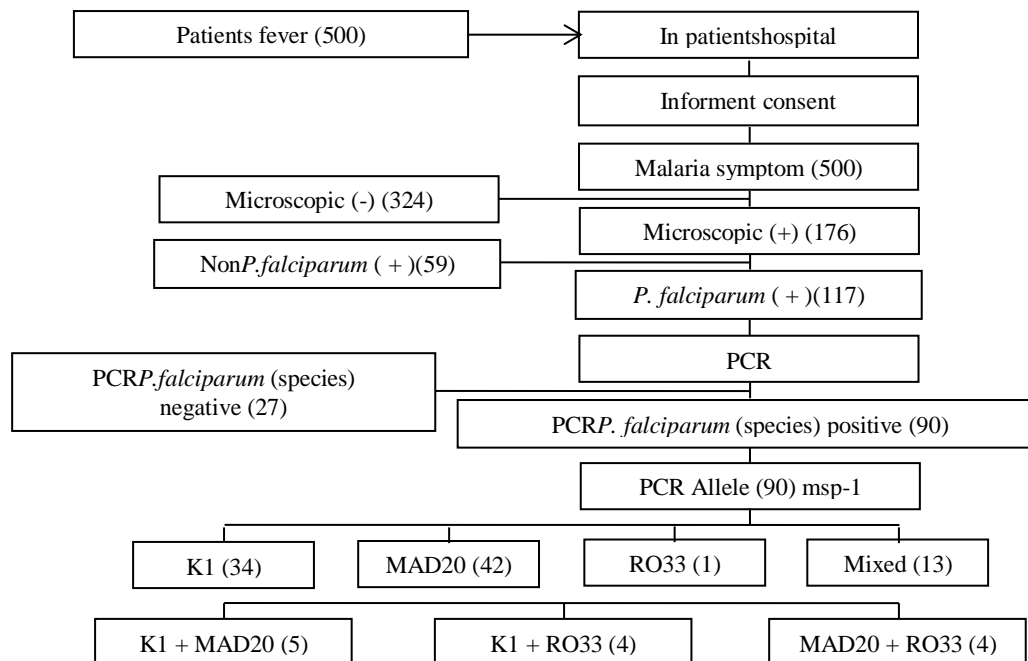


Figure 1. The study flow.

3. Result

Among 90 samples, 57.7% were male, and 42.3% were female with the most cases found between 21-30 years old (46.7%). Diverse allelic of msp-1 was identified in *P. falciparum* isolates from Aceh Province. Allele analysis of msp-1 revealed 3 different alleles.

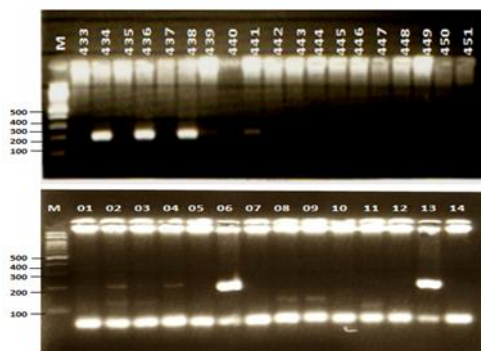


Figure 2. Nested PCR result in *Plasmodium falciparum* species (205 bp).

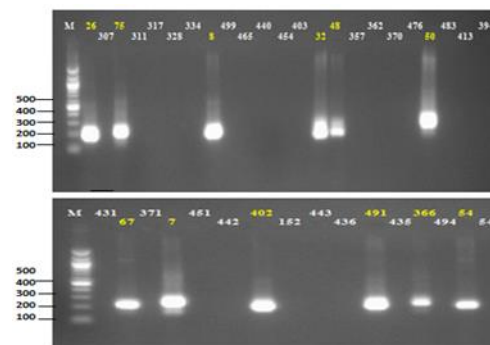


Figure 3. Genotyping allele result in msp-1 gene K1(176 bp).

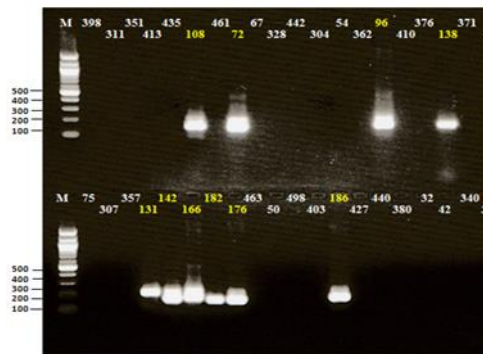


Figure 4. Genotyping allele result in msp-1 gene MAD20 (201 bp).

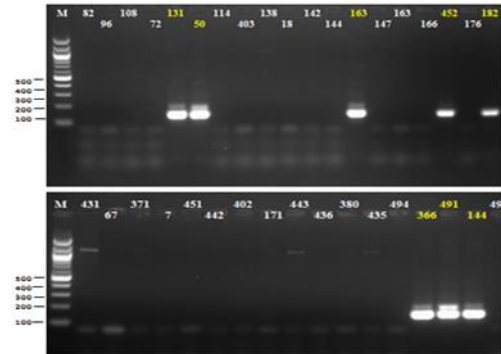


Figure 5. Genotyping allele result in msp-1 gene RO33 (215 bp).

From the allele typing analysis of *P. falciparum* from Aceh Province isolates, K1 and MAD20 allele types were identified, with a low number of the theRO33 allele. K1 allele type was identified in 43 (47.8%) blood samples with the majority (79.1%, 34/43) occurred as single infections. MAD20 type was the highest allele found in this study (56.6%), either as single or mixed infection.

Table 2. The distribution profile of msp-1 gene alleles.

Gene	Allele	Number	Percentage (%)	Amplifications (bp)
msp-1	K1	34	37.7	120-210
	MAD20	42	46.7	140-250
	RO33	1	1.1	150-215
	K1+MAD20	5	5.6	
	K1+RO33	4	4.4	
	MAD20+RO33	4	4.4	

4. Discussion

Analysis of the *P. falciparum* genetic profile may provide useful information on specific parasite characteristics to design intervention strategies targeting virulence factors.[28, 29] To our knowledge, this is the first study in Indonesia that provide information about the genetic diversity of msp-1 genotype in field isolates of *P. falciparum*. The first investigation which studies the genetic diversity of *P. falciparum* isolates was done in Libreville, Gabon. Extensive genetic polymorphism within the msp allelic families (30 alleles identified) is observed. This is consistent with the diversity found in Bakoumba (25 alleles) in 1999, in Senegal (33 alleles) in 1995, and in Mauritania (27 alleles) in 2010.[30, 31]

In our study, the distribution of msp-1 genotype was dominantly from K1 and MAD20 allele. Our study is similar to Kang et al.'s in Myanmar who have the same geographical areas, where they found MAD20 as the most predominant allele. The difference in a number of patients infected with mixed allele was higher in their study, whereas in our study, single infection from MAD20 was higher than mixed infection.[32]

5. Conclusion

The genetic diversity of MSP-1 genotype of *P. falciparum* from Aceh Province was identified in MSP-1 *P. falciparum* patients. The distribution of MSP-1 genotype found was the MAD20 type, which was the highest allele found in this study as single and mixed infection. A moderate level sign and symptoms of the mixed allele was also observed.

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