

Genetic diversity in *MSP-1* gene of *Plasmodium falciparum* and its association with malaria severity, parasite density, and host factors of asymptomatic and symptomatic patients in Papua, Indonesia

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Abstract

Background: Genetic diversity of *Plasmodium falciparum* represents a major issue in understanding the several aspects of malaria infection and diseases. The merozoite surface protein (MSP)-1 gene of *P. falciparum* codes for a major asexual blood-stage antigen, presently proposed as a major malarial vaccine candidate.

Objective: To determine the length of the DNA bands and genetic diversity of the *MSP-1* gene of *P. falciparum*, which is associated with malaria severity and host factors in population of the asymptomatic and symptomatic patients in Papua, Indonesia.

Materials and Methods: A total of 152 isolates from the blood samples of the patients infected with *P. falciparum* were analyzed by microscopic examination. DNA was extracted from *MSP-1* gene and nested PCR amplification carried out. The active case detection was conducted in Koya and Skow villages, whereas passive detection was obtained from general hospitals.

Result: The main allele of the gene *MSP-1*, which related to the severity of malaria, the parasite density, and host factors of the population with a single infection, was found to MAD20 that was higher in mild malarial cases. The family alleles K1, MAD20, and RO33 of the *MSP-1* gene of *P. falciparum* were found to be in large numbers in severe malaria and light malaria of the symptomatic patients. In the asymptomatic patients, it was found that the alleles of *MSP-1* (K1, MAD20, and RO33) were more numerous when the parasite density was less than 10,000/ μ L of blood.

Conclusion: There was no significant correlation between the genetic diversity of the *MSP-1* gene of *P. falciparum* with malaria severity, parasite density, age group, and gender of asymptomatic patients, but there was a significant correlation with the age groups and gender in the symptomatic patients. The K1, MAD20, and RO33 alleles showed a higher density in asymptomatic patients, but K1 and RO33 alleles were higher at severe and mild malaria and MAD20 was higher at mild malaria cases. Therefore, *MSP-1* gene allele could be used as a genetic marker to determine the genetic diversity of *P. falciparum* and as a candidate molecular vaccine.

KEY WORDS: *Plasmodium falciparum*, *MSP-1* gene, age, gender

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Introduction

Malaria is one of the most prevalent parasitic diseases and a major public health problem in 107 countries in the world. It is estimated that 3.2 billion of the world's population lived in areas at a risk of malarial transmission. The incidence of malaria worldwide is approximately 350–500 million clinical cases each year, with 1–3 million deaths.^[1]

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The study area was composed of two villages—Koya and Skow, and the two general hospitals as reference hospitals in Jayapura municipal. The two villages revealed a malaria outbreak from April to May 2007 and were provided assistance that boosted malarial control. The villages presented a high transmission of malarial infection because of the existence of breeding places of mosquitoes. *Plasmodium falciparum* is the most virulent of all the four parasites that cause malaria in humans. These malarial parasites are genetically diverse at all the endemic levels. The attached variability of *P. falciparum* is, especially, common in merozoite surface protein (MSP), which is being targeted for malaria vaccine.

The *MSP-1* gene of *P. falciparum* is a potential malarial vaccine candidate. Importantly, high levels of antibodies to *MSP-1* gene are reported to correlate with protection from clinical malaria.^[2–5] *P. falciparum* causes the most severe form of the disease and is responsible for the most malaria morbidity and almost all malaria mortality. Despite enormous efforts for malaria control and prevention, multiple factors such as insecticide resistance in the mosquito vectors, the lack of effective vaccines, and the emergence and rapid spread of drug resistant strain are contributing to the global worsening of the malaria situation.^[6]

The protein of the *MSP-1* gene is associated with the protection of the parasite, especially the highly conserved fragment of *MSP-1* gene with an approximate molecular size of 19 kDa of *P. falciparum*, which plays an important role in erythrocyte invasion by merozoite.^[7,8] The protein is a principal target of human immune responses and is a promising candidate for a blood-stage subunit vaccine.^[8,9] The *MSP-1* gene has seven variable blocks that are separated either through conserved or semi-conserved regions. The block 2, a region near the *N*-terminal part of the antigen, appears to be under the strongest diversifying selection within natural populations.^[8] Until now, four different allelic types of block 2 have been identified, namely: K1, MAD20, RO33, and MR alleles.^[10]

Genetic diversity is one of the prominent features of *P. falciparum* infection. Natural infection often contains a mixture of several genotypes, and the human and mosquito hosts are exposed to heterogeneous parasite populations. *P. falciparum* population diversity is commonly assessed by polymerase chain reaction (PCR)-based typing of the highly polymorphic parasite surface protein (MSP-1).^[11]

We have investigated the research about the allele dimorphic of erythrocyte binding antigen (*Eba-175*) gene of *P. falciparum* and its association with clinical manifestations and frequency distribution of *P. falciparum* *MSP-2* allele and with clinical manifestations and demographic factors in Jayapura municipal, Papua province. The *Eba-175* and *MSP-2* genes are virulence genes of *P. falciparum*, which could be potentially changed to become candidates for vaccine similar to *MSP-1* gene.^[12,13]

The block 2 *MSP-1* gene is, especially, polymorphic, and the three families of alleles are as follows: K1, MAD20, and RO33 alleles.^[14] Several studies have reported the

presence of these three alleles in the *MSP-1* gene,^[15–17] but the frequency of these alleles in *MSP-1* gene varied in different geographical areas, even in neighboring villages.^[18] Thus, the study of genetic diversity in malaria parasite is expected to provide a new concept for the spread of control measures. Although information on the frequency of genes consult, for example, resistance to a certain drug or a vaccine in a given area, has evident relevance to the implementation of control measures using such agents, a critical first step, that is, to obtain information on the genetic polymorphism of parasites in the human hosts and community, needs to be investigated.^[12,13,18]

MSP-1 and *MSP-2* genes act as genetic markers to determine the genetic diversity of *P. falciparum* and multiplicity of infection, to assess the level of malaria transmission, and to investigate the relationship of these factors with the acquisition of natural immunity against malaria.^[13,19]

In addition, *MSP-1* and *MSP-2* genes could elicit immune response in humans and have been identified as potential candidates for blood-stage malarial vaccine.^[13, 20,21]

Materials and Methods

Study Design

This cross-sectional, descriptive, and analytic study was carried out to determine the frequency of genotypes of the *MSP-1* of *P. falciparum* with malaria severity, parasite density, and host factors of the population of asymptomatic and symptomatic patients in Jayapura municipal.

Study Sites and Population

The habitants of Jayapura municipal were mainly Papuan and migrants from Java, Sulawesi, Maluku, and other parts of Indonesia. A total of 152 blood samples were collected in the following study sites: 41 samples from Koya village; 10 samples from Skow village; 41 samples from Jayapura General Hospital, and 60 samples from Abepura General Hospital. The age of the subjects ranged from 1 to 65 years. For the asymptomatic subjects, the selected villages represented the coastal regions, rice fields, and inland environmental settings of malaria. The climate was typically tropical with drier season during April to September and wet season during October to March.

The inclusion criteria were mild malaria cases in the village residents who joined voluntarily. During the survey, 7-day morbidity history of the subjects including ear (tympanic) temperature less than or equal to 39°C and parasite density below or above 10,000/μL of blood of children and adult subjects were taken. Malaria infectivity of the subjects were determined by microscopic identification of the parasite using Giemsa-stained blood smears. The inclusion criteria for severe malaria cases who showed the clinical manifestations were determined through the history of illness. Totally, 101 subjects were identified with the symptoms of malaria

(i.e., delirium, hyperpyrexia, and difficulty of breathing), of which 44 subjects were classified as cases with severe malaria with inclusion criteria such as body temperature above 39°C (tympanic temperature) and parasitemia above 10,000/μL; the remaining 57 subjects were classified as cases with mild malaria. The population character (i.e., age and gender) and clinical findings of each subject were also registered. All the bloods samples were collected after an informed consent, and the study was approved by the Research Health Ethics Committee, Faculty of Medicine of Hasanuddin University, Makassar, Indonesia.

DNA Extraction and PCR genotyping

Genomic DNA of *P. falciparum* from the asymptomatic and symptomatic subjects were extracted on the filter paper by using Chelex-100 methods.^[22] The PCR amplification using both primary and nested PCRs were performed with all the DNA samples. For the primary PCR, 2.5 μL of the extracted DNA was added to 90 μL of the reaction mix [including 0.4 units of Taq polymerase (Gibson BRL Life Technology), 50 mM KCl 134 and 0.1% Triton X-100, 10 mM Tris HCL (pH 9.0 at 25°C), 1.6 mM MgCl₂, and 125 mM of each primer]. The PCR conditions were 5 min at 95°C; 40 s at 94°C, 40 s at 58°C, and 1 min at 72°C; and 5 min at 72°C. The cycle conditions for the nested PCR were the same as those for the primary reaction with 25 cycles for the first PCR and 30 cycles for the second PCR. The PCR products were subjected to electrophoresis using 2% agarose gels and stained with ethidium bromide, and the DNA band was visualized by UV transillumination. The sequences of the primer used in PCR were worked in Eijkman Institute Molecular Biology, Jakarta, which were described earlier in the study by Snounou et al.^[15]

MSP-1 Block 2 Allele Typing

The positive samples of the *MSP-1* gene of *P. falciparum* from the asymptomatic and symptomatic subjects were analyzed for genetic diversity in the second PCR using primers of the K1, MAD20, and RO33 alleles.

DNA Sequencing

The single PCR products obtained were purified using Promega Kit and sequenced on ABI system 3730 using Big Dye® Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem). The single PCR products for K1, MAD20, and RO33 alleles were sequenced both forward and reverse, and the results were compared with the published sequence (accession number: AB827762, AB502795, and AB502750, respectively).

Statistical Analysis

The association between the genetic diversity of the *MSP-1* allele and malaria severity, parasitemia of density, age group, and gender of the asymptomatic and symptomatic patients were statistically analyzed by using Pearson's χ^2 -test.

Result

A total of 157 patients were enrolled for the study through the primary health centers (PHCs) in Skow and west and east Koya villages, and 51 asymptomatic patients were positive for the *P. falciparum* parasite. In the Jayapura and Abepura General Hospitals, 415 symptomatic patients were likewise enrolled in the study, and it was found that 101 patients were found positive and carried *P. falciparum* parasite. The clinical manifestations of malaria were examined from the blood smear by PCR amplification in patients who visited primary health services (20.4%) of Skow village and west and east Koya villages (76.6%) and Jayapura General Hospital (40.6%) and Abepura General Hospital (59.4%) in Jayapura municipal, Papua province.

Analysis of the *P. falciparum* *MSP-1* allele

Amplification of *P. falciparum* *MSP-1* allele by PCR

The *MSP-1* gene of *P. falciparum* was amplified by nested PCR from the blood samples of the asymptomatic and symptomatic patients with *P. falciparum* malaria in Jayapura municipal, Papua province, Indonesia.

The electropherogram of the nested PCR products showed that there were DNA bands with length variants of approximately 151–295 bp for the K1 allele [Figure 1], 106–259 bp for the MAD20 allele, as expected [Figure 2], and 153 bp for the RO33 allele [Figure 3].

On the basis of PCR products of the 51 asymptomatic malaria cases, it was found that 20.5% patients carried K1 allele, 22.7% MAD20 allele, and 6.8% RO33 allele. There were also patients who carried double infections [K1 and MAD20 alleles (20.6%), K1 and RO33 alleles (4.5%), and MAD20 and RO33 alleles (13.6%)] and triple infection [K1, MAD20, and RO33 alleles (11.4%)].

On the basis of statistic Pearson's χ^2 -test, there was no significant correlation between the genotypes of the *MSP-1* gene of *P. falciparum* with asymptomatic malaria ($P = 0.350$; $\alpha = 0.05$) [Table 1].

Of the 101 symptomatic malaria cases, the genetic diversity of the *MSP-1* with a single infection was found as follows: K1 allele, 12.8%; MAD20 allele, 21.3%; and RO33 allele, 6.4%. In double infections, the diversity were as follows: K1 and MAD20 alleles, 18.1%; K1 and RO33 alleles, 12.8%; and MAD20 and RO33 alleles, 9.6%. In triple infections, 19.1% carried all the three, K1, MAD20, and RO33, alleles.

On the basis of statistic Pearson's χ^2 -test, it was found that there was no significant correlation between the genetic diversity of the *MSP-1* alleles and symptomatic malaria ($P = 0.299$; 95%CI; $\alpha = 0.05$).

Malaria Severity and *MSP-1* Allele

Regarding the association of the alleles with malarial severity, it was found that K1 allele was higher in cases with severe malaria (13.6%) and MAD20 allele was higher in cases with mild malaria (34.4%) [Table 2].

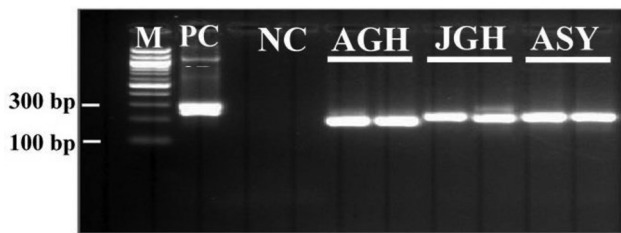


Figure 1: Electrophoregram of the K1 allele of *MSP-1* gene: PCR products of *P. falciparum* malaria from asymptomatic patients (ASY) (Koya and Skow villages) and symptomatic patients from Abepura General Hospital (AGH) were 295 bp, whereas that of symptomatic patients from Jayapura General Hospital (JGH) was 151 bp. M, 100-bp ladder marker; PC, positive control; NC, negative control

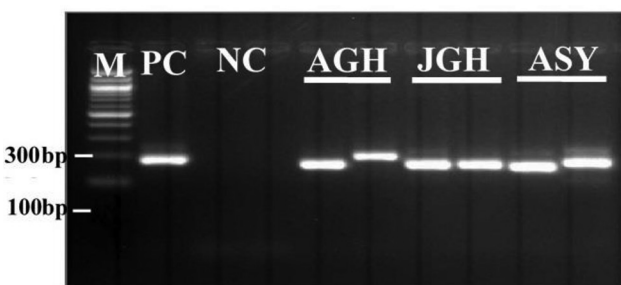


Figure 2: Electrophoregram of the MAD20 allele of *MSP-1* gene: PCR products of *P. falciparum* malaria from asymptomatic patients (ASY) (Koya and Skow villages) and symptomatic patients from Abepura General Hospital (AGH) were 106 bp and 259 bp, respectively, and that of symptomatic patients from Jayapura General Hospital (JGH) was 106 bp. M, 100-bp ladder marker; PC, positive control; NC, negative control

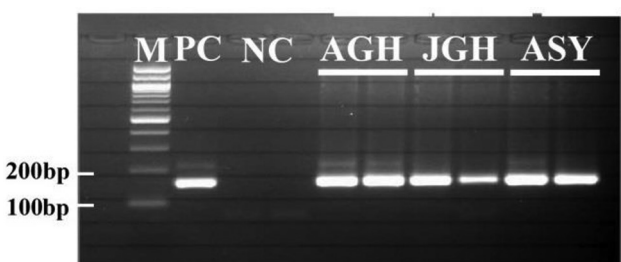


Figure 3: Electrophoregram of the RO33 allele of *MSP-1* gene: PCR products of *P. falciparum* from asymptomatic patients (ASY) (Koya and Skow villages) and symptomatic patients from Abepura General Hospital (AGH) and Jayapura General Hospital (JGH) were 153 bp. M, 100-bp ladder marker; PC, positive control; NC, negative control

From a total of 101 malaria symptomatic patients with the clinical signs and symptoms, 43.6% patients showed severe malaria and 56.4% of patients revealed mild malaria. The patients also revealed palpable spleen (34.6%), difficulty in breathing (2.97%), jaundice (29.7%), shock (0.99%), diarrhea (0.99%), and delirium (9.9%). The tympanic (ear) temperature

measurement was found above 39°C in 43.6% subjects with severe malaria and less than or equal to 39°C in 56.4% patients with mild malaria.

On the basis of age group, the patients with severe malaria were in the age groups 1–9 years (18.8%), 10–20 years (26.7%), and above 20 years (54.5%). On the basis of gender, the female patients presented with severe malaria (53.5%) higher than the male subjects (46.5%) [Table 3].

Patients with *P. falciparum* parasite who exhibited signs of delirium presented double infection with K1 and MAD20 alleles, and patients with diarrhea presented double infection with MAD20 and RO33 alleles.

The genetic diversity of the *MSP-1* gene of *P. falciparum* in the asymptomatic patients with single infection of and parasite density below 10,000/μL of blood was as follows: 77.8% patients carried K1 allele, 70.0% patients carried MAD20 allele, and 100% patients carried RO33 allele, which was higher than those found in patients with parasite density above 10,000/μL of blood. The patients with parasite density below 10,000/μL of blood carried mixed infections (double and triple infections): K1 and MAD20 alleles, 80.0%; MAD20 and RO33 alleles, 83.3%; K1 and RO33 alleles, 100%; and K1, MAD20, and RO33 alleles, 80.0% [Table 4].

In the symptomatic patients, based on malaria severity, 50% patients with severe and mild malaria carried K1 allele; 80.0% patients with mild malaria carried MAD20 allele [which was more than that found in cases with severe malaria (20.0%)]; and 50% patients with severe and mild malaria carried RO33 allele.

On the basis of mixed infection (double infections), the genetic diversity of the *MSP-1* gene of *P. falciparum* showed that 70.6% patients with severe malaria carried K1 and MAD20 alleles, which was higher than those with mild malaria (29.4%); 66.7% patients with severe malaria carried K1 and RO33 alleles, which was higher than those with mild malaria (33.3%); 77.8% patients with mild malaria carried MAD20 and RO33 alleles, which was more than those with severe malaria (33%).

On the basis of statistic Pearson's χ^2 -test, it was found that there was no significant correlation between the genetic diversity of the *MSP-1* allele of *P. falciparum* and the parasite density of asymptomatic patients ($P = 0.762$; 95%CI; $\alpha = 0.05$) [Table 5].

Pearson's χ^2 -test also showed that there was a significant correlation between the genetic diversity of the *MSP-1* of *P. falciparum* and the malarial severity of symptomatic patients ($P = 0.037$; 95%CI; $\alpha = 0.05$).

On the basis of age group of asymptomatic patients, of the children aged 1–9 years, 40% patients carried MAD20 allele, which was slightly lower than those who carried RO33 allele (66.7%) and higher than those who carried K1 allele (11.1%). In the age group 10–20 years, 44.4% patients carried K1 allele, which was more than those with MAD20 allele (11.0%) and those with RO33 allele (0%), whereas patients with mixed infection alleles (K1 and MAD20 alleles) showed the same values. In the age group older than 20 years,

Table 1: Genetic diversity of the *MSP-1* gene of *P. falciparum* among asymptomatic patients (Koya and Skow villages) and symptomatic patients (Jayapura and Abepura General Hospitals) in Jayapura municipal, Papua province

Symptomatology	Genotypes of the <i>MSP-1</i> of <i>P. falciparum</i> (%)							Total, <i>n</i> = 138
	K1 allele	MAD20 allele	RO33 allele	Mix K1 and MAD20 alleles	Mix K1 and RO33 alleles	Mix MAD20 and RO33 alleles	Mix K1, MAD20, and RO33 alleles	
Asymptomatic	9 (20.5)	10 (22.7)	3 (6.8)	9 (20.6)	2 (4.5)	6 (13.6)	5 (11.4)	44
Negative PCR	–	–	–	–	–	–	–	7
Total								51
Symptomatic	12 (12.8)	20 (21.3)	6 (6.4)	17 (18.1)	12 (12.8)	9 (9.6)	18 (19.1)	94
Negative PCR	–	–	–	–	–	–	–	7
Total								101

Table 2: Profile of symptomatic patients based on symptoms and signs, malaria severity, and genotype with a single infection of the *msp1* allele of *P. falciparum* at Jayapura and Abepura General Hospitals in Jayapura municipal, Papua province

Symptoms and signs	Number of patients with severe malaria	Percentage	Single genotype of the <i>msp1</i> allele of <i>P. falciparum</i>		
			K1	MAD20	RO33
Fever, chills, and headache	5	11.4	5	5	1
High fever, chills, headache, nausea, and vomiting	35	79	5	12	3
Splenomegaly	35	34.6	1	3	2
Difficulty of breathing	3	2.97	1	0	0
Jaundice	30	29.7	5	5	2
Shock	1	0.99	0	0	0
Diarrhea	0	0.0	0	0	0
Delirium	10	9.9	0	2	2
Temperature (°Centigrade)					
>39	44	43.6	5	5	3
≤39	57	56.4	7	15	3
Parasitemia					
>10,000 parasites/μL blood	44	43.6	6	4	3
≤10,000 parasites/μL blood	57	56.4	3	16	3
Gender					
Male subjects	47	46.5	6	10	1
Female subjects	54	53.5	6	10	5
Age (years)					
0–9	19	18.8	2	4	1
10–20	27	26.7	2	6	1
>20	55	54.5	8	10	4

Table 3: Frequency of genotypes of the *MSP-1* gene of *P. falciparum* according to parasite density of asymptomatic patients and malaria severity of symptomatic patients

Genotyping of the <i>MSP-1</i> gene of <i>P. falciparum</i>	Asymptomatic patients			Symptomatic patients		
	Parasite density		Total, <i>n</i> = 54	Malaria severity		Total, <i>n</i> = 101
	>10,000, <i>n</i> = 13	<10,000, <i>n</i> = 41		Severe malaria, <i>n</i> = 44	Mild malaria, <i>n</i> = 57	
K1 allele (1)	2 (22.2)	7 (77.8)	9	6 (50.0)	6 (50.0)	12
MAD20 allele (2)	3 (30.0)	7 (70.0)	10	4 (20.0)	16 (80.0)	20
RO33 allele (3)	0	3 (100)	3	3 (50.0)	3 (50.0)	6
Double (1 and 2)	4 (44.4)	5 (55.6)	9	12 (70.6)	5 (29.4)	17
Double (1 and 3)	0	2 (100)	2	8 (66.7)	4 (33.3)	12
Double (2 and 3)	1 (16.7)	5 (83.3)	6	2 (22.2)	7 (77.8)	9
Triple (1, 2, and 3)	1 (20.0)	4 (80.0)	5	7 (38.9)	11 (61.1)	18

Table 4: Frequency of genotypes of the *MSP-1* gene of *P. falciparum* based on age as host factor of population of the asymptomatic and symptomatic patients in Jayapura municipal, Papua province

Genotypes	Age of asymptomatic patients (years)			Total, n = 54	Age of symptomatic patients (years)			Total, n = 101
	1–9, n = 14	10–20, n = 15	>20, n = 25		1–9, n = 19	10–20, n = 27	>20, n = 55	
K1 allele (1)	1 (11.1)	4 (44.4)	4 (44.4)	9	2 (16.7)	2 (16.7)	8 (66.7)	12
MAD20 allele (2)	4 (40.0)	1 (11.0)	5 (50.0)	10	4 (20.0)	6 (30.0)	10 (50.0)	20
RO33 allele (3)	2 (66.7)	0	1 (33.3)	3	1 (16.7)	1 (16.7)	4 (66.7)	6
Double (1 and 2)	1 (11.1)	2 (22.2)	6 (66.7)	9	5 (27.8)	4 (22.2)	11 (61.1)	18
Double (1 and 3)	0	0	2 (100)	2	0	4 (33.3)	8 (66.7)	12
Double (2 and 3)	2 (33.3)	2 (33.3)	2 (33.3)	6	3 (33.3)	2 (22.2)	4 (44.4)	9
Triple (1, 2, and 3)	0	2 (40.0)	3 (60.0)	5	3 (17.6)	5 (29.4)	7 (41.2)	17

Table 5: Frequency of genotypes of the *MSP-1* gene of *P. falciparum* based on genders of host factors of population of the asymptomatic and symptomatic patients in Jayapura municipal, Papua province

Genotypes	Genders of asymptomatic patients (age in years)			P	Genders of symptomatic patients (age in years)			P
	Male subjects, n = 37	Female subjects, n = 17	Total, n = 54		Male subjects, n = 47	Female subjects, n = 54	Total, n = 101	
K1 allele (1)	7 (77.8)	2 (22.2)	9	0.865	6 (50.0)	6 (50.0)	12	0.362
MAD20 allele (2)	7 (70.0)	3 (30.0)	10		10 (50.0)	10 (50.0)	20	
RO33 allele (3)	1 (33.3)	2 (66.4)	3		1 (16.7)	5 (83.3)	6	
Double (1 and 2)	3 (60.0)	2 (40.0)	5		9 (50.0)	9 (50.0)	18	
Double (1 and 3)	2 (10.0)	0	2		4 (33.3)	8 (66.7)	12	
Double (2 and 3)	4 (66.7)	2 (33.3)	6		7 (77.8)	2 (22.2)	9	
Triple (1, 2, and 3)	6 (66.7)	3 (33.3)	9		8 (47.1)	9 (52.0)	17	

50.0% patients carried MAD20 allele, which was slightly higher than those with K1 allele (44.4%), whereas for double infections, 100% patients carried K1 and RO33 alleles, which was higher than those with K1 and MAD20 alleles (66.7%) and those with MAD20 and RO33 alleles (33.3%).

On the basis of statistic Pearson's χ^2 -test, it was found that there was no significant correlation between the age group of asymptomatic patients and the genetic diversity of the *MSP-1* of *P. falciparum* ($P = 0.350$; $\alpha = 5\%$).

On the basis of age group of symptomatic patients, 4 (20%) children carried MAD20 allele, which was slightly higher than the subjects who carried K1 and RO33 alleles (16.7%). For the double infections, it was found that 5 (27.8) subjects carried K1 and MAD20 alleles, which was higher than those who carried K1 and RO33 alleles. In the age group of 10–20 years, 30.0% patients carried MAD20 allele, which was more than those with K1 and RO33 alleles (16.7%), whereas patients with triple infections (K1, MAD20, and RO33 alleles), were slightly more than those with double infections (K1 and MAD20 alleles and K1 and RO33 alleles). For those who were older than 20 years, 50.0% patients carried MAD20 allele, which was slightly lower than K1 allele (66.7%) and RO33 allele (66.7%), whereas in the double infections, 66.7%

patients carried K1 and MAD20 alleles, which was slightly more than the triple infections of K1, MAD20, and RO33 alleles.

On the basis of statistic Pearson's χ^2 -test, it was found that there was no significant correlation between the age group of symptomatic patients and the genetic diversity of the *MSP-1* of *P. falciparum* ($P = 0.874$, $\alpha = 5\%$).

On the basis of gender, it was found that the genotypes of the *MSP-1* gene of *P. falciparum* with single infection was as follows: 77.8% patients carried K1 and 70.0% patients carried MAD20 alleles, and was found to be slightly high in the asymptomatic male than that in female patients. However, the patients who carried triple infections (K1, MAD20, and RO33 alleles) were more of male than female patients.

On the basis of statistic Pearson's χ^2 -test, it was found that there was no significant correlation between the genetic-diversity of the *MSP-1* allele and the gender of asymptomatic patients ($P = 0.865$; $\alpha = 5\%$).

On the basis of gender of the symptomatic patients, the male and female subjects were found to be equal in number for K1 and MAD20 alleles (50% each). Similar results were also obtained for mixed infections in people who carried both K1 and MAD20 alleles, i.e., 50.0% male subjects and 50.0% female subjects.

On the basis of statistic Pearson's χ^2 -test, it was found that there was no significant correlation between the genetic diversity of the *MSP-1* allele and the gender of asymptomatic patients ($P = 0.362$; $\alpha = 5\%$).

Discussion

This study provided the estimate of genetic diversity of the *MSP-1* (block 2) of *P. falciparum* infections in Jayapura municipal region. Malaria transmission in this region is still endemic and seasonal, with mainly asymptomatic infections seen in children to adults at the PHCs and Jayapura and Abepura General Hospitals in Jayapura municipal.

Molecular epidemiological studies can be used to study the genetic diversity of infection in relation to various factors such as transmission intensity, diseases phenotype, and host immunity.^[23] Such cross-sectional studies are conducted mainly with asymptomatic and symptomatic patients and not done in the highly endemic regions with the disease outbreak.

On the basis of PCR, it was found that *MSP-1* gene (block 2) of *Plasmodium falciparum* in populations of asymptomatic and symptomatic patients in Jayapura municipal showed a highly complex genetic diversity, almost similar to that found in Myanmar.^[6] The PCR fragment size of the K1 allele of *MSP-1* gene was found to be 151–295 bp, MAD20 allele 106–259 bp, and RO33 allele 153 bp. They were identified as shown in Figures 1–3, respectively. The DNA length of PCR products of K1, MAD20, and RO33 alleles were found to be different in asymptomatic and symptomatic patients in Papua.

The result obtained from unicipal Jayapura was not concurrent with that reported from Myanmar.^[6] This study showed that genetic heterogeneity within the parasite population may influence the ultimate clinical signs and symptoms of malaria disease.

We further investigated the allele's diversity of *MSP-1* gene of *P. falciparum*, and the sequence analysis of *MSP-1* was performed. The sequence analysis of *MSP-1* block 2 showed a total of 48 alleles of *MSP-1* gene: 16 samples of each K1, MAD20, and RO33 alleles were identified. Allele diversity of *MSP-1* gene in block 2 of *P. falciparum* Papua isolates was owing to the different numbers of unique tripeptides repeats, which is similar to the reported studies [Figure 4].^[6]

The recombination rate could be assumed through examining linkage disequilibrium between the diversity loci in *MSP-1* at the blood stage in the natural populations. In an area of high-transmission intensity, linkage disequilibrium was seen in immune populations.^[24]

For the asymptomatic patients, the frequency of types of the *MSP-1* allele of *P. falciparum* was found as follows: in patients with single infection, MAD20 allele was more dominant than K1 and RO33 alleles from Koya and Skow villages. The patients with double infections revealed that combination of K1 and MAD20 alleles was more than the combinations of MAD20 and RO33 alleles and K1 and RO33 alleles. Certain

regions of the sample did not contain these alleles as they coded *MSP-2* alleles (block 3).^[25]

The sample of asymptomatic patients carried K1, MAD20, and RO33 alleles. In the asymptomatic patients with severe malaria, the cases occurred because, although patients have been cured, recurrence was seen as the patients blood still contained *P. falciparum* parasite of above 10,000 parasite/ μ L of blood in PHCs of Koya and Skow villages. According to Tanabe and coworkers,^[20] the mean number of *MSP-1* alleles per infected person reflects the intensity of malaria transmission. In high-transmission areas, the mean number would be higher than in low-transmission areas, because inoculations of new *MSP-1* allele type result through repeated mosquito biting.

In the symptomatic patients, it was found that the patients with single infection with MAD20 allele, more dominant than K1 and RO33 alleles, were found at Jayapura and Abepura General Hospitals in Jayapura municipal, Papua province. The malarial cases were almost similar to those reported from Myanmar^[6] and consistent with the same situations that prevail in Thailand, Iran, Pakistan, and Colombia.^[11,15,24,25] In the patients with mixed infections, K1 and MAD20 alleles were higher than MAD20 and RO33 alleles, and K1 and RO33 alleles, whereas in the symptomatic patients, triple infection was found slightly higher.

Difference in the prevalence of *MSP-1* gene could be influenced by different geographic areas, longitudinal changes in frequency distribution of *MSP-1* gene in determining the immune response, and frequency of heterozygosis in oocytes, in which meiotic recombination of *MSP-1* gene is in a large number of field samples than that in hospital samples in Jayapura municipal.^[25]

On the basis of parasitemia of density above 10,000/ μ L of blood, it was found that RO33 allele of the *MSP-1* gene (block 2) of *P. falciparum* was not present in asymptomatic patients. However, RO33 allele was found in symptomatic patients with the number of parasite density above 10,000/ μ L of blood or less than 10,000/ μ L of blood.

The study showed that there was no significant correlation between the genetic diversity of the *MSP-1* gene of *P. falciparum* and the malaria severity, parasite density, and age groups and genders of asymptomatic patients, besides malaria severity and parasite density of symptomatic patients; but there is no significant correlation with age groups and gender in Jayapura municipal, Papua. The difference in the number and frequency of genotypes of the *MSP-1* allele at different age groups with or without clinical signs and symptoms of malaria may suggest the presence of such immune responses.

The ratio between asymptomatic (immune) and symptomatic (nonimmune) patients would be of particular interest. This study was similar to that reported by Tanabe et al. from Osaka, Japan.^[23]

The *MSP-1* alleles that were more dominant was MAD20 allele for single infection and K1 and MAD20 alleles for mixed infection. The RO33 allele was found in symptomatic patients. In the symptomatic population of the Jayapura municipal,

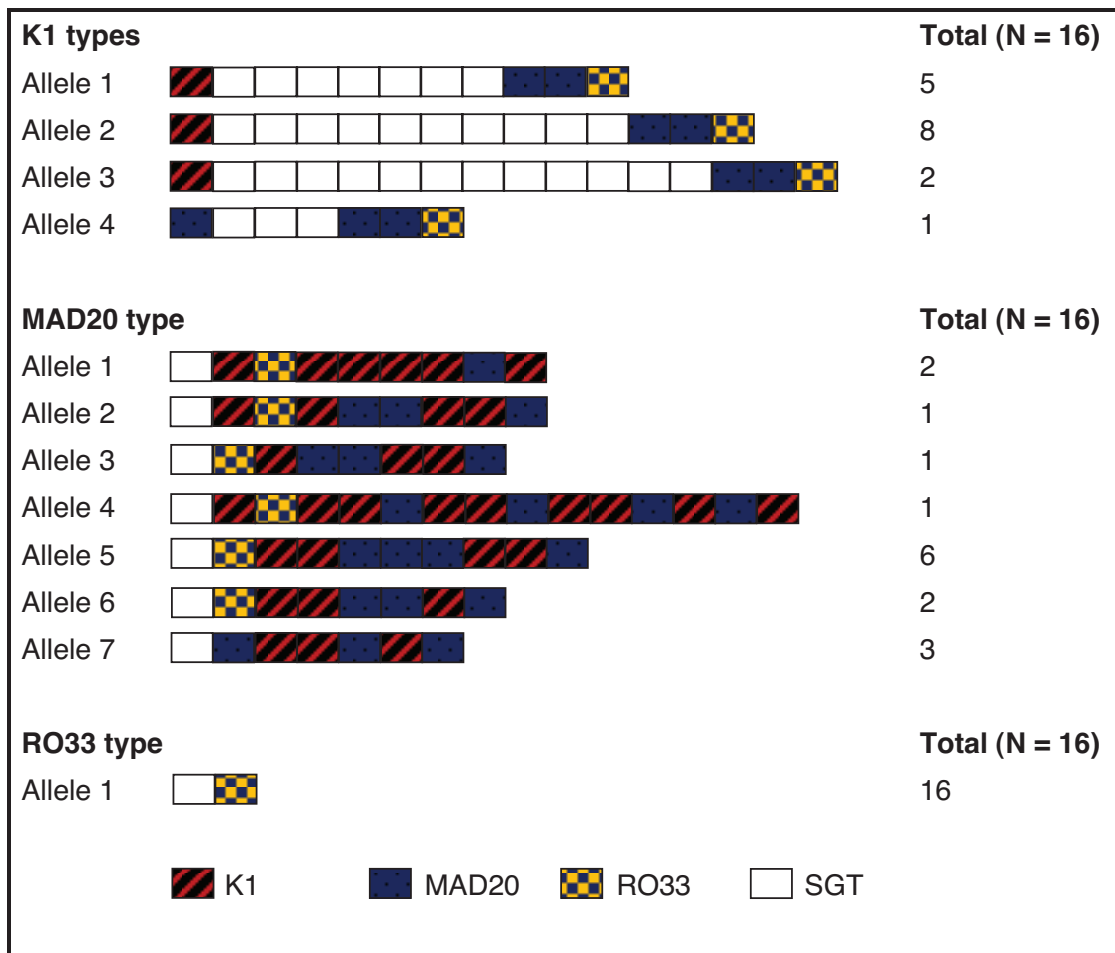


Figure 4: Scheme of the *MSP-1* gene block 2 of *P. falciparum* isolates from Jayapura municipal, Papua. A total of 16 different alleles with parasitemia of density above 10,000/ μ L of blood was identified by sequence analysis of the *msp1* allele in block 2. K1 allele type showed difference in the number and arrangement based on amino acid as follows: SAQ, SGT, SGA, and SGP. MAD20 allele type showed difference in the number and arrangement based on amino acid as follows: SGG, SVT, SKG, and SVA. RO33 allele showed only SGT and SVT. Depending on the position type, the results were as follows: K1 allele, SGG and SAQ; MAD20 allele, SGP and SVA; RO allele, SGA and SVT.

severe malaria was found in patients with single infection with K1 and MAD20 alleles, and in symptomatic patients with mixed infection, K1 and MAD20 alleles combination was higher than the combinations of K1 and RO33 alleles and K1, MAD20, and RO33 alleles. This study was similar to those in the other places, but in Senegal study, it was found that RO33 allele type showed a higher incidence of severe malaria.^[25] However, *MSP-1* gene could be used as candidate molecular vaccine in Jayapura municipal, Papua province.

Clinical manifestations of malaria are influenced by various factors in human host, parasite, and environment. In humans, age, immunity, pregnancy, and genetic factors have been shown to determine the malaria clinical outcome; in the malaria parasite, drug resistance, multiplication rate, invasion pathways, cytoadherence and resetting, antigenic variation

and polymorphism, and malaria toxin are among the other factors that have been identified.^[26]

The genetic structure of *P. falciparum* plays a highly important role in the natural acquisition of immunity in malaria infection.^[27,28] The knowledge of the genetic structure of *P. falciparum* is necessary to develop to control the disease, including the design of effective vaccines against *P. falciparum* parasite. The study showed that genetic polymorphism of *MSP-1* of 152 bp of *P. falciparum* isolate collected in Jayapura municipal, where malaria is endemic to being of meso- to holo-endemic status in the northeastern coast and highland parts of the province.^[29-31]

In hyperendemic areas such as Jayapura municipal, the patients tend to get affected with more infection with alleles single parasite infection, which favors genetic recombination

and generates a higher genetic diversity, which is similar to that reported from Colombian village.^[32] In addition to transmission intensity, other factors such as selective sweeps, resulting of vaccines, and antimalaria drugs can greatly influence genetic diversity as a similarity which was found in China–Myanmar.^[33]

Conclusion

The study showed that the length of DNA bands found was different with another country, and there was no significant correlation between genetic diversity of the *MSP-1* gene of *P. falciparum* and its malaria severity, parasite density, and age group and gender of asymptomatic and symptomatic patients. There was a significant correlation with the age group and gender of symptomatic patients. Allele genetic of the *MSP-1* gene had found that more dominant was MAD20 allele for single infection of mild malaria, whereas mixed infection of K1 and RO33 alleles were the same or higher of severe and mild malaria of symptomatic patients. The malaria severity increased as K1 allele increased than MAD20 allele of severe malaria and mild malaria. The parasite density, age group, and gender found MAD20 allele to be higher for single infection, and K1 and MAD20 alleles combination was higher in the asymptomatic and symptomatic patients. The asymptomatic patients who carried K1, MAD20, and RO33 alleles showed a higher parasite density below 10,000/μL of blood. The RO33 allele of *MSP-1* block 2 was found in symptomatic patients with parasite density above 10,000/μL of blood. However, *MSP-1* gene could be used as a genetic marker to determine the genetic diversity of *P. falciparum* as candidate molecular vaccine in Jayapura municipal, Papua province, Indonesia.

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