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FREQUENCY DISTRIBUTION OF *Plasmodium falciparum* MSP-2 ALLELE AND ASSOCIATION WITH CLINICAL MANIFESTATIONS AND DEMOGRAPHIC FACTORS IN JAYAPURA MUNICIPAL, PAPUA PROVINCE, INDONESIA

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Abstract- The merozoite surface protein-2 (*msp2*) of *Plasmodium falciparum* is a polymorphic glycoprotein expressed on the surface membrane of the merozoite, and plays major role in the erythrocyte invasion. Certain *msp2* allele has been associated with malaria severity. The present study aims to explore the association between the msp2 allele with the clinical manifestations and demographic factors in Papua, Indonesia. Malaria asymptomatic cases were obtained from the community health centers in Koya and Skow villages, whereas symptomatic cases were obtained from Jayapura and Abepura General Hospitals. A total of 152 blood samples, 51 asymptomatic and 101 symptomatic subjects, were analyzed by microscopic examination and PCR amplification. The results indicated that the IC1/3D7 allele was the dominant allele in both groups, with the frequency distribution in asymptomatic malaria was 52.9% for IC1/3D7 allele, 19.6% for FC27 allele, and 27.5% for mix of IC1/3D7 and FC27 alleles. In symptomatic malaria cases, the frequency distribution was 50.5% for IC1/3D7allele, 22.8% for FC27 allele, and 26.7% for mix of IC1/3D7 and FC27 alleles. Similarly in the symptomatic subjects, IC1/3D7 allele was shown to be more frequently detected than FC27 allele in both level of parasite density. Statistical analysis using Pearson Chi-square showed that there is no significant correlation between the *P. falciparum* msp2 allele and clinical manifestation, parasite density, age and gender. FC27 allele seemed to be increased in frequency in severe malaria and females subjects.

Keywords- Clinical manifestation, Malaria severity, msp2 allele, *Plasmodium falciparum*

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Introduction

Malaria poses 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 risk of malaria transmission. The incidence of malaria worldwide is estimated to be 350-500 million clinical cases of malaria each year, and most of them are caused by *Plasmodium falciparum* and *P. vivax* [1]. It is also estimated that the parasite causes illness in 500 million individuals and approximately 1.2 million deaths per year. Children below five years of age and pregnant women make up the majority of individuals that succumb to this infection [2,3]. In Indonesia, malaria represents a major public health problem especially in eastern islands, including Papua Province. In Papua, 1.9 million people live in risk area and all of the four species of human malaria; *P. falciparum*, *P.vivax*, *P. malariae*, and *P. ovale* existed. In stable transmission areas, high proportions 50% of population are observed to be of intermediate risk [4]. Merozoite surface protein-2 (MSP2) is a glycoprotein expressed on the surface membrane of the merozoite stage and is produced during schizogony stage and released at the sporulation stage, particularly during merozoite invasion into erythrocyte. This protein in *Plasmodium falciparum* is highly polymorphic and the encoding *msp2* gene has two allelic forms, IC1 and FC27. In Senegal and Kenya, the IC1 allele is more frequently found in severe malaria patients [5] whereas FC27 allele is more frequent in asymptomatic malaria [6]. By contrast, in Papua New Guinea, the FC27 allele is more frequent is severe malaria cases [7]. In West Irian Jaya, FC27 allele was found in the majority of individuals who were infected by parasite *P. falciparum* [7]. Before this study, we had been doing research about allele dimorphic of Eba-175 gene of *Plasmodium falciparum* and associated with clinical manifestations in Jayapura District, Papua Province [8]. The development about malaria cases we will be doing it in Papua Province. The present study aims to investigate the association between the *msp2* alleles and the clinical manifestations, severity, parasite density and demographic factors.

Material and Methods

Study Site and Population

The study was conducted in Jayapura Municipal, Papua Province, Indonesia [Fig-1], with total population in 2013 approximately 120,000 people. It is located in Latitude 2°31'58,8" North and

longitude 140°43'1,2" East. The climate is typically tropical with drier season during April to SeAtember and wet season during October to March. The habitants of Jayapura Municipal is mainly Papuan and migrant from Java, Sulawesi, Mollucas, and other parts of Indonesia. A total of 152 blood samples were collected in the following study sites: Koya (41 samples); Skow village (10 samples); Jayapura General Hospital (41 samples) and Abepura General Hospital (60 samples). The age of the subjects ranged from 1-65 years old. For the asymptomatic subjects the selected villages represented the coastal, rice field, and inland environmental setting of malaria. The target population included the village resident who joined voluntarily. During the survey, 7 days morbidity history of the subjects including ear (tympanic) temperature of children and adult subjects were taken. Malaria infectivity of the subjects were determined by microscopic identification of the parasite with using Giemsa- stained blood smears. The clinical manifestations were determined by history of illnes and phisical subjects were 101, 44 subjects with the symptom of severe malaria (i.e., delirium, hyperpirexia, difficulty of breathing) and were classified as severe malaria whereas 57 were classfied as mild malaria. The demographic character (i.e., age, gender) and clinical findings of each subjects were also registrated. All the bloods samples were collected after informed consent and use of the samples for this study was approved by Research Health Ethic Committee, Faculty of Medicine of Hasanuddin University, Makassar, Indonesia.

Fig. 1- Study site in Jayapura District, Papua, Indonesia. Circle 1 = Skow village; Circle 2= West Koya village; East Koya Village; Triangle 1 = Jayapura General Hospital; Triangle 2 = Abepura General Hospital.

DNA Extraction and PCR Amplification

Genomic DNA of *P. falciparum* was extracted of the filter paper from asymptomatic and symptomatis subjects using Chelex-100 methods [9]. PCR amplification using both a primary and nested PCR were performed with each DNA sample. For the primary PCR, 5µL of the extracted DNA were added to 95µL of reaction mix including 1.5 units of Taq polymerase (Gibson BRL Life Technology), 50mM KCl, 10mM Tris HCL (pH 8.8), 1.5mM $MgCl_2$, 0.2 mM of each primer pair. The primer pair for the primary PCR corresponded to nucleotides 3-23 and 781-811 from the 5' and 3' conserved region of the MAD71 sequence of *msp2* gene [10]. The PCR conditions were 5 min at 95ºC, 2 min at 58ºC, and 2 min at 72ºC. The amplification of the nested reaction (total volume 100µL) with nested primers corresponding to the nucleotides 111-129 and 709-728 of the same sequence of the *msp2* gene. The cycle conditions for the nested PCR were the same as those for the primary reaction with 25 cycles of the first PCR and 35 cycles of second PCR. The PCR products using 2 % agarose gels and stained with ethidium bromide and the DNA band visualized by ultra violet transillumination.

MSP-2 Block 3 Allele Typing

The positive samples of the *msp2* gene of *P. falciparum* from asymptomatic and symptomatic subjects as a second PCR analysis using primers of the IC1/3D7 allele and FC27 allele as described [10].

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 IC1/ 3D7 and FC27 alleles were sequence forward and reverse and the results were compared with the published sequence (Accession no: GQ890871 and U72948).

Statistical Analysis

To determine the association between frequency distribution of the msp2 allele with clinical manifestations, malaria severity, parasitemia of density, age, and gender a statistical analysis was performed by using Chi- square (X^2).

Results

Through active case detection in the community health centers in Skow and Koya villages, 157 subjects were enrolled and 51 asymptomatic subjects was found positive for *P. falciparum.* By passive case detection in two hospitals in Jayapura, 415 symptomatic subjects were likewise enrolled in the study and 101 were found positive for *P. falciparum.* The clinical manifestations of malaria that came out from the interview were also presented [Table 1].

Based on active case detection, a high prevalence of malaria cases was found at Koya village. Koya village had more malaria cases 41 (80.4%) than in Skow 10 (19.6%).

The symptomatic subjects were selected from 415 malaria cases admitted to Jayapura and Abepura General Hospital in Jayapura Municipal. After doing the semi-quantitative microscopic examination, it was found that 101 subjects suffered from falciparum malaria, single infection. The PCR examination targeting the *msp-2* gene indicated that 101 subjects had positive DNA bands, 60(59.4%) from Abepura General Hospital and 41(40.6%) from Jayapura General Hospital. Analysis of the 101 malaria symptomatic subjects revealed 43.6% subjects suffered from severe malaria and 56.4% subjects had mild malaria. The spleen was palpable in 35 subjects (34.6%), difficulty of breathing in 3 (2.97%), jaundice in 30 (29.7%), shock in 1(0.99%), diarrhea in 1 (0.99%), and delirium in 10(9.9%), respectively. The ear (tympanic) temperature was >39ºC in 44 (43.6%) who have severe malaria and temperature was ≤ 39ºC in 57 (56.4%) who have mild malaria.

Table 1- Prevalence of Malaria based on Blood Smear and PCR at Community Health Centers and General Hospitals in Jayapura Municipal, Papua Province

Clinical Manifestations	Study sites	Malaria cases n=572	Number of blood smears positive subjects (%) n =155	Number of PCR positive subjects (%) n= 152
Asymptomatic	Skow	57	11 (20.4)	10 (19.6)
	Koya	100	43 (79.6)	41 (80.4)
	Total	157	54	51
Symptomatic	Jayapura General Hospital	120	41 (40.6)	41 (40.6)
	Abepura General Hospital	295	60 (59.4)	60 (59.4)
	Total	415	101	101

Table 2- Profile of symptomatic subjects based on clinical signs and symptoms of malaria at the Jayapura and Abepura General Hospital in Jayapura Municipal, Papua Province

Clinical signs and symptoms	Number of subjects with severe malaria	(%)	Number of subjects with mild malaria	(%)
Fever, chills, headache	5	(11.4)	34	(60)
High fever, chills, headache, nausea, vomiting	35	(79)	23	(40)
Splenomegaly	35	(34.6)	0	(0.0)
Difficulty of Breathing	3	(2.97)	0	(0.0)
Jaundice	30	(29.7)	14	(13.9)
Shock	1	(0.99)	0	(0.0)
Diarrhea	0	(0.0)	1	(0.99)
Delirium	10	(9.9)	0	(0.0)
Temperature (ºC) >39ºC	44	(43.6)	0	(0.0)
Temperature (ºC) ≤ 39ºC	0	(0.0)	57	56.4)
Parasitemia >10,000 parasites/µL blood	44	(43.6)	0	(0.0)
Parasitemia ≤ 10,000 parasites/µL blood	0	(0.0)	57	(56.4)

Analysis of the *P. falciparum msp2* Allele

Amplification of *P. falciparum msp2* Allele of by PCR

The *msp2* gene of *P. falciparum* was amplified by nested PCR from asymptomatic and symptomatic blood samples from subjects with falciparum malaria in Jayapura, Papua, Indonesia.

Fig. 2- Electropherogram of the PCR products showing the DNA band of IC1 or 3D7 allele.

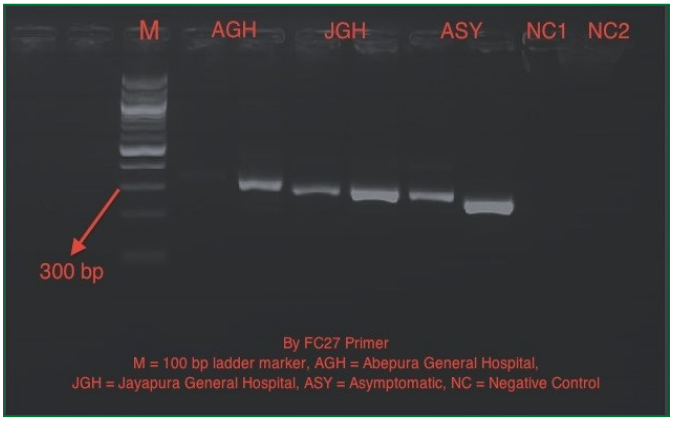

Fig. 3- Electropherogram of the PCR products showing the DNA band of FC27 allele from Abepura General Hospital (AGH), Jayapura General Hospital (JGH) and asymptomatic (ASY) from Skow and Koya villages with the Negative Control (NC) and molecular mass Marker (M) ladder only in Jayapura Municipalities, Papua, Indonesia.

The electropherogram of the nested PCR products showed that

there are DNA bands with length variants of approximately 470 - 600bp for the IC1/3D7 allele [Fig-2] and 260 - 385bp for the FC27 allele, as expected [Fig-3], respectively and in amino acid sequence alignment for the IC1/3D7 allele [Fig-3] and FC27 allele [Fig-4]. 3D7 allele of two subjects each from Abepura General Hospital (AGH), Jayapura General Hospital (JGH) and asymptomatic (ASY) from Skow and Koya villages with Positive Control (PC), Negative Control (NC) and Moleculer Mass Marker (M) ladder in Jayapura Municipal, Papua, Indonesia.

In amplifying the *msp2* gene, specificity of the PCR products was ensured by including distilled water (ddH2O) as negative control. To ensure that the DNA of the PCR products corresponds to the target DNA, DNA sequencing was performed.

Based on the PCR products of the 51 asymptomatic malaria cases, it was found that 27(52.9%) subjects carried the IC1/3D7 allele, 10 (19.6%) subjects had FC27 allele, and 14 (27.5%) carried mix IC1/3D7 and FC27 alleles, respectively.

Using prevalence ratio (PR), it was found that the association of IC1/3D7 and asymptomatic malaria is statistically significant (PR=1.5>0.05; CI=95%; α = 0.05) [Table 3].

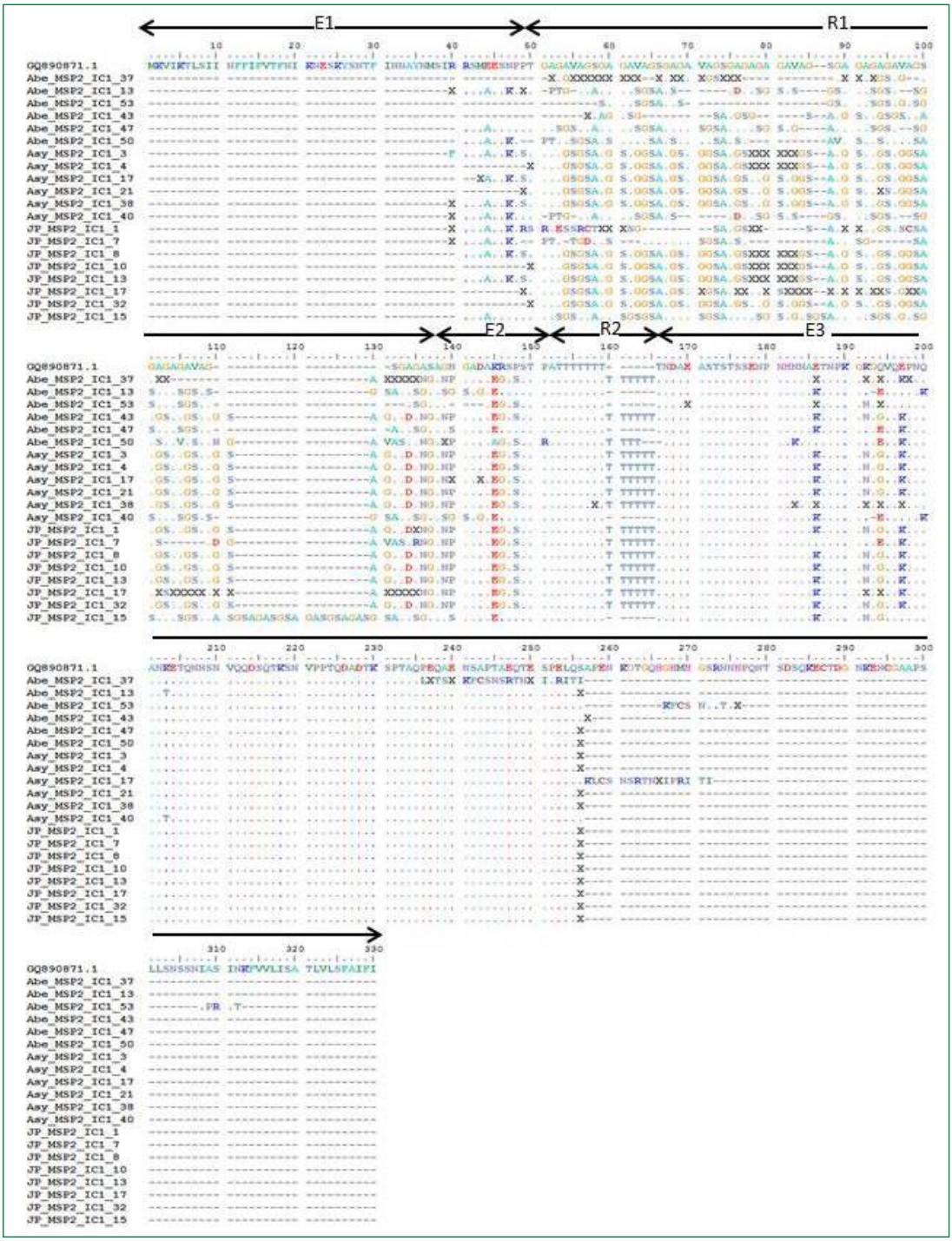

Fig. 4- Sequence alignment of the predicted amino acid sequences of IC1/3D7 allele type of the *msp*-2 gene of *P. falciparum* from Jayapura Municipal,Papua, Indonesia.

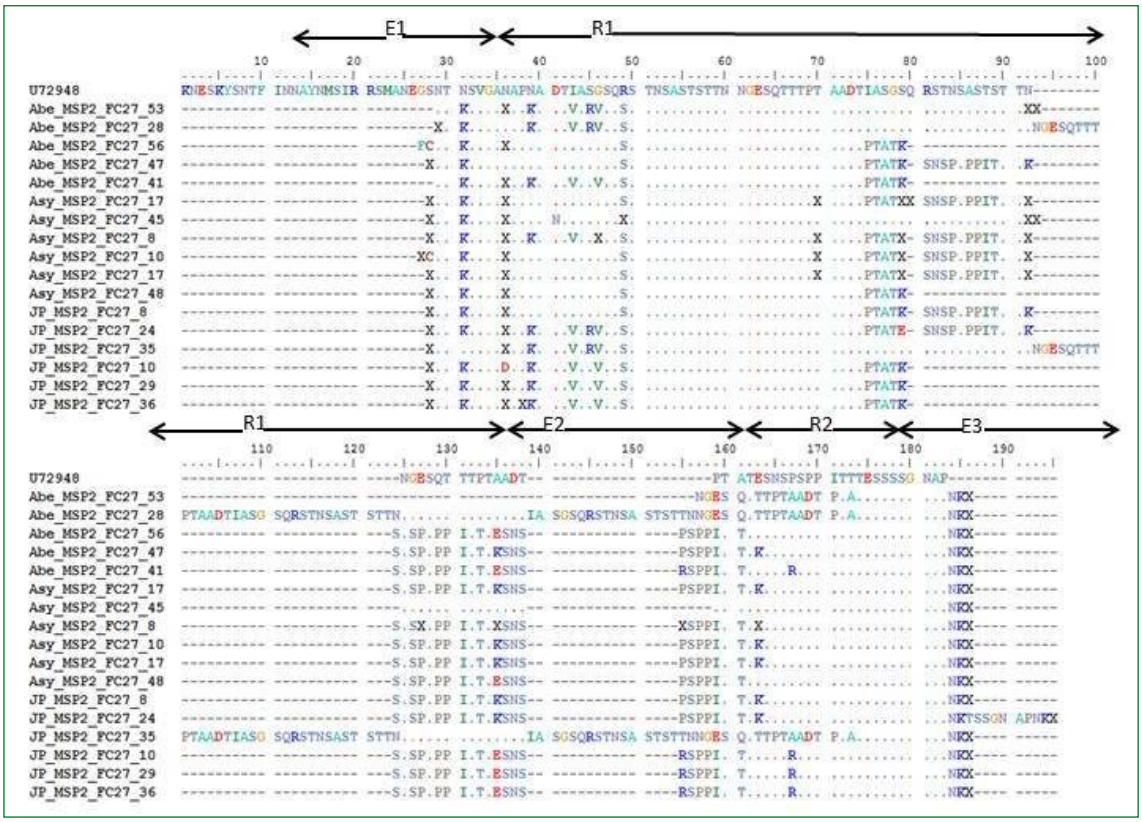

Fig. 5- Sequence alignment of the predicted amino acid sequences of FC27 allele type of the *msp-2* gene of *P. falciparum* from Jayapura Municipal, Papua, Indonesia.

Table 3-Frequency distribution of geno- types of the *msp2* gene of *P. falciparum* among asymptomatic subjects and symptomatic subjects in Jayapura Municipalities.

Clinical manifestations	Genotypes (%)			Total n= 152
	IC1/3D7 allele	FC27 allele	Mix of IC1/3D7 and FC27 alleles	
Asymptomatic	27 (52.9)	10 (19.6)	14 (27.5)	51
Symptomatic	51(50.5)	23 (22.8)	27(26.7)	101

From the 101 symptomatic malaria cases, 51(50.5%) subjects had IC1/3D7 allele, 23 (22.8%) subjects had FC27 allele, and 27(26.7%) subjects carried mix IC1/3D7 and FC27 alleles. Likewise, the association of IC1/3D7 and symptomatic malaria is statistically significant (PR=1.38>0.05; CI=95%; α=0.05). [Table 3].

Malaria Severity and *msp2* Allele

Among the 101 symptomatic cases with severe malaria, it was found that 20 (45.4%) carried IC1/3D7 allele, 12 (27.3%) carried FC27 allele, and 12(27.3%) carried mix IC1/3D7 and FC27 alleles, respectively. Whereas those with mild malaria, it was found that 31 (54.4%) carried IC1/3D7 allele, 11(19.3%) carried FC27 allele, and 15(26.3%) carried mix IC1/3D7 and FC27 alleles [Table 4].

Table 4- Frequency distribution of genotypes of the *msp2* gene of *P. falciparum* according to malaria severity

Malaria Severity	Genotypes of the msp2 gene of P. falciparum (%)			Total
	IC1/3D7 allele	FC27 allele	Mixed of IC1/3D7 and FC27 alleles	
Severe	20 (45.4)	12 (27.3)	12 (27.3)	44(100)
Mild	31(54.4)	11(19.3)	15(26.3)	57(100)

Statistical analysis indicated that there is no significant correlation between clinical manifestations (severe or mild malaria) and genotypes of the *msp2* gene of *P. falciparum* (P-value = 0.579 > 0.05; CI = 95%; α = 0.05).

Association of the *P. falciparum msp2* Gene and Parasite Density in Asymptomatic Subjects

Parasite Density *and msp2* Allele

Among the 51 asymptomatic malaria cases, IC1/3D7 allele was more frequently detected in low density of parasitemia (less than 10,000 parasites/µL of blood) at 18 (66.7%) as well as high parasitemia (above 10,000 parasite/µL of blood) at 9 (33.3%). FC27 allele was less frequently distributed in both levels of parasitemia density. Statistical analysis indicated that there is no significant correlation between parasite density of asymptomatic subjects and genotypes of the *msp2* gene of *P. falciparum* (P-value 0.323 > 0.05; CI= 95%; α = 0.05). (For severe malaria, the cut-off is >10,000 µL/blood).

Association of *P. falciparum msp2* Gene and Demographic Factors

Age and *msp2* Allele

Among the 51 asymptomatic malaria cases, it was found that IC1/3D7 allele was more frequently detected than FC27 allele in ages less than 10 years at 7 (26%) and, 10 - 20 years old at 9 (33%). For the age group 21-30 years old, it was found that 8 (80%) had FC27 allele. For the age group above 30 years old, it was found that 3 (11%) carried IC1/3D7 allele and 2 (14.2%) carried mix IC1/3D7 and FC27 alleles. Statistical analysis indicated that there is no significant correlation between age of asymptomatic subjects

and genotypes of the *msp2* gene of *P. falciparum* (*P value*=0.171 > 0.05; CI= 95%; α = 0.05).

Gender and *msp2* allele. Regarding gender and genotypes among 51 asymptomatic malaria cases, it was found that more males 18 (66.7%) had IC1/3D7 allele compared to females with 9 (33%). Statistical analysis indicated that there is no significant correlation between gender of asymptomatic subjects and genotypes (*P-value* = 0.958 > 0.05; CI, 95%; α = 0.05).

Table 5- Frequency distribution of *msp2* gene of *P. falciparum* based on parasite density and demographic factors of asymptomatic subjects in Jayapura Municipal

Variables		Genotypes of the msp2 gene of P. falciparum (%)			Total	P-value
		IC1/3D7 allele	FC27 allele	Mixed of IC1/3D7 allele		
Parasite Density (parasites/ µL blood)	>10,000	9 (33.3)	1 (10)	3(21.4)	13	
	≤10,000	18 (66.7)	9 (90)	11(78.6)	38	0.323
	Total	27 (100.0)	10 100)	14(100.0)	51	
Age (years old)	0-9	7 (26)	1 (10)	4 (28.6)	12	
	10-20	9 (33)	1 (10)	4 (28.6)	14	
	21-30	8 (30)	8 (80)	4 (28.6)	20	0.171
	>30	3 (11)	0	2 (14.2)	5	
	Total	27 (100)	10 (100)	14(100.0)	51	
Gender	Male	18 (66.7)	7 (70)	9 (64.3)	34	
	Female	9 (33.3)	3 (30)	5 (35.7)	17	0.958
	Total	27 (100.0)	10 (100)	14(100.0)	51	

Table 6- Association of *msp2* gene of *P. falciparum* and malaria parasite density and demographic factors in symptomatic subjects at Jayapura and Abepura General Hospital in Jayapura Municipal Association of the *P. falciparum msp2* gene and parasite density in symptomatic subjects

Variables		Genotypes of the msp2 gene of P. falciparum (%)			Total	P-value
		IC1/3D7 allele	FC27 allele	Mixed of IC1/ 3D7 and FC27 alleles		
Parasite Density (parasites/µL blood)	>10,000	20 (39.2)	12 (52.2)	12(44.4)	44	
	≤10,000	31 (60.8)	11 (47.8)	15(55.6)	57	0.579
	Total	51 (100)	23 (100)	27(100)	101	
Age (years old)	0- 9	6(11.8)	6 (26.1)	5 (18.5)	17	
	10-20	14(27.4)	8 (34.8)	7(25.9)	29	
	21-30	15(29.4)	6 (26.1)	8(29.6)	30	0.639
	>30	16(31.4)	3 (13.0)	7(26.0)	25	
	Total	51(100)	23(100)	27(100)	101	
Gender	Male	29 (56.9)	8 (34.8)	10 (37)	47	
	Female	22(43.1)	15 (65.2)	17(63)	54	0.106
	Total	51(100)	23 (100)	27 (100)	101	

Parasite Density and *msp2* Allele

Among the 101 symptomatic malaria cases, IC1/3D7 allele was more frequently detected in parasitemia above 10,000 parasite/µL blood at 20 (39.2%), and those with parasitemia less than 10,000 parasite/µL blood at 31 (60.8%). FC27 allele was slightly more frequently detected among subject with parasitemia above 10,000 parasite/µL of blood. Statistical analysis indicated that there is no significant correlation between malaria severity of symptomatic subjects and genotypes of the *msp2* gene of *P. falciparum* (*P-value* 0.579 > 0.05; CI= 95%; α = 0.05).

Association of the *P. falciparum msp2* allele and demographic factors:

Age and *msp2* allele. Among the 101 symptomatic malaria cases, IC1 /3D7 allele was more frequently detected than FC27 in all age groups: 6 (11.8%) in less than 10 years old; 14 (27.4%) in 10 - 20 years old; 15 (29.4%) in 21 - 30 years old; and 16 (31.4%) in above 30 years old. Based on statistical analysis, it was determined that there is no significant correlation between age of asymptomatic patients and genotypes of the *msp2* gene of *P. falciparum* (*P-value* 0.639 > 0.05; CI= 95%; α = 0.05).

Gender and *msp2* Allele

Regarding gender and genotypes of 101 symptomatic malaria cases, it was found that 29 (56.9%) males had IC1/3D7 allele. Whereas in females, it was found that 22 (43.1%) had IC1/3D7 allele. However, FC27 allele was more frequently detected among females at 15 (65.2%). Based on statistical analysis, it was determined that there is no significant correlation between gender of symptomatic subjects and genotypes of the *msp2* gene of *P. falciparum* (*P value* = 0.106 > 0.05; CI, 95%; α = 0.05).

Discussion

Malaria exhibits a wide spectrum of clinical manifestation in human, from asymptomatic infection to severe and fatal cerebral complication. Several factors in the malarial parasite, human host and environment have been implicated. Factors in the malarial parasite include drug resistance, multiplication rate, invasion pathway, cytoadherence, rosetting, antigenic polymorphisms, antigenic variation (PfEMP1), and malaria toxin [11].

The role of MSP2 protein has been elaborated in the parasite invasion pathway into erythrocyte [10] and subsequently two allelic

forms of the gene encoding the MSP2 protein, *msp2*; IC1/3D7 and FC27 alleles were reported [12] IC1/3D7 allele was more frequently found in severe malaria patients in Senegal and Kenya whereas the FC27 allele was more frequent in asymptomatic malaria cases [5,6].

The result of this study showed that the IC1/3D7 allele was predominant in both asymptomatic and symptomatic subjects and no correlation between the *msp2* allele with the malaria clinical manifestation. This result is different than those reported in Senegal and Kenya [5], where the FC27 allele is more frequently present in asymptomatic malaria [6].A report from Oksibil (Papua) showed that in the field there was more even distribution of FC27 and 3D7 alleles [7]. Of the asymptomatic malaria cases, IC1/3D7 allele was found in the majority of the subjects with lower parasitemia of density. Molecular epidemiological studies in highly endemic areas are often conducted in asymptomatic subjects, but in low endemic areas, however, genetic diversity is usually studied in individuals with clinical disease. Therefore, the difference in allelic frequency could not be associated. Genetic diversity of the *msp2* gene has indeed been reported to be lower in individuals with clinical disease than in asymptomatic infections [13].

The IC1/3D7 allele found in this study had an extremely polymorphic character, consisting of multiple insertions of repeat tandem nucleotide that differed to those reported from Myanmar [4, 11] and had family alleles that were more heterogeneous. Analysis of several regions of the *msp2* gene indicated that the dominant amino acid for region E1, E2 and E3 of the IC1/3D7 allele are A, K and E, G, S and T, respectively. For the regions R1 and R2 are S, G, A, and T, respectively. In general, the dominant amino acids in block 3 of PfMSP2 are T, G, K and S, respectively. The FC27 allele had repetitive sequences that were related, but not identical to each other. The dominant amino acid for region E1, E2 and E3 of the FC27 allele are K, V, S, I, T, N, and K, respectively. Generally the dominant amino acids in block 3 of PfMSP2 are T and S. The region R1 and R2 of the FC27 allele has P, T, A, S, I and T, respectively. Several non synonymous amino acid substitution where identified in the family specific region (E1, E2 and E3) of IC1/3D7 and FC27 type alleles and the variation make the genetic diversity of IC1/3D7 allele type much greater that FC27 type allele.

The frequency of IC1/3D7 in the asymptomatic and symptomatic subjects is higher and more heterogeneous, with insertion and deletion of nucleotides, than FC27 allele in Jayapura District, Papua Province, Indonesia. Similar frequency patterns were also observed in Thailand, Iran, Pakistan and Cameroon [12, 14-16]. Therefore, there are different sequences of the genetic diversity of *P. falciparum* from Papua, Indonesia isolates. The results of the sequence analysis could be a basis for the development of effective malaria vaccine by different geographic regions but it depends on the primers and sensitivity of the technique optimized in the laboratory.

Among the 51 asymptomatic malaria subjects, IC1/3D7 allele was more frequently detected in both low (below 10,000 parasite/µL of blood) at 66.7% and high parasite density (above 10,000 parasite/ µL of blood) at 33.3%. FC27 was less frequently distributed in both levels of parasite density. Statistical analysis indicated that there is no significant correlation between parasite density in asymptomatic subjects and genotypes of the *msp2* gene of *P. falciparum* (*P-value* 0.323 > 0.05; CI= 95%; α = 0.05). The study showed no association between genotype or allele family of *P. falciparum* msp2 and level of parasitemia of malaria. An explanation is that genetic diversity depends on various factors, and studies about transmission intensity, disease phenotype, host immune status and genetic factors will provide more insight into the parasite virulence mechanism in humans. This is similar with the results reported by Kiwuwa from Kampala, Uganda [13].

Looking at the allele distribution among the age groups of asymptomatic subjects, IC1/3D7 was more commonly detected compared to FC27 in the age groups 0-9 years old (26%), 10-20 years old (33%), and above 30 years old (11%). However, FC27 was more commonly detected than IC1/3D7 in the age group 21-30 years old (80%). Statistical analysis indicated that there is no significant correlation between age of asymptomatic subjects and genotypes of the *msp2* gene of *P. falciparum* (*P-value* 0.171 > 0.05; CI= 95%; α = 0.05). The results are similar with those reported in Congolese, where it was revealed that 3D7 allele was found more than FC27 allele [17]. But they are different with the report in Senegal, that FC27 allele was slightly higher in frequency in asymptomatic subjects aged 15 years, during the transmission season [6], and in South of Benin, where FC27 allele was more highly detected than IC1/3D7 allele in uncomplicated malaria cases [18].

Based on distribution of *msp2* allele and gender among asymptomatic subjects, the results indicated that IC1/3D7 allele was more frequently detected in males (66.7%) than females (33.3%). Similarly, FC27 allele was more frequently detected in males (70%) than females (30%). Statistical analysis indicated that there is no significant correlation between gender of the asymptomatic subjects and msp2 allele (*P-value* = 0.958 > 0.05; CI, 95%; α = 0.05). There were more males among those infected because of occupation as males have to work and provide for the family. Thus, they have more opportunity to be bitten by mosquitoes.

From 101 symptomatic malaria cases, IC1/3D7 allele was more frequently detected among subjects with mild malaria (60.8%) than those with severe malaria (39.2%). However, FC27 was more frequently detected among subjects with severe malaria (52.2%) than those with mild malaria (47.8%). Statistical analysis indicated that there is no significant correlation between clinical manifestations (severe or mild malaria) and *msp2* allele of *P. falciparum* (*P-value*=0.579 > 0.05; CI = 95%; α = 0.05).

In this is study, IC1/3D7 allele was more frequently detected among severe malaria than FC27 allele in Jayapura Municipal, Papua Province. This result is similar to that reported in Senegal and Kenya [5] and in Sub-Saharan Africa where 3D7 allele was found more in patients than FC27 allele and that reported from Dakar and Senegal [19]. Comparing with another study, the FC27 allele found in Jayapura Municipal, Papua Province tend more to be similar to what was reported from Oksibil, Papua [7]. The *msp2* gene cannot be used as a marker of malaria symptomatology because both alleles were detected in asymptomatic and symptomatic subjects. In southeastern regions of Iran, it was found that 3D7/IC1 allele was more frequently detected than FC27 allele in patients who suffered falciparum malaria. This means that 3D7/IC1appear to have a higher genetic diversity than expected for an area of low transmission. This situation in this area is perhaps emerging, possibly because of reduced efficacy of first-line malaria treatments [17].

From 101 symptomatic malaria cases, IC1/3D7 allele was more frequently detected in subjects with parasitemia below 10,000 parasites/µL of blood (60.8%). Conversely, FC27 was slightly more frequently detected among subjects with parasitemia above 10,000 parasites/ µL of blood (52.2%). Based on statistical analysis, the results indicated that there is no significant correlation between

level of parasitemia of symptomatic patients and msp2 allele of *P. falciparum* (*P-value* 0.579 > 0.05; CI= 95%; α = 0.05). This study is similar as reported from Brazzaville in Congo where *msp2* allele did not show any impact on the parasite density [20].

From 101 symptomatic malaria cases, the distribution of msp2 allele among the age groups showed that IC1/3D7 allele was more frequently detected in the age groups 21-30 years (29.4%) and above 30 years (31.4%) compared to FC27 allele. However, FC27 allele was more frequently detected in the age groups 0-9 years (26.1%) and 10-20 years (34.8%). Statistical analysis indicated that there is no significant correlation between age of asymptomatic patients and msp2 allele of *P. falciparum* (*P- value* 0.639 > 0.05; CI= 95%; α = 0.05). This study is similar to that reported from Brazzavile in Congo and Bangui where the number of parasite genotypes of the *msp2* gene among subjects with symptomatic falciparum malaria was not influenced by age [20,21]. Among the symptomatic subjects, *P. falciparum* infection in this population is associated with the presence and growth of recently inoculated parasite strains to which subjects have either not previously been exposed to or have not fully developed immunity especially of the group age less than 10 years, similar to what was reported in Mozambique [22].

Based on distribution of *msp2* allele and gender among the 101 symptomatic malaria cases, results indicated that IC1/3D7 allele was more frequently detected among males (56.9%) while FC27 was more frequently detected among females (65.2%). Statistical analysis indicated that there is no significant correlation between gender of symptomatic subjects and *msp2* allele (*P- value* = 0.106 > 0.05; CI, 95%; α = 0.05). This study similar with was reported from Brazzaville in Congo that there is no statistically significant difference between msp2 allele and gender [20].

The frequency distribution of the msp2 allele among the asymptomatic and symptomatic subjects of malaria cases were shown previously. The results indicate that IC1/3D7 allele is higher in distribution in both groups. However, malaria cases with mix allele infection were higher in symptomatic than asymptomatic cases, but based on statistical analysis to determine the correlation between the IC1/3D7 allele and FC27 allele with malaria symptomatology, there is no significant correlation between msp2 allele and clinical manifestations (*P- value* 0.904 > 0.05; CI= 95%; α = 0.05). The IC1/3D7 allele dominance in the study was found in both asymptomatic and symptomatic subjects, and in both mild and severe malaria. Therefore, the *msp2* allele of *P. falciparum* could be used as candidate molecular vaccine for falciparum malaria [23].

Conclusions

The frequency distribution of the msp2 allele showed that IC1/3D7 allele of the *msp2* gene of *P. falciparum* was more dominant than FC27 allele in both the asymptomatic and symptomatic subjects in Jayapura District, Papua Province, Indonesia. However, there is no significant correlation between IC1/3D7 and FC27 alleles of the *msp2* allele of *P. falciparum* with clinical manifestations and severity. Therefore, *msp2* allele could be used as candidate molecular vaccine for falciparum malaria. IC1/3D7 and FC27 alleles of the *msp2* allele of *P. falciparum* did not show significant correlation with parasite density, age and gender, in Jayapura Municipal, Papua Province, Indonesia.

Abbreviations

PCR: Polymerase Chain Reaction

PfEMP1: *P.falciparum* Erythrocyte Membrane Protein-1

MSP-2: Merozoite Surface Protein-2

IC1/3D7 and FC27: Family alleles of the *msp2* gene of *P. falciparum*.

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