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Plasmodium Falciparum Gene Polymorphisms Pfmdr1 N86Y and Drug Self-medication in the Endemic Areas of West Papua Region, Indonesia

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Abstract

West Papua Province of Indonesia has the highest malaria incidence (5.1%) and prevalence (12.5%) and the population is used to Self-medication (2.8%). Incidence of tropical malaria in the district of Manokwari in 2015 based on laboratory results tests registry were 1,672 cases, most cases is from Prafi Health Center (960 cases). The study aims to determine the relationship of Self-medication with the N86Y Pfmdr1 gene polymorphisms in Plasmodium falciparum in Prafi district, Manokwari, West Papua. This study utilized cross-sectional design, with purposive sampling technique. Detection of gene polymorphisms of Pfmdr1 N86Y using PCR-RFLP technique using two pairs of the forward primer and the reverse of Nested I and Nested II. Data were analyzed using chi-square. Total samples collected were 43 respondents. Results of PCR-RFLP test were found mutant allele 86Y were 81.4% and mutant allele N86 were 18.6%. Analysis of Chi Square test obtained significant relationship with Self-medication with *p-value* = 0.046 ($p<0.05$). This study found that Self-medication have influence on the occurrence of gene polymorphisms Pfmdr1 N86Y.

Keywords: malaria; Falciparum; Gene Mutation; Pfmdr1; N86Y; polymorphisms; Self-medication.

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1. Introduction

Approximately 3.2 billion people, nearly half of the world's population at risk of malaria. In 2015, an estimated 214 million new cases of malaria and 438,000 deaths, mainly in sub-Saharan Africa. Millions of people have not been able to access the health services they need to prevent and treat malaria [1]. The heaviest burden is in the African region, where approximately 90% of deaths from malaria, and in children under the age of 5 years (78%) of all deaths [2]. In the area of Southeast Asia 70% of the total population, or about 1216 million people, residing in malaria endemic areas. Approximately 96% of the population at risk of contracting malaria in areas of Southeast Asia are in Bangladesh, India, Indonesia, Myanmar, and Thailand and led to 95% of cases of malaria (either ill or deceased) in the area [3].

Riskesdas survey in 2013 in West Papua Province entered on the order of four provinces in Indonesia which has the highest malaria incidence and prevalence (5.1% and 12.5%) and the population is treating itself malaria (Self-medication) sustained (2.8%) [4]. Self-medication is one's efforts in treating the symptoms of illness or disease without consulting a physician first [5]. Self-medication is taken alternative society to increase the affordability of treatment. In practice, Self-medication can be a source of error treatment (medication errors) due to limited public awareness of the drug and its use [15].

Cases of malaria parasite resistance to chloroquine were first discovered in East Kalimantan in 1973 to P.falcifarum, and 1991 for P. vivax in Nias. Since 1990, cases were reported increasingly widespread resistance in all provinces in Indonesia. In addition, reportedly also their resistance to Sulfadoxine-Pyrimethamine (SP) in several places in Indonesia [6].

One factor that may inhibit the malaria control is the emergence of resistance to antimalarial. Pfmdr1 and Pfatp6 gene is a gene associated with resistance to anti-malarial Plasmodium used in ACT [7]. Mutations to Pfmdr1 also associated with chloroquine resistance, including N86Y [20]. The study aims to determine the relationship Self-medication with N86Y Pfmdr1 gene polymorphisms in Plasmodium falciparum malaria in the Prafi District Manokwari, West Papua.

2. Materials and methods

2.1. Location and Research Design

The study was observational epidemiologic studies with cross-sectional methods utilized purposive sampling technique. The location of the research is in West Papua Province, Manokwari Regency, Prafi District.

2.2. Population and Sample

The population in this study were all people (patients) who came for treatment at the health center of Prafi. The sample in this study were all patients who come for treatment at the health center were uncomplicated malaria based on microscopic examination with the discovery of Plasmodium falciparum parasite. The sample size in this study was 43.

Collecting data using questionnaires and blood samples. How Sampling is done by Passive Case Detection (PCD) is waiting for patients to come to the health center for a blood sample taken in the finger then on Make a slide staining to be checked under a microscope by a microscopic attendant Puskesmas. If the parasite Plasmodium falciparum was found on microscopic examination, the patient is taken back his blood sample for further input into Buffer solution L6 and the research samples until it reaches the target sample

2.3. Sample collection and DNA extraction using Boom's methods

The blood samples of 100 ul of positive malaria were added to 500 mL of lysis buffer L6 on the tube that has a lid. This mixture is then centrifuged at 12,000 rpm for 10 minutes. Sediment samples were concentrated is homogenized for 30 minutes. Before the suspension was added diatoms, L6 buffer mixture which already contains DNA extraction yield was centrifuged for 2-3 minutes at a speed of 12,000 rpm, with the aim that the DNA extraction yield settles at the bottom of the tube. 20 mL diatom suspension was added to the tube, diatom suspension should always be rotated and stirred by using a gyratory shaker, 100 rpm for 10 minutes. L6 buffer mixture of diatoms and rotated back using microcentrifuge Eppendorf's centrifuge at a speed of 12,000 rpm for 15 seconds. The supernatant formed from each separated by using a suction tube made of a Pasteur pipette and connected with a vacuum pump, to prevent loss of diatoms in suspension earlier. About 10 mL of the suspension remains.

The supernatant was washed two (2) times using 1 ml of buffer washers L2. L2 washing buffer is added 1 ml, rotated and centrifuged at 12,000 rpm for 15 seconds, then the supernatant was discarded. The precipitate was washed again with 1 ml of 70% ethanol for 2 (two) times, and then rotated and centrifuged at 12,000 rpm for 15 seconds. The supernatant was discarded, the precipitate was washed again with 1 ml acetone, rotated and centrifuged at 12,000 rpm for 15 seconds, then the supernatant back discarded. Acetone remaining in the sediment (sediment) is evaporated by opening the cover tube and heated by an oven at a temperature of 50-55 ° C for approximately 10 minutes. After the sediment dries, TE elution buffer was added about 60 ml, then rotated evenly so that the suspension of sediments and soluble.

Then the tube was incubated in an oven at a temperature of 56oC for 10 minutes. Then, the mixture is then centrifuged at a speed of 12,000 rpm for 30 seconds. The supernatant was taken with caution as many as 40-50 mL of the supernatant and put in a new tube. Extraction results stored at - 80 ° C [10, 11].

2.4. Detection of gene polymorphisms Pfmdr1 N86Y with RFLP - PCR technique

Primers, restriction enzymes, and PCR products for gene polymorphisms Pfmdr1 see N86Y by using nested PCR. This process is performed on DNA samples which have been isolated. First made-mix PCR with primers 1 M1 which will be amplified as much as 22.5 mL of 2.5 mL of 10X PCR buffer, 0.1 mL of Taq polymerase, dNTPs 0.1 mL, 19.6 mL of distilled water, then added Primer M1 forward: AAGAGGTTGAAAAAGAGTTGAAC and Reverse Primer:

CCGTTAATAATAAATACACGCAG each 0.1 mL. Furthermore, as much as 2.5 mL of DNA extract added to a mixture of 22.5 mL PCR Primer Forward / Reverse and carried out the first stage of amplification with 94 ° C for

3 min followed by 45 cycles of 30 seconds at 94 ° C respectively; 30 seconds at 56oC; and 30 seconds at 60 ° C. This process was followed by immersion at 60oC 3 minutes. Then made back-mix PCR Primer 2 with M2 to be amplified as much as 22.5 mL of 2.5 mL of 10X PCR buffer, 0.1 mL of Taq polymerase, dNTPs 0.1 mL, 19,6µl distilled water, then added Primer M2 Forward:

AGAGTACCGCTGAATTATTTAG and M2 Primer Reverse: CCTGAACTCACTTGTTCTAAAT each 0.1 mL. Furthermore, as much as 2.5 mL of DNA extract added to a mixture of 22.5 mL PCR Primer Forward / Reverse and carried out the first stage of amplification with 94 ° C for 3 min followed by 40 cycles each of 30 seconds at 94 ° C; 30 seconds at 47oC; and 1 min at 68oC.

This process was followed by immersion 64oC 3 minutes. By using PCR machine (Applied Biosystems Thermal cycler 2720). After the results of nested PCR done cutting with restriction enzymes cutting where the results will form the second band of 239 bp and 179 bp. While the nested PCR product of 418 bp which is read by 2% gel electrophoresis been below the ultraviolet [12,13].

2.5. Research ethics

Research conducted has received approval from the ethics committee of human biomedical research Medical Faculty of Hasanuddin University.

2.6. Data analysis

The statistical analysis used in this study is an analysis Bivariate and univariate analysis. The used statistical test Chi Square test with a confidence level of 95% and the value of $\alpha \leq 0.05$. Data were analyzed using SPSS version 21.0.

3. Results

Characteristics of respondents found the majority of respondents aged > 18 years is 58.1%, most respondents Gender male gender of 69.8%, the distribution of most respondents education on high school education amounted to 55.8%, while most respondents work in employment as a farmer of 30.2% and the highest rate of respondents from non-Papuans tribe by 65.1%.

Distribution of respondents who do Self-medication amounted to 51.2% and that does not do Self-medication 48.8%

Analysis by PCR technique in 43 blood samples of respondents get their gene polymorphism allele 86Y Pfmdr1 in 35 samples and allele N86 in 8 samples. Pfmdr1 gene codon 86 polymorphism was formed 2-type allele is a mutant allele type (two DNA bands of 239 bp and 179 bp) and the wild-type allele (form a single DNA band size 418 bp)

Table 1: Characteristics of Respondents

Characteristic	Frequency (n = 43)	%
Age		
0-5 years	7	16.3
6-12 years	5	11.6
13-18 years	6	14.0
> 18 years	25	58.1
Gender		
Man	30	69.8
woman	13	30.2
Education		
No School	7	16.3
Elementery School	5	11.6
Junior High School	6	14.0
Senior High School	24	55.8
College	1	2.3
Work		
Farmer	13	30.2
Palm planters	8	20.9
Trader	2	4.7
Government Employee	1	2.3
Teacher	11	25.6
No work	7	16.3
Tribe		
Papuan	15	34.9
Non Papua	28	65.1

Source: Primary Data

Table 2: Variable Self-medication done by respondents

Variables	Frequency (n = 43)	%
Self-medication		
Yes	22	51.2
No	21	48.8

Source: Primary Data

Description: codon 86 gene polymorphisms Pfmdr1 formed two types of alleles are mutant allele type (two DNA bands of 239 bp and 179 bp) and the wild-type allele (form DNA-sized single 418 bp) as shown in the sample image No. 1, 2, 3, 4, 5, 7 , 8, 10, 12, 13, 15, 16 is the wild-type allele and sample No. 6, 9, 11, 14 is the mutant allele type. There are no mix-type (mutant and wild-type allele) in this study.

The results indicate that the effect of the polymorphism Self-medication Pfmdr1 was found 86Y mutant alleles of 81.4% and N86 mutant alleles of 18.6%. Chi Square test results obtained by *p-value* = 0.046 which mean that there is an influence on the occurrence of gene polymorphisms Self-medication Pfmdr1.

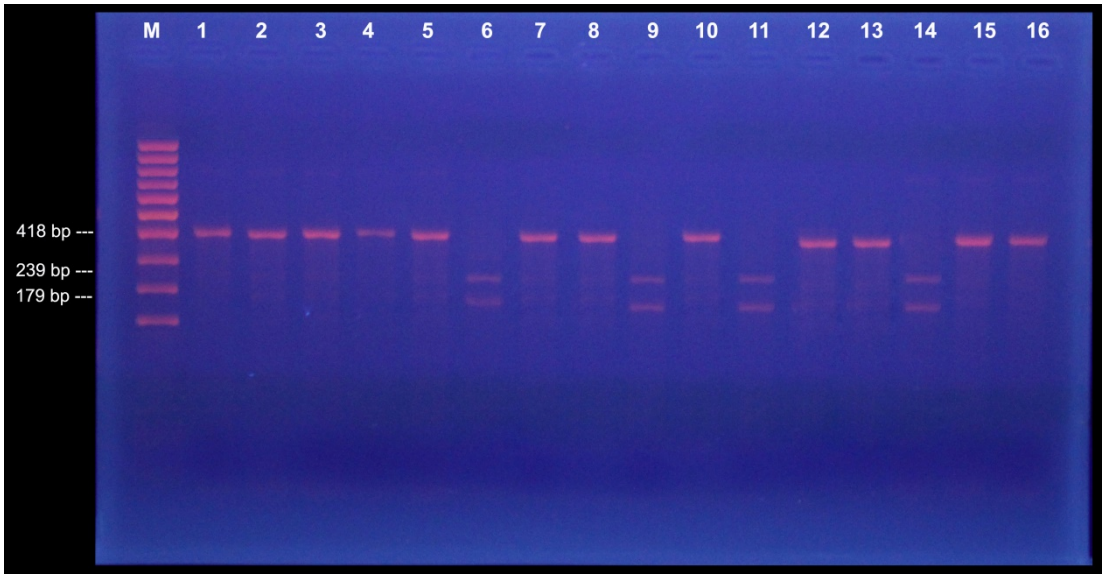

Figure 1: Visualization of the PCR amplification results in patients with falciparum malaria

Table 3: Relationship Self-medication with Gene Polymorphism Pfmdr1 N86Y

	Pfmdr1 gene polymorphisms N86Y				
Self-medication	86Y	%	N86	%	*p-value*
Yes	15	68.2	7	31.8	0.046
No	20	95.2	1	4.8	
Total	35	81.4	8	18.6	

Source: Primary Data

4. Discussion

Riskesdas Survey in 2013 in West Papua Province entered on the order of four provinces in Indonesia which has the highest malaria incidence and prevalence (5.1% and 12.5%) and the people treat themselves malaria (Self-medication) sustained (2.8%) [4]. Community efforts to treat himself known as Self-medication. Self-medication usually did to address complaints and mild illness which many experienced people, such as fever, aches, dizziness, cough, influenza, stomach ulcers, intestinal worms, diarrhea, skin diseases and others [8]. Inspired by the desire to take care of themselves, take care of sick relatives, less satisfied with the health services available, and many choices of drugs is a factor that supports of Self-medication practices [16]. The decline in drug

sensitivity can occur as a result of ongoing treatment and inadequate, resulting in mutations parasites, in addition, it is also thought to be taken from areas that are resistant [14].

Polymorphisms in pfmdr1 also associated with resistance to chloroquine, mefloquine, quinine and artemisinin [17]. Molecular studies in recent decades have identified several mutations in the P. falciparum genes associated with CQR. mutations in Plasmodium falciparum multidrug resistance 1 (Pfmdr1), especially in codon 86, in which asparagine is converted to tyrosine [19].

At the Chi Square test obtained the result that the influence of gene polymorphisms Pfmdr1 Self-medication against mutant allele is found at 81.4% and the 86Y mutant alleles of N86 of 18.6%. Chi Square test results obtained by value p = 0,046 which mean that there is an influence on the occurrence of gene polymorphisms Self-medication Pfmdr1. From the research results on 43 samples were analyzed using Primer M1F and M1R in the PCR mix 1, formed ribbon single DNA size of 418 bp is allele wild-type at codon 86Y in 35 samples (1, 2, 3, 4, 5, 7, 8, 10, 12, 13, 15, 16, 17, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43). Irsan research results which occur Polymorphisms in pfcrt 76-Thr and 86Y pfmdr1 found in all isolates. These findings suggest that treatment failure with chloroquine in recent years in South Sumatra [19].

Next on PCR2 using Primer M2F and M2R input method cutting enzyme restriction fragment length polymorphism (RFLP) using restriction enzymes from Fermentas Apoi. Cutting with a restriction enzyme to form two bands ApoiDNAof 239 bp and 179 bp which type mutant alleles at codon N86 in 8 samples (6, 9, 11, 14, 18, 23, 31, 33). It shows that in 43 samples in carefully all the samples have undergone gene polymorphism at codon N86Y Pfmdr1. This study is different from that performed by carolia where results found 7 sample mix-type (mutant and wild-type). Sample type mix indicates the formation of ribbonDNA at 418 bp, 239 bp and 179 bp [9].

Most of the people in the district of West Papua province Prafi when exposed to malaria them more buying their own medicine in pharmacies to treat the disease. Results riskesdas 2013 say that of the five provinces in Indonesia, West Papua highest population self-treatment of malaria by 5.1% and the public get effective treatment with ACT in the first 24 hours only 49.6% [4]. The use of drugs that do not fit the standards will encourage the emergence of resistant Plasmodium against drugs consumed. When this happens the trend of increased parasite resistance to drugs in malaria endemic region, which is one cause of the high morbidity and mortality of malaria [21]. This indicates that there are still problems in the implementation of the provision of counseling on Prafi District of antimalarial drugs, such as behavioral and public knowledge about the treatment of malaria.

5. Conclusion

The study found gene polymorphisms Pfmdr1 at codon 86. The type of mutation that was found was a mutant allele 86Y amounted to 81.4% and N86 mutant alleles of 18.6%. Health education should be improved to patients about the impact of Self medication / self-treatment of malaria.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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