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Genetic Polymorphism of Plasmodium Falciparum Merozoite Surface Protein-1 (Pfmsp-1) in Closed and Opened Community at South Buru District, Maluku Province

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Abstract
Malaria is an infectious disease that is dominant either in tropical area or sub-tropical area and it can cause death in million people even more for every year. Plasmodium falciparum Morozoite Surface Protein-1(PfMSP-1) is very important in the process of parasite invasion into erythrocyte and it is also a candidate of malaria vaccine. Furthermore, this protein is needed to be researched further, particularly regarding the polymorphism of encoding gene due to having high polymorphism geographically. PfMSP-1 has three types of alleles which are K1, MAD20, and RO33. However, this research aimed to observe the polymorphism of PfMSP-1 in either closed community or opened community at South Buru district. The sample of this research was collected in purposive sampling which was 128 respondents who were from either closed community or opened community by utilizing blood spot and DNA isolation. The result of the identification and DNA isolation was tested in single step PCR method. Hence, it was found 10 samples which were positive P. Falcifarum. According to nested PCR test, it was found that the genetic polymorphism of PfMSP-1 on K1, MAD20, and RO33 allele which were 100bp, 200bp, 300bp were in opened community. However, for the 100bp and 200bp were not found in closed community. Polymorphism was influenced by citizen migration from endemic area to non-endemic area or from non-endemic area to endemic one. The irregular use of anti-malaria drugs could cause a resistance to the plasmodium, hence, it could be occurred a mutation in host's body. The malaria illness history caused relapse even repetitive infections to Plasmodium falciparum that was in the host's body and it had an ability of immune system so that it could survive. It was found that the genetic polymorphism of PfMSP-1 on K1, MAD20, and RO33 allele was in opened community. Nonetheless, it was not found in closed community at South Bulu District, Maluku Province.

Keywords: *Polymorphism, PfMSP-1, PCR, closed community and opened community*

I. INTRODUCTION

Malaria is an infectious disease that is dominant either in tropical area or sub-tropical area and it can cause death in million people even more for every year. According to WHO data (2015), it was found that there were 214 million of malaria new cases around the world with a range of 149 million to 303 million cases. Moreover, in Africa (88%), Southeast Asia (10%), and Middle East (2%) in 2015 were found 438.000 deaths due to malaria with the range of 236.000 to 438.000 deaths around the world.

The genetic polymorphism was influenced by several factors: the irregular use of anti-malaria drugs which could cause the occurrence of parasite resistance to the drugs, the mobilization or the citizen migration from malaria endemic area to the non-endemic one which had a risk of malaria transmission, the citizen either came or went to and from malaria endemic area in certain time, having a potency to be infected by malaria (Depkes RI, 1999; Jordan *et al*, 2001; Weraman, 2013).

Plasmodium falciparum merozoite surface protein-1 (PfMSP-1) was a surface protein that was on the surface stage of merozoite from *P. falciparum.* MSP-1 has three types of alleles which were K1, MAD20 and RO33. However, the genotype frequency was different based on the geographical area although it was only a nearby village (Arwati, 2015). MSP-1 was a protein that was used while conducting invasion into the erythrocyte after merozoite entering and staying into the erythrocyte. Besides, MSP-1 was a candidate of malaria vaccine. MSP-1 represented the process of parasite evolution that was more researched and used for learning the genetic polymorphism of parasite. A research of PfMSP-1 on K1 allele in malaria had been conducted more, such as at Pacitan district (Arwati *et al*,

2010) that was in import malaria and indigenous malaria, at Mentawai Islands-West Sumatera (Irawati *et al*, 2009) that was in mountains and lowlands, and South Sumatera (Handayani *et al*, 2015).

II. METHOD

The Sampel of this research was obtained in purposive sampling. The numbers of the respondents were 128 respondents who were detected positive malaria by RDT and microscopic test. Afterwards, the result of the test was conducted a confirmation by using single step PCR (Patsoula et. al.2002). The ingredients of the blood spot were isolated by DNA. In order to identify the plasmodium, it was needed a primer set of PL3, PL4, and PL5 which were included in the table below:

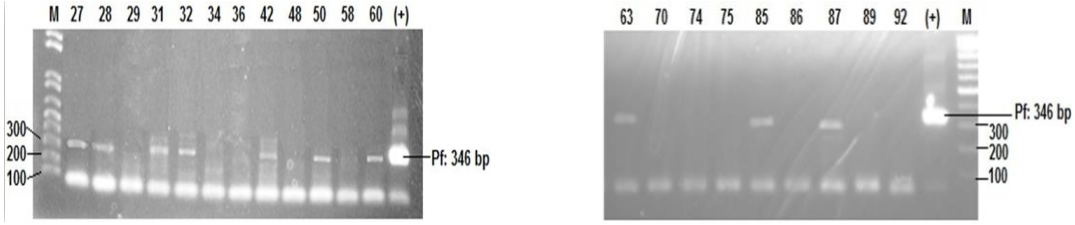
Table 1. Primer Used for Single Step PCR

Primer Name	***Sequence***	***Product Size (bp)***	**Decision**
PL3	5ATG GCC GTT TTT AGT TCG TG3	266	*P. vivax, P. falciparum & mix*
PL4	5GGA AAC GGT ACG ATA AGC CA3	266	*P. vivax*
PL5	5ACG CGT GCA GCC TAGTTT AT3	346	*P. falciparum*

III. RESULTS

The detection result of plasmodium species by using single step PCR as followed:

(a) (b)



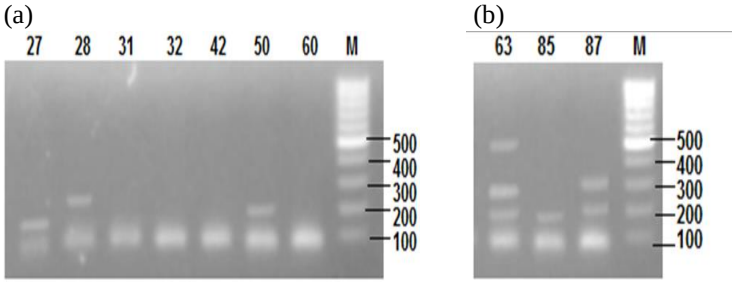
Picture 1. Identification of P. Falciparum species (a) in closed community by using single step PCR method. The samples which were positive were the sample in number: 27, 28, 31, 32, 42, 50, and 60. Meanwhile, (b) in opened community were in number: 63, 85, and 87. Note: M : Marker (Invitrogen 100 bp). (+) : positive control as a comparer from Invitrogen of P. Falciparum.

Table 2. *Recapitulation of Single Step PCR Result*

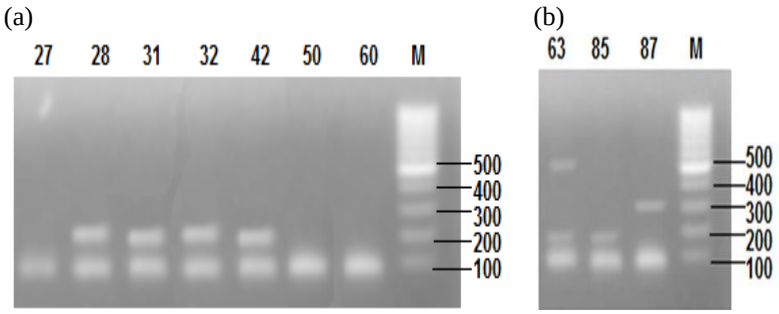
Community	**The Sample Number**	**Total**
Closed	27, 28, 30, 31, 32, 42, 60	7
Opened	63, 85, 87	3
Total		10

The result of genetic polymorphism test of PfMSP-1on K1, MAD20, and RO33 allele by using nested PCR method as followed:

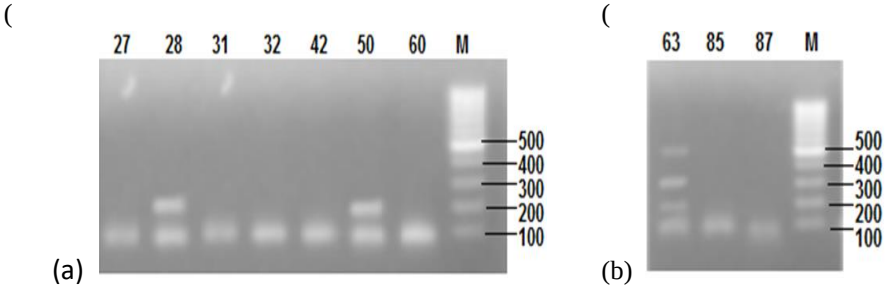
(a) (b)



Picture 2. The polymorphism of PfMSP-1 on K1 allele in (a) Closed community and (b) Opened community by using Primary K1a and K1b with nested PCR method. Note: M: Marker (Invitrogen, 100bp). Polymorphism on K1 allele was found in (b) opened community which was showed on the sample number: 63. It had 4 tape sizes which were 100bp, 200bp, 300bp, and 500bp and it was known as polymorphic with multiple of infection (MOI)-having more than three tape sizes. Meanwhile, the sample number: 87 had three tape sizes which were 100bp, 200bp, and 300bp and it was also known as polymorphic. However, there was only one sample that was known as monomorphic and it was in number 85 which was 100bp. In (a) closed community had been found that the sample number of 27, 28 were 100bp, 200bp and the sample number of 50 was 100bp and 300bp (dimorphic). Whether, the sample number of: 31, 42, and 60 was 100bp (monomorphic).



Picture 3. Polymorphism of PfMSP-1 on MAD20 allele in (a) Closed Community and (b) Opened Community by using Primary MAD20a and MAD20b with nested PCR method. Note: M: Marker (Invitrogen, 100bp). On the picture above, it was showed that in (a) closed community with the sample number: 28, 31, 32, and 42 had tape sizes which were 100bp and 200bp (dimorphic) and the sample number: 27, 50, and 60 were monomorphic. Meanwhile, the sample in (b) opened community with number 63 had tape sizes which were 100bp, 200bp, and 500bp (polymorphic). The sample number of 87 had tape sizes which were 100bp, 300bp, and the sample number of 85 had tape sizes which were 100bp, 200bp and they were known as dimorphic.



Picture 4. Polymorphism of PfMSP-1 on RO33 allele in (a) Closed Community and (b) Opened Community by using Primary RO33a and RO33b with nested PCR method. Note: M: Marker (Invitrogen, 100 bp). The sample number: 28 and 50 were dimorphic which had tape sizes of 100bp and 200bp. It meant that it was occurred parasite infection with one genotype. The sample number of 27, 31, 32, 42, and 60 were monomorphic and it meant that it was occurred one infection with one genotype. Whether, in opened community, the sample number of 63 was polymorphic which had more than three tape sizes.

IV. DISCUSION

According to the result of genetic polymorphism test of MSP-1 on K1, MAD20, and RO33 allele, in closed community was not occurred a change of genetic polymorphism. However, this case was occurred because most of closed community had not known more even had not contacted more with the outside of their environment. They had not been exposed to vegetative treatment and also they did not go anywhere to other areas, particularly to opened areas. Meanwhile, in opened community, there was a polymorphism change and it was found that most of tape size had more than three sizes which meant that it was occurred parasite infection with more than three genotypes. This was occurred because the opened community accessed more easily to the health services and the irregular use of anti-malaria drugs could cause the resistance of the drugs.

In addition, there were some similar researches which were conducted. A research conducted by Irawati (2011) who proposed the occurrence of polymorphism according to geographical location of plateau and lowland. A

research of MSP-1 was also conducted by Handayani et al., (2015) who used the sample of *P.falciparum* from South Sumatera Province. She found three alleles of K1 with the band length of 130-210 bp and MAD20 allele in seven alleles with band length of 140-240 bp. Whether, for RO33 allele was not found the band. Besides, there were other researches which were conducted by Arwati et al, 2010 at Pacitan District and Ferreira *et al*, (2002) in Brazil, Tanzania, and Vietnam. Arwati et al found that in symptomatic and asymptomatic malaria had been found 2 band length in each allele of K1, MAD20, and RO33. Whether, Ferreira *et al*, (2002) showed the MSP-1 that there was variety of genetic MSP-1 to each malaria endemic area. However, the difference of external environment was a main factor of the causes of variety in MSP-1 gene. This variety caused the difference of genetic MSP-1 among each malaria endemic areas.

V. CONCLUSION

1. Polymorphism of PfMSP-1 on K1, MAD20, and RO33 allele was found in opened community and it was not found in closed community.
2. Polymorphism change was influenced by the citizen mobilization and the irregular use of anti-malaria drugs which caused the resistance of the drugs.
3. There was genetic recombination in the vector's body.
4. The difference of genetic polymorphism of PfMSP-1 was influenced by geographical location either in closed community or in opened community.

References

1. Aubouy Agnès, F. M.-N. (2003). Polymorphism in two merozoite surface proteins of Plasmodium Isolat from Gabon. *Malaria Journal.Vol.2* , 1-6.
2. Arwati.H, Sri Hidayati, YP. Dachlan (2010) Karakteristik polimorfisme protein permukaan merozoit plasmodium falciparum pada malaria kasus impor dan indigenus di kabupaten Pacitan sebagai upaya mendesain konstruksi vaksin lokal Indonesia, *onesearch.id/Record/IOS1-INLIS000000000315428*
3. Handayani, Nindela, Saleh 2015 Genetic Diversity of Merozoite Surface Protein 1 (*MSP-1*) in Plasmodium falciparum Dield Isolates from South Sumatera. *eprints.unsri.ac.id/5473/1/Artikel_Oral.pdf*
4. Holder, B. M. (2005). What is the Function of MSP-1 on the Malaria Merozoit? *Focus* , 1-3. *http://www.ncbi.nlm.nih.gov/pubmed/15275468*
5. Irawati, N (2010) Genetic polymorphism of merozoite surface protein-1 (MSP-1) block 2 allelic types in *Plasmodium falciparum* field isolates from mountain and coastal area in West Sumatera, Indonesia. *Medical Journal Of Indonesia Vol.20.No.2010*
6. Jordan, A.M Nzila, E.K Mberu, E Nduati, A Ross, W.M Watkins, C.H, 2001 Sibley Genetic diversity of *Plasmodium falciparum* parasites from Kenya is not affected by antifolate drug selection *International Journal for Parasitology*, Volume 32, Issue 12, Pages 1469-1476
7. Ferreira Marcelo U.,W. (2002). Sequence diversity and evolution of the malaria vaccine candidate merozoite surface protein-1 (MSP-1) of Plasmodium falciparum. *GENE* , 65-75.
8. Fitri A.P, Jamsari, Irawati (2011) Identifikasi Variasi Sekuen Dan Analisis Hubungan Kekerabatan Gen Merozoite Surface Protein 1 (Msp-1) Plasmodium falciparum Isolat Kepulauan Mentawai
9. The world malaria report 2015 www.who.int/**malaria//world-malaria-report-2015**
10. Patsoula E, Spanakos G, Sofianatou D, Parara M Vakalis NC (2003). A single -step PCR-based method for the detection and differentiation of Plasmodium vivax and P. falciparum. Annals of Tropi Med &Parasito 97,15-21
11. Wiraman.P (2013) Indeks klinis epidemiologis Penemuan dini Kasus Malaria Bagi Kader Kesehatan di wilayah Kepulauan Provinsi nusa Tenggara Timur. Disertasi Universitas Airlangga Surabaya