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Genetic Diversity in Merozoite Surface Protein.1 of *Plasmodium falciparum* in Highlands: Wamena Papua Indonesia

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Abstract: Malaria is the first killer as well as endemic disease in highland, lowland and coastal areas of Papua. In 2016 there were 160 thousands cases noted and 80% of the cases were caused by *Plasmodium falciparum*. Total sample for this study were provided by a clinical trial conducted from May to June 2018 in the regional hospital of Puncak Jaya, Wamena, located in the highland region of Papua. Positive blood samples was collected in a *ethylene diaminetetra acetic acid* (EDTA) tube. DNA was extracted using method of *Geneaid Genomic DNA Mini Kit* (*Blood/Cultured Cell*). The primary reactions used a set of primer corresponding to the conserved regions of block 2 of *msp*1. The second reactions primer set targets specific allelic families of *msp*1 (K1, MAD20 and RO33). All polymerase chain reaction amplicons were analyzed by electrophoresis in a 1.0% agarose gel. A total of 26 blood samples of positive *P. falciparum* were analyzed, 24 (93.2%) were successfully amplified for *msp*1. The MAD20 allelic family was predominant with 20.8% (8/24), followed by the RO33 allelic family with 8.2% (2/24) and the K1 allelic family with 1/24 (4.2%). The frequency of single allele is 11/24 (45.8%) while multi-allele are 16/24 (66.7%) with K1/RO33, MAD20/RO33, K1/MAD20/RO33 respectively 2/24 (8.3%), 8/24 (33.3%) and 6/24 (25%).

Keywords: *Plasmodium falciparum*, *MSP*-1, Malaria, Highland Papua

Introduction

Malaria is one of social health problems in the world. This disease influences the high death rate of pregnant women, babies and children under the ages of 5 years old (Permenkes Malaria, 2009). Every year there are about 500 million people infected malaria and more than 1 million people die (Ernawati *et al*., 2011). High cases of malaria parasite happen in some countries in Africa and Asia, including Indonesia. In Indonesia, until 2009, 80% re- agencies/municipalities were regarded as malaria endemic, about 45% people who lived in endemic areas are at risk to being infected with malaria parasite. There are 1.143.023 cases reported in 2009 (Harliani and Nurhadi, 2015). This number of cases were maybe not the exact number of cases due to the fact that endemic malaria cases in remote villages were not easily reached by transportation and low health services.

Of half Indonesian population, about 90 millions people live in endemic area of malaria (Bapenas, 2005). It is predicted that there are about 30 million cases every year and only 10% are well treated and have health facilities (Bapenas, 2005). Papua is a province in Indonesia that has the highest malaria case. Data from Provincial Health Department showed 294 cases in 2016, lower than previous cases in 2015 where only 497 thousands cases reported and this number is still regarded high (Papua, 2017). The most dominant causes of malaria parasite is *Plasmodium falciparum* (80%) (Papua, 2017).

Genotyping of malaria parasite population is an important tool to determine the types and number of parasitic clone in a *falciparum* malarial infection. The most widely used techniques for genotyping malaria

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infection is based on amplification of the polymorphic gene encoding Merozoite Surface Protein (MSP) (Hoffmann *et al*., 2003; Kang *et al*., 2010; Sorontou and Pakpahan, 2015; Muhammed *et al*., 2015; Some *et al*., 2018). The *MSP*1 gene of *P. falciparum* is a potential malaria vaccine candidate. The protein of the *MSP*1 gene is associated with the protection of the parasite (Sorontou and Pakpahan, 2015).

Gene *MSP*1 is the most conserved gene fragment that can be used as genetic marker to identify *P. falciparum*. The size of *MSP*1 is about 19kDa and this protein has important role in erythrocyte invasion. Gene *MSP*1 is located in chromosome 9 and contains of 17 blocks of sequences flanked by conserved region. Block 2 is the most polymorphic part and grouped into three allelic families namely MAD20, K1 and RO33 (Some *et al*., 2018). This study aimed to establish genetic polymorphism of MAD20, K1 and RO33allelic types of the *msp*1 gene among *P. falciparum* clinic isolates from the regional hospital of Puncak Jaya, Wamena Papua.

Materials and Methods

All subjects aged 12 years or more attending the regional hospital of Puncak Jaya, Wamena Papua with fever or history of fever in the last 24 h were referred by a clinician for screening of malarial infection by using Giemsa-stained thick and thin blood smears. Written informed consent was obtained for all donors and 3 ml venous blood sample in EDTA tube that contain anticoagulant was taken from 26 *P. falciparum* infected individuals. All *P. falciparum* malaria patients who enrolled in this study met the following criteria (i) they presented symptoms, (ii) their thick blood smears contained only *P. falciparum*, (iii) *P. falciparum* infection at parasites densities >10000 parasites/µl) (iv) they did not use chemoprophylaxis and had not taken anti-malarial drugs (self-treatment) (v) if female, they were neither pregnant nor breast feeding and (vi) their blood was collected on the day of diagnosis before initiating of malaria treatment. After the collection of the blood samples, the patients were immediately treated according to Indonesian Ministry of Health standards for malaria therapy (Ricio *et al*., 2013; Some *et al*., 2018). Blood samples in EDTA tubes was then sent to Medical Laboratory of Genetic Science of PT. Genetika Science Indonesia for DNA extraction in Jakarta. DNA extraction was carried out using method of Geneaid Genomic DNA Mini Kit (Blood/Cultured Cell). PCR Amplifications were perform using KOD FX Neo (Toyobo). The primary and nested (second amplifications) PCR reactions were carried out 50µL reaction volume using 5µL of template DNA and 1µL primary PCR product respectively in two reactions. The allelic family-specific primer were used in the nested reaction for block2 of *msp*1 corresponding to MAD20, K1 and RO33 allelic families. The primer is used as follows:

Results

P. falciparum parasites were detected in a total of 26 samples by using Giemsa-stained thick and thin blood smears based on microscopic diagnostic. PCR genotyping was done for 26 selected parasitaemia positive sample. PCR amplification was successful for *MSP*1 in 92.3% (24/26) of the samples.

This study also identified polymorphism of allelic families of *MSP*-1, namely K1, MAD20, RO33 and combination of three alleles. In *MSP*-1, the MAD20 allelic family was predominant with 20,8% (8/24), followed by the RO33 allelic family with 8.2% (2/24) and the K1 allelic family with 4.2% (1/24). The frequency of single allele is 11/24 (45.8%) while multi-allele are 16/24 (66.7%) with K1/RO33, MAD20/RO33, K1/MAD20/RO33 respectively 2/24 (8.3%), 8/24 (33.3%) and 6/24 (25%). This study did not find multi-allele with K1/MAD20.

Discussion

Genetic diversity of Merozoite Surface Protein are currently being recommended in antimalarial clinical trials as standard markers to distinguish recrudescent from newly infecting malaria parasites (WHO, 2007; Some *et al*., 2018). However, very few studies have investigated the genetic diversity of merozoite surface protein in malaria parasites circulating in many endemic country including Indonesia especially in Papua.

Table 1: Primer Arrangement that is used for amplification *MSP*-1

Locus	Primer	Nucleotide Arrangement
Reaction PCR I		
Primer PCR	*msp*1	CTAGAAGCTTTAGAAGATGCAGTATTG
		CTTAAATAGTATTCTAATTCAAGTGGATCA
Reaction PCR II		
*msp*1	K1-MAD20	AAATGAAGAAGAAATTACTACAAAAGGTGC
		GCTTGCATCAGCTGGAGGGCTTGCACCAGA
		AAATGAAGGAACAAGTGGAACAGCTGTTAC
		ATCTGAAGGATTTGTACGTCTTGAATTACC
	RO33	TAAAGGATGGAGCAAATACTCAAGTTGTTG
		CATCTGAAGGATTTGCAGCACCTGGAGATC

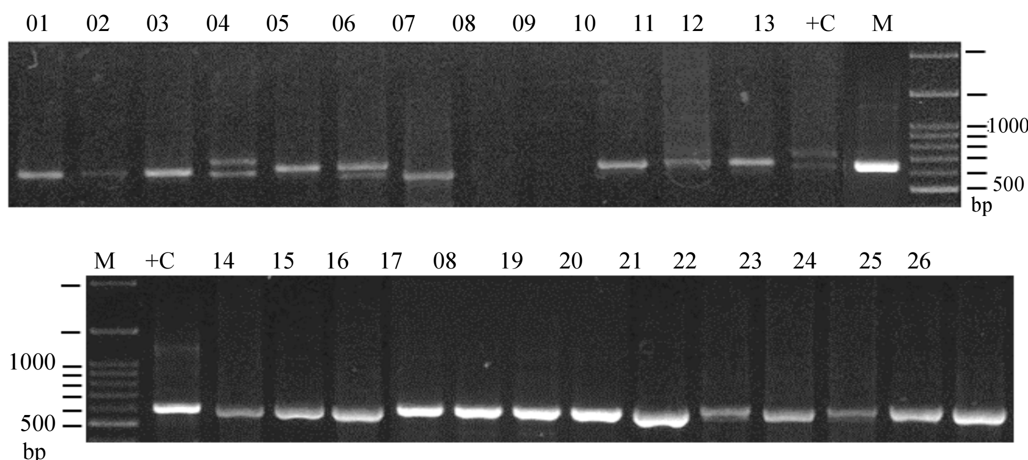

Fig. 1: Electrophoregram of *MSP1* PCR products of *P. falciparum*. Lanes 01-07, 10-26 shows pattern of PCR fragments in genetic marker display by these isolates. Lanes 08,09 showed no presence of DNA fragmen. +C= positive control, M= Marker (100 bp)

This is the first study that gives information about genetic diversity of Merozoite Surface Protein.1 of *P. falciparum* in the highland region of Wamena, Papua. According to Kolawole *et al*. (2016) merozoite Surface Protein 1 of *P. falciparum* is a major surface protein. The protein is a principal target of human immune response and is a promising candidate for a blood stage sub unit vaccine. Thus, Some *et al*. (2018) also highlighted that genotyping of malaria parasite population remain an important tool to determine the types and number of parasites clones in an infections.

Our study showed that PCR typing of *MSP*-1 (block 2) of *P. falciparum* in Puncak Jaya Wamena Papua have a highly complex genetic diversity. All third types of *msp*1 allelic families MAD20, RO33 and K1, were identified. Our further study of *MSP*1 allelic families MAD20, RO33 and K1 showed high recombination. From 24 isolate sample of gene *MSP*1 found is poly-allele MAD20/RO33 (33,3%) (Fig. 1). In contrast with previous study in Brazil, Myamnar, Thailand and West Sumatera, where the highest frequency is mono allele (Atroosh *et al*., 2011; Kolawole *et al*., 2016; Khaminsou *et al*., 2011; Oyebola *et al*., 2014; Mohammed *et al*., 2015). According to Oyebola *et al*. (2014) areas with high malaria spread generally have high parasite diversity with multi allele or polyclonal character.

Recombinatios of *msp*1 allelic families MAD20, RO33 and K1 in the study in contrast with Sorontou and Pakpahan's report (2015) in lowlands and coastal areas of Papua (Koya & Skouw). In this study, variation of poly allele K1/MAD20 was not detected.

Various genes of *P. falciparum* were expressed as surface protein, especially *MSP*1 is a form of genetic diversity that exist in a population. Geographically, different locations also influence genetic diversity. This is due to some cases, two of them are mutation and recombination (Nurwidayati, 2010).

Conclusion

Genetic polymorphism with various allele types can be identified in *MSP*-1 block 2 *P. falciparum* that include K1, MAD20 and RO33.

This study result showed that the highest frequency of infection was caused by polyclonal (poly allele) i.e. MAD20/RO33, while the highest frequency of monoclonal infection (mono allele) is MAD20. This study did not find combination of variation allele K1/MAD20.

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Author's Contributions

Rosye H.R.Tanjung: Fully involved in all phases of the study, including in laboratory during molecular Analysis, data analysis, interpretation and write-up of the manuscript.

D.Y.P Runtuboi: Designed the study project critical revised the manuscript. Fully involved in all phases of the study including in laboratory during molecular Analysis, data analysis, interpretation, and write-up of the manuscript.

Y. Sarungu: Involved in statistical analysis of data and critical revision of manuscript for publication.

M.J Imbiri and A.I Resmol: Contributed to write up, read and approved the final manuscript.

J. Suyono: Contributed to interpretations molecular data, write up, read and approved the final manuscript.

Competing Interest

The authors declare that they have no competing interests.

Ethics

The aims and objectives of the study was discussed with Head of the hospital and staffs of regional Hospital Laboratory of Puncak Jaya Regency. Ethical clearance of Ethics Committee and permission to undertake the study were obtained from these stakeholders. Each study participant after being briefed and offered the opportunity to ask questions about the study, was provided with individual informed written consent form to sign or thumb print. The written consent forms and participant information form were kept separately from the data collections tools.

References

Atroosh, W.M., H.M. Al-Mekhlafi, M.A.K. Mahdy, R. Saif-Ali and A.M. Al-Mekhlafi *et al*., 2011. Genetic diversity of *Plasmodium falciparum* isolates from Pahang, Malaysia based on *MSP*-1 and *MSP*-2 genes. Parasit Vectors, 4: 1-9. DOI: 10.1186/1756-3305-4-233.

Bapenas, 2005. Summary of reports on progress towards achieving. Indonesia's Millennium Development Goals. pp: 24-26.

Ernawati, K., B. Soesilo, A. Duarsa and Rifqatussa'adah, 2011. Individual and Housing Environment Risk Factors Related to Malaria in Punduh Pedada, Pesawaran District of Lampung Province in 2010. Makara Kesehatan, 15: 51-57.

Harliani, N.M. and Nurhadi, 2015. Factors Related to Malaria Incidence in the Working Area of Pangkep District Kalmas Liukang Health Center. J. Ilmiah Kesehatan Diagnosis, 6: 749-746.

Hoffmann, E.H.E., P.E.M. Ribola and M.U. Ferreira, 2013. Genetics relatedness of *Plasmodium falciparum* isolates and the origin of allelic diversity at the merozoites surface protein -1 (*MSP*-1) locus in Brazil and Vietnam. Malaria J.

Kang, J.M., S.U. Moon, J.Y. Kim, S.H. Cho and K. Lin *et al*., 2010. Genetic polymorphism of merozoite surface protein-1 and merozoite surface protein-2 in *Plasmodium falciparum* field isolates from Myanmar. Malaria J. DOI: 10.1186/1475-2875-9-131

Khaminsou, N., O. Kritpetcharat, J. Daduang, L. Chaererntanyarak and P. Kritpetcharat, 2011. Genetic analysis of the merozoite surface protein-1 block 2 allelic types in *Plasmodium falciparum* clinical isolates from Lao PDR. Malaria J., 10: 1-14.

Kolawole, O.M., O.A. Mokuolu, Y.A. Olukosi and T.O. Oloyede, 2016. Populations genomic diversity of *Plasmodium falciparum* in malaria patients attending Okelele Health Cendtre, Okelele, Ilorin, Kwara Stete, Nigeria. Afrika Health Sciences, 16: 704-711.

Muhammed, H., T. Mindaye, M. Belayneh, M. Kassa and A. Assefa, 2015. Genetic diversity of *Plasmodium falciparum* isolates based on *MSP*-1 and *MSP*-2 genes from Kolla-Shele area, Arbaminch Zuria District, southwest Ethiopia. Malaria J., 14: 1-7.

Nurwidayati, A., 2010. Article review: Response of antibodies to Plasmodium falciparum merozoite protein in determining malaria transmission. J. Vektor Penyakit, 4: 17-25.

Oyebola, M.K., E.T. Idowu, Y.A. Oluka, B.A. Iwalokun and C.O. Agomo *et al*., 2014. Genetic diversity and complexity of *Plasmodium falciparum* infections in Lagos. Nigeria, 4: 587-591.

Papua, D.K.P., 2017. The health profile of the Papua province in 2016. pp: 13-15.

Permenkes Malaria, 2009. Health Ministry Decree of Republic of Indonesia No 293/MENKES/SK/IV/2009. 28 April 2009 Concerning the Elimination of Malaria in Indonesia. Director General of P2PL, Department of Health.

Ricio, L.R.P., D.S.P. Silva., J.C.L. Junior, M. Theisen and F. Santos *et al*., 2013. Genetic polymorphism in the glutamate-rich protein of *Plasmodium falciparum* field isolates from a malaria-endemic area of Brazil. Mem. Isnt. Oswaldo Cruz, Rio de Janeiro, 108: 523-528.

Some, A.F., T. Baziè, I. Zongo, S. Yerbanga and F. Nikièma *et al*., 2018. *Plasmodium falciparum msp*1 and *msp*2 genetic diversity and allele frequencies in parasites isolated from symptomatic malaria patients in Bobo-Diolasso, Burkina Faso. Parasites Vector, 11: 323.

Sorontou, Y. and A. Pakpahan, 2015. Genetic diversity in *MSP*-1 gene of *Plasmodium falciparum* and its association with malaria severity, parasites density and host factor of asymptomatic and symptomatic patients in Papua, Indonesia. Int. J. Medical Science Public Health, 4: 1584-1593.

WHO, 2007. Methods and Techniques for Clinical Trial on Antimalarial Drug Efficacy: Genotyping to Identify Parasite Population. Amsterdam, The Netherlands, pp: 13-16. ISBN: 978924159630