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Research article

Genetic diversity of merozoite surface protein-1 in *Plasmodium falciparum* field isolates from a mountain and coastal area in West Sumatera, Indonesia.

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Abstract :

Aim: To know the diversity of allelic type of MSP-1 block 2 among *Plasmodium falciparum* isolates collected in a mountain area and a coastal area in West Sumatera, Indonesia, and compare them in a mountain and coastal area.

Methods: A total of 56 *P. falciparum* infected blood samples, which were collected from 27 patients attending a local health facilities in South Solok district is a mountain region and 29 patients attending a local health facilities in South Coastal district is a coastal region, West Sumatera, Indonesia were used in this study. The regions flanking the highly polymorphic characters, block 2 for MSP-1, were genotyped by allele-specific nested-PCR to analyse the population diversity of parasite. Sequence analysis of the polymorphic regions of MSP-1 was also conducted to identify allelic diversity in the parasite population.

Results : Diverse allelic polymorphism of MSP-1 was identified in *P. falciparum* isolates from a mountain area and a coastal area in West Sumatera, Indonesia,West Sumatera, Indonesia and most of the infections were determined to be mixed infections. Sequence analysis of MSP-1 block 2 revelaled that 16 different alleles for MSP-1 (3 for K1 type, 2 for MAD20 type and 2 for RO33 type) were identified.

Conclusion: Extensive genetic polymorphism with diverse allele type was identified in MSP-1 in *P. falciparum* field isolates from a mountain area and a coastal area in West Sumatera, Indonesia. A high level of mixed infections was also observed, as was a high degree of multiplicity of infection.

Key words: Plasmodium falciparum, MSP-1 block 2, allelic types, mountain area, coastal area.

Introduction:

Malaria is a major public health problem and is associated with 300-500 million clinical cases world wide as well as 0.5 – 3 million deaths annually, almost all of them are caused by *P. falciparumi*[1,7,12]. Genetic diversity presented by P. Falciparum field isolates, the occurence of variant forms of the parasite in different geographic areas, and occultation of multiple genotypes during a single mosquito, constitute one of the main obastacles to the design of a malaria vaccine[10,13].

Merozoite surface protein (MSP)-1 and merozoite surface protein-2 (MSP-2) are 2 protein causing immune responses in humans [15,2] and are important candidates for development of blood stage malaria vaccines[11]. The block 2 MSP-1 is particularly polymorphic and 3 distinct allelic families have been described as Mad 20, K1 and Ro33[4]. The polymorphic central domain of the gene encoding MSP-2 belongs to 2 distinct families; Ic and Fc27[14]. Allelic forms of these antigen genes have been reported from different parts of the world[3,5,6,8] and further characterization of the degree of polymorphism in these antigens will be of interest for appropriate design of malaria vaccine[9].

The study was conducted in South Solok district, an area located in Bukit Barisan Mountain and in Pesisir Selatan, an area located in west coast in West Suimatera province, Indonesia. The area has a farming of cocoa and sawit coconut in South Solok and majority was fisihing community in Pesisir Selatan. All area is infested a primary vector, *Anopheles balabacensis* in South Solok and *Anopeheles sundaicus* in Pesisir Selatan and is characterized by high altitude, relative high humidity, constant rain, and an average of temperature 23^{0}C in Pesisir Selatan. The analyzed samples were collected during an aoutbreak in which the

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prevalence of malaria was 60%, measured as the presentage of people with parasites among those who presented with malaria symptoms at a health service.

Methods:

Clinical Samples:

The samples was collected by finger puncture in the form of thick smears on slides. The slides were stained with Giemsa, and the presence of *P. falciparum* was detected under microscopic observation. The slides were then sent to the Medical Faculty, Andalas University in Padang, and parasitemia was determined and normalized for 100 leucoytes. This information was converted into the number of parasites per microliter, assuming a leucocyte count of 8,000/µL. To prepare DNA of the clinical samples, the thick smear , moistened previously with 1% saponin, the incubatred for one hour at 4°C. The sample was centrifuged at 12,000 x g for five minutes, and the supernatant discarded. The precipitate eas resuspended in 40 µL of 5% Chelex-100 and boiled for 10 minutes. Fianlly the sample was centrifuged at 12,000xg for five minutes, and the supernatant was recovered and stored at 4°C . We used 8 µL of the extract to amplify the MSP-1 gene through polymerase chain reaction (PCR).

The first amplification were used as a template for respective nested PCR assays. We took 5 µL to identify the allotypes in block2 of MSP-1.

Allotype detection in block 2 of MSP-1:

In the PCR-based amplification for MSP-1 gene and the nested PCR to identify the allotypes in block 2 of this gene, we used an amplifiaction profile with an initial denaturation at 94°C/2 min followed by 72°C/2 min, time during which the Taq DNA Polynerase enzyme was added. Then, 35 cycles at 94°C/30 sec, 55°C/30 sec, and 72°C/2 min were performed, ending with a final extension at 72°C/2 min. The following primers were used in amplifying the MSP1 gne: OK11 (5' TAG AAG ATG CAG TAT TGA CAG GTT A 3') and OK12 (5' ATT CTA ATT CAA GTG GAT CAG TAA ATA A 3'). To define the allele present in block 2 of the MSP-1 gene, the primers OK1 (5'CTT AAA TGA AGA AGA AAT TAC TAC AAA AGG TGC 3' and OK2 (5' GAG GGC TTG CAC CAG ATG AAG T 3') were used for the K1 allelic type. The primers OK3 (5' GTA TTA AAT GAA GGA ACA AGT GGA ACA 3') and OK4 (5' TAT CTG AAG GAT TTG TAC GTC TTG AAT T 3') were used to typify the MAD20 allotype. The primers OK5 (5' ATT AAA GGA TGG AGC AAA TAC TCA AFT TGT 3' and OK6 (5' TC GAA GGA TTT GCA GCA CCT GGA GA 3') were used to amplify the RO33 allelic type. Both the primary and the nested PCR were conducted in a final volume of 50 µL, using 200 µM of each dNTP, 1 µM of each primer, 2,5 U of TaqDNA polymerase (Promega) per reaction and 1 mM of $MgCl_2$ in the enzyme buffer (50 mM KCL, 10 mM Tris-HCl, pH 9,0, 0,1 % Triton-X100).

Allelic distribution and complexity of infection:

The prevalence of each allelic type analyzed was determined as the presentage of PCR fragments for the type in the total number of amplified bands for the corresponding locus. The complexity of infection, which is the average number of PCR banmds per infected individual, was determined as described earlier. The presentage for type and complexuty if infection were calculated independently for each genetic marker.

Sequencing analysis of MSP-1:

For sequence analysis of MSP-1, all PCR products (128 for MSP-1) obtained by allelic typing PCR were purified from the gel and cloned into pGEM-T Easy vector (Promega, Madison, WI,USA). Each ligation mixture was transformed into *E. coli* DH5α competent cells and positive clones were screened for the presence of plasmid with the appropriate insert. Sequencing reactions were performed using the BigGye Terminator Cycle Sequencing Ready Reaction Kit in an ABI 377 automatic DNA sequencer (Applied Biosystems, Foster City,CA,USA). To verify the sequences, sequence analysis was performed by analysing at least two plasmid clones containing each gene insert. Analysis of the primary structures of the deducted amino acid sequences was done with DNASTAR (DNASTAR,madison, WI,USA). Nucleotide sequences reported in this papaer are available in the GenBank database under accession numbers EU445555-EU445557 EU445559-EU445566, GO861442-GO861443, and GO861445 for MSP-1.

Discussion:

The genetic structure of *P. falciparum* population plays a highly important role in the natural acquisition of immunity in malarial infections. Therefore,

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knowledge of the genetic structure of these populations is necessary to develop strategies to control the disease, including the design of effective vaccines against *P. falciparum*. In this study, genetic polymorphism of two merozoite surface proteins, MSP-1 of 63 *P. falciparum* siolates collected in Myanmar, where malaria is endemic or hypoendemic, was analysed. To our knowledge, no such study has been done in Myanmar to date, and therefore this study provides the first estimate of the genetic diversity of a *P. falciparum* wild-type isolates circulating in Myanmar.

Allele-specific PCR typing of MSP-1 (block 2) showed that P. Falciarum population in Myanmar have a highly complex genetic diversity. For MSP-1, both types of K1 and MAD20 with different length of amplified products (120-210 bp for K1 and 140-250 bp for MAD20) were identified. Most of the isolates (63,5%) were mixed infections which harbored both allele types. Several similar studies in different geographic areas which used block 2 of MSP-1 as a polymorphic marker reported inportant variations in the frequency of the genotypes. MAD20 (57/63, 90,5%) was predominant allele in the *P. falciparum* population in Myanmar, which is concistent the situation in Thailand, Iran, Pakistan and Colombia. On the other hand, in studies in French Guaiana, Kenya and peru, MAD20 is the less frequent type and K1 is the most frequent.

To further investigate the allelic diversity of MSP-1 in *P. falciparum* isolates from Myanmar, sequence analysis of MSP-1 was performed. Sequence analysis of MSP-1 block2 showed that a total of 14 alleles of MSP-1, 5 for K1 and 9 for MAD20 type, were identified. Allelic diversity of MSP-1 block 2 in *P. falciparum* Myanmar isolates was due to different numbers of unique tripeptide repeats, which is similar to previous studies.Interestingly, duplication of PT otif at the 3' end of block 2 was identified in 3D7 type alleles (alleles 10 and 11). This proliferation of the PT motif had been identified in non-Asian parasite previously, but it is the first description of PT duplication in Asian parasite.

Although it seems likely that nonreciprocal recombination events, such as replication slippage and gene conversion, durting the mitotic (asexual) replication of the parasite also play a plausible role in creating allele variation., allelic diversity *P. falciparum* MSP-1 is mainly generated by meiotic recombination events involving genetically parasite clone that infect the same mosquito vector. Therefore, the proportion of mixed infections and the number of clones per individual is one of the pre-requisites to generate new genotypes and to increase the diversity of the parasitic population.

Conclusion:

A major finding of this study was that P. *falciparum* field isolates in Myanmar exhibited a high degreee of genetic polymorphism in MSP-1. Moreover, most of the infection were mixed with a high level of MOI. Theses result collectively suggested the highly complex population structure of parasite in Myanmar.

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