No antimalarial resistance of *Plasmodium falciparum* varian West Indonesia using molecular markers screening

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Abstract. Artemisin-based combination therapy (ACT) were widely used in Indonesia for the treatment of Malaria *Plasmodium falciparum*. Previous study in East Indonesia, located in Irian showed no prevalence of resistance, suggesting the treatment still effective in the area. This study aimed to provide baseline data of antimalarial drug resistance markers on *P. falciparum* isolates in Lampung West Indonesia. Mutation on genes associated with ACT (PfRBP9) were assessed by PCR amplification. Mutation on the marker related with artemisin resistance were determined by DNA sequencing technique. Isolates of 14 samples revealed no mutation in the D56V codon which is usually confined. This study has demonstrated a low prevalence resistance allele in the study area. Continuous surveillance of antimalarial drug efficacy is recommended and the findings provide information for stakeholders ensuring proper policy controlling malaria.

1. Introduction

The emergence of artemisin resistance in south-east Asia threatens the global to control malaria [1]. *Plasmodium falciparum* resistance to artemisin, the most potent and latest anti-malarial, endangers malaria elimination strategies [2]. Ring-stage susceptibility to artemisinin were reduced, and parasite clearance in patients treated with ACTs were slow [3].

RPB9-propeller polymorphism inaugurate a useful molecular marker for wide-scale monitoring efforts [4]. Non-synonymous propeller mutants reported signify emerging artemisin resistance, such as mutations D56V is common [4]. Parasites with mutations associated with artemisin resistance were widely present along the Thai-Cambodia and Thai-Myanmar borders [5]. No evidence of artemisinin resistance was found outside Southeast Asia and China, where resistance-associated mutations were confined [6]. Here we describe the genetic features of *P. falciparum* RPB9 from Lampung Indonesia, and analyse the possibility of artemisin resistance evidence.

2. Methods

2.1. Study location
The study was carried out in Bandar Lampung South Sumatra, Indonesia, between February until June 2017. Previous research in 2000 showed the clinical resistance to chloroquine by *P. malariae* [7].
2.2. Study design
This was a prospective efficacy study of artemisin for P. falciparum in adults with uncomplicated symptomatic malaria. Fast screening using the rapid diagnostic test (SD BiolineTM) and confirmed with microscope examination.

2.3. Patients
Patients were eligible to enroll if they had a fever or history of fever in the preceding 24 hours, with slide-confirmed malaria for P. falciparum or mixed asexual parasites for P. vivax. They were aged between 1 and 65 years, weighed more than 5 kilograms. Pregnant and lactating women, patients with signs of severe malaria, were all excluded. Informed consent was obtained from patients aged more than 18 years old and in those less than 13 years old.

2.4. Research procedure
DNA from venous or capillary blood samples collected in Vacutainers™ was extracted using the Wizard Genomic DNA Purification Kit (Promega Inc) as per the manufacturer’s instructions. Speciation was done by using an 18sRNA gene-based polymerase chain reaction (PCR) method for the detection of P. falciparum. The presence of polymorphisms in the propeller domains of the P. falciparum (RBP9) 3D7 gene, associated with artemisinin derivatives resistance, was determined by Sanger sequencing using the 1stBASE protocol.

The band size is 673bp, with nested and semi nested size are 184 – 287 and 572 bp. The forward primers: pfRPB9-R: 5’ – TTG AGC TTC TTT TTC CCA ATA ATG GC - 3'; and the gene 746: 5’ – TCT TCA CAG AAA TAT TCC TCC ACG T - 3'; the reversed primers of: pfRPB9-F: 5’ – TGA TAT ATG TTT GTA GGA GCT GTG AG – 3'; and the gen 177: 5’ – ATA TAT CCA CCC CGA AAC CAA AAA C – 3’. After an initial 5-min hold at 94°C, 5 or 10 μL were PCR amplified for 40 cycles: 94°C for 30 s, 60°C for 20 s, and 72°C for 40 s, followed by a final extension 72°C for 10-min.

2.5. Sequence analysis
Sequence data were examined using software Geneious 8.1.9, with tools for mutation analysis MAFFT v7.017. Furthermore, the data obtained were analysed using SPSS program and displayed in tabular form.

3. Results
Between February until June 2017, 71 positive samples were collected. Those included with mix other than P. falciparum (26 patients) and single infection (45 patients). Nearly all the samples contained a single RBP9 allele. Amplicon of 14 isolates were detected with PfRBP9, namely isolated number: 6-19-23-29-34-36-39-42-45-58-69-70-71-72.

4. Discussions
Artemisinin resistance was first detected in western Cambodia in 2007, and is now confirmed in the surrounding countries in Greater Mekong region [8–11]. In Indonesia, previous study for east Indonesia artemisin resistance surveillance showed that no mutation gene [12]. Suggested that IV artesunate was treatment of choice in Indonesian adult (OD 3.2, 95% CI 1.3–7.8) [13–16]. Artemisinin (ART) and its derivatives are the most potent and fastest acting anti-malarial, firstly introduced in Indonesia around 1980s [2,17,18]. Almost half of the country's population of 250 million live in
malaria-endemic areas [19]. Furthermore, most half of them seeking treatment for clinical malaria each year [20].

No resistance of artemisin in Africa [21]. Concern has been raised while 90% of the global mortality from malaria occurs in Africa [21–23]. However, other marker of the resistance was identified in the propeller region of RBP9 in 92 (5.5%) isolates in 2013 and 2014 from Senegal [24]. In central Vietnam, the efficacy of DHA (dihydroartemisin) is still satisfactory, but the parasite clearance time and rate are indicative of emerging artemisinin resistance [25]. First study in India to document the presence of F446I NS mutation that region close to the Myanmar border, urged to undertake systematic monitoring [26]. Continuous monitoring is performed by neighbouring countries close border to Cambodia-Thailand-Myanmar.

**Picture 2.** Electrophoresis detection of 14 isolates with fullnested primers in band size of 182 bp.

**Picture 3.** Electrophoresis detection of 14 isolates with seminested primers in band size of 287 bp.
**Figure 1**: No detection of amino acid change from Aspartic acid into Valin codon 56; No nucleotide changes, still GAC in sequence base 518-520.

5. Conclusion

No SNP (single nucleotide polymorphism) on the marker of the RBP9 allele found from west Indonesia. Continuous surveillance of antimalarial drug efficacy is recommended as for information to the stakeholders ensuring proper policy controlling malaria.

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