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Clinical features of severe malaria: Protective effect of mixed plasmodial malaria

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ABSTRACT

Objective: To investigate clinically severe malaria patients with *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*) and mixed species infections.

Methods: This study was conducted at Dr. Saiful Anwar General Hospital, Malang, Indonesia, from December 2011 to May 2013. Twenty nine patients (mean age of 41 years, 22% female), who suffered from severe malaria according to World Health Organization criteria (major and minor) and other criteria based on previous studies, were selected by consecutive sampling. Blood samples were obtained at admission from peripheral blood for microscopic diagnostic, nested PCR and laboratory examination of blood chemistry. Laboratory results were compared between the groups and correlated to each other.

Results: From 29 samples, eight (28%) were diagnosed as *P. falciparum* mono-infection, 12 (41%) as *P. vivax* mono-infection and nine (31%) as mixed infections, confirmed by PCR. Cerebral malaria occurred in *P. falciparum* or mixed species infection only. Parasitaemia was highest in *P. falciparum* mono-infection. Mean haemoglobin was significantly lower in *P. falciparum* than *P. vivax* infection ($P = 0.01$). Mean thrombocyte count (77 138/μL) was low in all groups. Mean urea, creatinine, total and direct bilirubin were significantly higher in *P. falciparum* mono-infection compared to other groups, whereas aspartate aminotransferase and alanine aminotransferase showed no significant differences. Parasitaemia was positively correlated with an increase in urea, creatinine, bilirubin and leucocytosis in all species.

Conclusions: Both *Plasmodium* species can solely or in combination cause severe malaria. Mixed infection was generally more benign than *P. falciparum* mono-infection and seemed to have some protective effects.

1. Introduction

Malaria, as an infectious disease, remains a major health problem in the world, especially in tropical and developing countries. In Indonesia, malaria is one of the top ten infectious diseases with an incidence of 1.9% and a prevalence of 6.0% in 2013 [1]. The World Health Organization estimated that about 198 million people worldwide suffered from malaria with a mortality rate of approximately 584 000 cases in 2013 [2].

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The study protocol was performed according to the Helsinki declaration and ethical approval was obtained from the Ethical Committee Medical Research of Faculty of Medicine, University of Brawijaya. Informed written consent was obtained from the 29 severe malaria patients.

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Malaria is caused by protozoan of *Plasmodium* genus, and transmitted by *Anopheles* sp. mosquito as vector. There are five human-pathogenic species of *Plasmodium* known: *Plasmodium vivax* (*P. vivax*), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium ovale* (*P. ovale*) (*P. ovale wallikeri* and *P. ovale curtesi*), *Plasmodium malariae* (*P. malariae*) and *Plasmodium knowlesi* (*P. knowlesi*) [3,4]. Each species has different clinical symptoms. *P. falciparum* is known to cause the most severe clinical symptoms with cerebral malaria as a major complication. However, some recent researches suggested that other *Plasmodium* such as *P. vivax* and *P. knowlesi* were also responsible for severe manifestations. *P. vivax* can cause severe clinical outcome because of the simultaneous increase of the tumour necrosis factor, interferon-γ and interleukin-10 [5]. In a case series of severe vivax malaria conducted by O'Brien *et al.*, jaundice and severe thrombocytopaenia were the major complications [6].

Parasite density may affect the severity of clinical symptoms of malaria and the laboratory results. Based on a study conducted by Tangpukdee *et al.*, high parasite density was associated with severe clinical illness, complications and mortality [7].

Further investigation of the relationship between parasite density and elevation of laboratory blood chemistry parameters in severe malaria infection could help to reveal differences and common features in *P. falciparum* and *P. vivax* infections. Therefore, this study on parasite density and blood chemistry parameters such as urea, creatinine, bilirubin (direct, indirect, and total), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in severe *P. falciparum* and *P. vivax* patients was conducted.

2. Materials and methods

The study was conducted from December 2011 to May 2013 at Dr. Saiful Anwar General Hospital, Malang, Laboratory of Parasitology and Biomedical Central Laboratory, Faculty of Medicine, University of Brawijaya, Indonesia. The area is not endemic for malaria. Severely ill patients are referred to the University Hospital from rural areas of Java and other nearby islands. A total of 29 severe malaria patients (positive malaria parasite slide or rapid diagnostic test (RDT) later-confirmed by nested PCR) were selected through consecutive sampling technique after written informed consent. Each patient showed at least one sign or symptom of severe malaria according to World Health Organization criteria and other criteria that had been reported previously for severe or complicated *P. vivax* [8–10]. Severity criteria of samples are shown in Table 1. Blood samples were taken from patients at admission. Patients had been ill for 3–14 days before admission. Patient follow-ups were done by a doctor in charge, but data were not included in this research. Diagnosis of malaria was based on thick and thin blood film and/or RDT and later-confirmed by nested PCR. Detailed history was taken and complete physical examination was performed. Patients were treated based on guidelines from Ministry of Health of Indonesia. Severe malaria patients were treated with artesunate injection, while uncomplicated malaria patients were treated with oral dihydroartemisinin + piperaquine [11].

Five millilitres peripheral venous blood (medial cubital vein) from each patient were collected in an ethylene diamine tetraacetic acid vacutainer. Blood samples were processed for thick and thin blood smears for microscopic examination, nested PCR to confirm the diagnosis, and laboratory examination of blood chemistry. Thick and thin blood smears were prepared according to standard procedures. Slides were stained with 10% Giemsa for 30 min and independently read by two experienced parasitologists. Asexual parasites were counted on thin blood films among 1 000 red blood cells to attain percentage of parasitized red blood cells. Slides were declared negative if no parasites were seen in 200 fields of the thick film.

Table 1
Criteria of severe malaria.

Clinical criteria	Parameter value
Impaired consciousness	Glasgow coma scale < 15
Prostration	(+)
Multiple convulsions	> 2 episodes within 24 h
Deep breathing and respiratory distress	(+)
Acute pulmonary oedema and acute respiratory distress syndrome	(+)
Circulatory collapse or shock	Systolic blood pressure < 80 mmHg in adults and < 50 mmHg in children
Abnormal bleeding	(+)
Acute kidney injury	Creatinine > 2.5 mg/dL or < 400 mL/24 h)
Clinical jaundice plus evidence of other vital organ dysfunction	Bilirubin > 3 mg/dL; ALT/AST > 3 times elevated
Metabolic acidosis	Plasma bicarbonate < 15 mmol/L, pH < 7.25
Severe normocytic anaemia	Haemoglobin < 5 g/dL, packed cell volume < 15% in children; < 7 g/dL, packed cell volume < 20% in adults
Haemoglobinuria	(+)
Hyperlactatemia	Lactate > 5 mmol/L
Hypoglycaemia	< 2.2 mmol/L or < 40 mg/dL
Pulmonary oedema	(+) Radiological
Parasitaemia	Low and moderate: hyperparasitaemia (> 100 000/μL ~ 2.5%)
Thrombocytopaenia	< 50 000/mm^3
AST	> 38 IU/L in male; > 32 IU/L in female
ALT	> 41 IU/L in male; > 31 IU/L in female
Creatinine	> 1.4 mg/dL in male; > 1.1 mg/dL in female
Urea	> 50 mmol/L
Jaundice	Serum bilirubin > 50 mmol/L or > 3 mg/dL

Chemical laboratory values were measured at the central laboratory of Dr. Saiful Anwar General Hospital using automatic blood cell counter and automatic analyser laboratory unit. Normal values were blood urea ≤ 50 mmol/L; creatinine ≤ 1.4 mg/dL in men, ≤ 1.1 mg/dL in women; AST ≤ 38 IU/L in men, ≤ 32 IU/L in women; ALT ≤ 41 IU/L in men, ≤ 31 IU/L in women; total bilirubin < 1.1 mg/dL; direct bilirubin ≤ 0.125 mg/dL; indirect bilirubin ≤ 0.8 mg/dL.

DNA isolation was performed using PureLink™ Genomic DNA Kits (Invitrogen®), nested PCR was performed using 2× PCR Master Mix (Norgen®), and primers for genus and species identification of *Plasmodium* was used as described previously [12] after optimization.

The mean, minimum and maximum of parasite density and laboratory alterations were calculated. Proportions were calculated and analysed by *Chi*-squared test. Means were compared by student's *t*-test and Mann–Whitney–Wilcoxon test if