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

Bagus Hermansyah[1], Loeki Enggar Fitri[2*], Teguh Wahju Sardjono[2], Agustina Tri Endharti[2], Samsul Arifin[3], Niniek Budiarti[4,5], Didi Candradikusuma[4,5], Erma Sulistyaningsih[1], Nicole Berens-Riha[6]

[1]*Department of Parasitology, Faculty of Medicine, University of Jember, Jember, East Java, Indonesia*

[2]*Department of Parasitology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia*

[3]*Department of Biochemistry and Biomolecular, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia*

[4]*Division of Tropical Medicine and Infectious Diseases, Department of Internal Medicine, Dr. Saiful Anwar Hospital, East Java, Indonesia*

[5]*Division of Tropical Medicine and Infectious Diseases, Department of Internal Medicine, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia*

[6]*Division of Infectious Diseases and Tropical Medicine, Medical Centre of the University of Munich (LMU), Leopoldstrasse 5, 80802, Munich, Germany*

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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].

*Corresponding author: Loeki Enggar Fitri, Department of Parasitology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia.
Tel: +62 8125285531
E-mail: lukief@ub.ac.id

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

appropriate. Correlations between parasite density and laboratory parameters were analysed using logistic regression models. A significance level of $P < 0.05$ was chosen. The statistical program used was Stata version 11.0 (StataCorp, Texas, USA).

Ethical approval was obtained from the Ethical Committee Medical Research of Faculty of Medicine, University of Brawijaya.

3. Results

Thirty five malaria patients were admitted at Dr. Saiful Anwar General Hospital during the study and six patients were excluded due to clinically uncomplicated cases, so 29 subjects with severe malaria were enrolled in this study. Six (20.7%) patients were females. Microscopy showed 18 *P. falciparum* mono-infections, 10 *P. vivax* mono-infections and one mixed species infection. Molecular diagnosis by PCR confirmed eight *P. falciparum* mono-infections (Group 1) and 12 *P. vivax* infections (Group 2), whereas the remainder (nine) were mixed species infections (Group 3). None of the subjects was infected with *P. malariae*, *P. ovale* or *P. knowlesi*. Baseline, clinical and laboratory data are shown in Table 2.

The degree of parasitaemia varied between 0.01% and 6.90%. There was one microscopically negative sample that was positively tested for *P. falciparum* by RDT and confirmed by PCR. Mean parasitaemia in *P. falciparum* malaria was 3.04% compared to 0.35% and 1.58% in *P. vivax* and mixed species infection, respectively. Patients with *P. falciparum* malaria (Group 1) presented with a significantly higher parasitaemia than patients in Group 2 ($P \leq 0.01$) or Group 3 ($P \leq 0.04$) in logistic regression models controlled for gender and age. A student's *t*-test showed similar results ($P < 0.01$ when *P. falciparum* compared to *P. vivax*, $P < 0.01$ when *P. vivax* compared to mixed species, $P = 0.13$ when *P. falciparum* compared to mixed species).

Cerebral malaria and seizures only occurred in *P. falciparum* mono-infection and mixed species infection. Results showed that 62.5% of Group 1 and 22.2% of Group 3 showed cerebral symptoms.

Mean haemoglobin was significantly lower in *P. falciparum* than that in *P. vivax* infection ($P = 0.02$, controlled for gender $P = 0.01$). Haemoglobin in Group 3 was higher than that in Group 1 but the difference was not significant. Females had in general lower haemoglobin (8.7 mg/dL, SD = 1.8) than males (10.8 mg/dL, SD = 2.7, $P = 0.08$).

Mean leucocyte count was significantly higher in *P. falciparum* than *P. vivax* infection ($P = 0.06$, controlled for gender $P = 0.04$). Leucocytes were ranging between 2 380 and 23 500/μL in Group

Table 2
Baseline, clinical and laboratory data from all species.

Data	All	*P. falciparum* (Group 1)	*P. vivax* (Group 2)	Mixed species (Group 3)
Species (by PCR) [*n* (%)]	*N* = 29	8 (27.6)	12 (41.4)	9 (31.0)
Species (by microscopy) [*n* (%)]		18 (62.1)	10 (34.5)	1 (3.4)
Gender [*n* (%)]				
Male	23 (79.3)	6 (75.0)	8 (66.7)	9 (100.0)
Female	6 (20.7)	2 (25.0)	4 (33.3)	0 (0.0)
Mean age in years (SD; range)	41.4 (12.3; 22.0–69.0)	40.6 (10.5; 22.0–60.0)	39.4 (10.2; 26.0–59.0)	41.6 (14.2; 22–69)
Parasitaemia (%) (SD; range)	1.47 (1.75; 0.01–6.90)[a]	3.04 (2.38; 0.21–6.90)[b]	0.35 (0.18; 0.01–0.60)[b]	1.58 (1.20; 0.10–3.50)[b]
Cerebral malaria [*n* (%)]				
No	22 (75.9)	3 (37.5)	12 (100.0)	7 (77.8)
Yes	7 (24.1)[c]	5 (62.5)	0 (0.0)	2 (22.2)
Seizure [*n* (%)]				
No	27 (93.1)	7 (87.5)	12 (100.0)	8 (88.9)
Yes	2 (6.9)	1 (12.5)	0 (0.0)	1 (11.1)
Mean haemoglobin (mg/dL) (SD; range)	10.4 (2.7; 6.5–15.3)	8.7 (2.3; 6.5–13.4)[b]	11.4 (3.0; 6.5–15.3)[b]	10.6 (1.9; 7.9–11.3)
Mean leukocytes/μL (SD; range)	7 788 (4 511; 2 380–23 500)	10 535 (7 248; 2 380–23 500)	6 598 (2 527; 3 340–12 500)	6 934 (2 446; 4 000–11 080)
Mean thrombocytes/μL (SD; range)	77 138 (44 775; 12 000–207 000)	93 875 (57 270; 12 000–207 000)	75 333 (36 235; 25 000–138 000)	64 667 (43 379; 28 000–166 000)
	N = 28	7	12	9
Mean urea (mmol/L) (SD; range)	63 (65; 19–278)	124 (97; 39–278)[b]	30 (17; 19–78)[b]	47 (16; 26–77)[b]
Mean creatinine (mg/dL) (SD; range)	1.5 (2.0; 0.6–9.6)	2.8 (3.5; 0.7–9.6)[b]	0.9 (0.3; 0.6–1.5)[b]	1.1 (0.2; 0.9–1.5)[b]
Mean AST (IU/L) (SD; range)	76 (67; 19–356)	79 (31; 40–121)	84 (96; 23–356)	64 (38; 19–116)
Mean ALT (IU/L) (SD; range)	57 (30; 13–124)	48 (26; 13–83)	68 (33; 18–124)	49 (27; 22–98)
	N = 25	7	9	9
Mean total bilirubin (mg/dL) (SD; range)	4.3 (7.5; 0.2–24.2)	10.1 (12.7; 1.5–34.2)[b]	2.1 (2.3; 0.2–6.9)[b]	2.0 (2.0; 0.3–6.6)[b]
Mean direct bilirubin (mg/dL) (SD; range)	2.9 (5.9; 0.0–26.9)	7.6 (9.9; 0.5–26.9)[b]	1.1 (1.4; 0.0–3.5)[b]	1.2 (1.3; 0.2–4.0)[b]
Mean indirect bilirubin (mg/dL) (SD; range)	1.4 (1.7; 0.2–7.2)	2.5 (2.8; 0.3–7.2)	1.0 (1.0; 0.2–3.4)	0.9 (0.7; 0.2–2.5)

[a]: $P < 0.05$ by student's *t*-test compared with mean parasitaemia of *P. falciparum* and *P. vivax* as well as of *P. vivax* and mixed infection; [b]: Logistic regression shows $P < 0.05$ between marked groups; [c]: $P < 0.05$ by *Chi*-squared test compared with all groups.

Table 3
Leucocyte differentiation and ML-ratio by species.

Parameter	All (n = 19)	*P. vivax* (n = 10)	Mixed (n = 9)
Mean absolute lymphocytes (SD; range)	1 338 (875; 125–2 886)	1 175 (952; 125–2 886)	1627 (748; 628–2 538)
Mean absolute monocytes (SD; range)	457 (406; 48–1 518)	356 (256; 122–954)	655 (490; 149–1 518)
ML-ratio (SD)	0.414 (0.286)	0.413 (0.279)	0.449 (0.308)
NLCR (SD)	4.52 (4.03)	5.53 (5.13)	3.51 (2.44)
Mean relative eosinophiles (SD; range)	1.6 (2.0; 0.0–9.0)	1.9 (2.6; 0.0–9.0)	1.5 (1.4; 0.0–4.0)
Mean relative basophiles (SD; range)	0.3 (0.6; 0.0–2.2)	0.4 (0.7; 0.0–2.2)	0.3 (0.4; 0.0–1.0)
Mean relative lymphocytes (SD; range)	22.3 (14.8; 1.0–56.0)	20.5 (16.4; 1.0–55.0)	25.0 (14.2; 11.0–56.0)
Mean relative monocytes (SD; range)	7.5 (5.3; 1.0–22.0)	6.0 (3.7.0; 1.0–11.0)	9.8 (6.3; 2.0–22.0)

1. Leucocyte differentiation was only consistently performed in Groups 2 and 3. Lymphocytes were low in both groups, other values were relatively normal and showed no differences between the groups. The overall monocyte-lymphocyte-ratio (ML-ratio) was 0.414. The neutrophil-lymphocyte count ratio (NLCR) was 4.52 on average. Group 2 showed a higher NLCR than Group 3 but the difference was not significant (Table 3).

Thrombocytes were low in all groups ranging between 12 000 and 207 000. Groups 2 and 3 showed lower mean trombocytaemia than Group 1 but the difference was not significant.

Mean urea (124 mmol/L) and mean creatinine (2.8 mg/dL) were highly elevated in *P. falciparum* mono-infection and significantly higher than those in both other groups.

Total bilirubin (10.1 mg/mL) was highly elevated in Group 1 and only moderately elevated in the other groups (2.1 mg/mL in Group 2 and 2.0 mg/mL in Group 3). Logistic regression showed significant differences between Group 1 and Group 2 as well as Group 1 and Group 3 (controlled for gender $P = 0.04$ and 0.02, respectively). The same was observed for direct bilirubin (7.6 mg/mL) between the groups (controlled for gender $P = 0.03$ and 0.02, respectively), whereas indirect bilirubin was relatively normal and showed no significant differences between the groups. AST and ALT showed only moderately elevated levels in all patients, there was no difference observed.

Parasitaemia was positively correlated with an increase of urea, creatinine, bilirubin and leucocytosis (Table 2). The correlation was significant for urea, creatinine and leucocytosis in all patients (controlled for gender $P \leq 0.01$, $P \leq 0.01$ and $P \leq 0.01$, respectively). Further analysis of the different groups was difficult regarding the low sample size. Relationship between creatinine and parasitaemia was most pronounced in Group 1. Increase in bilirubin (total, indirect and direct) was significantly correlated with parasitaemia in the mixed species group only.

On average, parasitaemia in females was higher than in males but the overall difference was not significant ($P = 0.496$). However, the *P. vivax* group showed significantly higher parasitaemia in females than in males ($P = 0.043$). Group 1 consisted of 6 males and 2 females, Group 2 consisted of 8 males and 4 females, and Group 3 consisted of 9 males only.

4. Discussion

In this study, 29 severe malaria cases were recruited from December 2011 to May 2013. Malaria was caused by *P. vivax* (41.4%), by mixed infection of *P. falciparum* and *P. vivax* (31.0%) and by *P. falciparum* alone (27.6%). Microscopy and PCR correlated poorly, especially in mixed infections. If available, molecular confirmation is therefore recommendable as it might have important treatment consequences due to the different regimens in *P. falciparum* and *P. vivax* malaria.

The high number of severe malaria cases caused by *P. vivax* is an interesting phenomenon since this species rarely causes severe malaria compared with *P. falciparum*. This result is consistent with several reports that *P. vivax* is not as benign as recently thought [5,13,14]. A change in the pattern of *Plasmodium* species that cause severe malaria was observed at the Dr. Saiful Anwar General Hospital Malang, Indonesia. Our previous study reported two cases of severe *P. vivax* malaria from Malang, Indonesia. Patients exhibited anaemia, thrombocytopaenia, jaundice, renal disturbance, and melaena [15].

None of the patients was infected with *P. malariae*, *P. ovale* or *P. knowlesi* infection. The reason might be the small sample size, the rareness of those three species in Indonesia, especially in Java Island, and their normally benign character [4,16].

No cerebral symptoms occurred in *P. vivax* mono-infection. Although the paradigm that *P. vivax* is incapable of cyto-adherence is questioned nowadays, the pathology seems to be different to *P. falciparum* [17]. Also, the degree of mean parasitaemia varied between the three groups and was highest in *P. falciparum* mono-infection with 3.04% compared to 0.35% and 1.58% in *P. vivax* mono-infection and mixed infection, respectively. Higher parasitaemia in *P. falciparum* is expected as it attacks all erythrocytes but *P. vivax* only invades reticulocytes. However, mixed infection showed significantly lower parasitaemia than *P. falciparum* mono-infection ($P < 0.01$). A protective effect of *P. vivax* was discussed and observed in a recent study but mechanisms are unclear so far [18]. Relative *P. vivax* parasitaemia was higher and haemoglobin in general was lower in females than in males, the latter might be the explanation for the higher parasitaemia.

Mean haemoglobin was lowest in Group 1 and significantly higher in Group 2. Mixed infections showed higher mean haemoglobin than Group 1, but the difference was not significant. Anaemia correlates closely with parasitaemia due to destruction of parasitized erythrocytes.

Bacterial infections and septicaemia are associated with both malaria species as recent studies reported [19,20]. In this study, leucocytosis in Group 1 was higher than that in Groups 2 or 3, the difference was distinct but not significant ($P = 0.08$ or 0.18, respectively). Parasitaemia and leucocytosis were positively correlated ($P < 0.01$) but blood culture was not performed for confirmation of bacterial infection. As described before, mean thrombocythemia was generally low [21].

ML-ratio was introduced by Warimwe *et al.* as parameter for the risk of clinical manifestation of malaria [22]. The ML-ratio in severe malaria patients from Indonesia was high compared to children with asymptomatic parasitaemia in Kenya. Acute malaria infection probably increases the ML-ratio but no baseline

data before infection were available for comparison. There was no difference between Groups 2 and 3.

The ratio of NLCR was proposed as a parameter of systemic inflammation and stress. Wolfswinkel *et al.* found that the NLCR correlated with parasitaemia in imported malaria patients [23]. NLCR was highest in severe malaria falciparum compared to uncomplicated falciparum and non-falciparum malaria but the difference was not significant. Mean NLCR was comparable in Indonesian patients with no significant difference between the groups.

Increased level of blood urea (> 50 mmol/L) were found in all but one patient with *P. falciparum* mono-infection, in only one patient with *P. vivax* mono-infection and in 55.6% of Group 3. Urea concentration correlated positively with parasitaemia ($P < 0.001$, $R^2 = 0.41$).

Mean creatinine was significantly higher in Group 1 than Groups 2 or 3 ($P = 0.01$), correlation controlled for gender with parasitaemia was highly positive as well ($P = 0.001$, $R^2 = 0.41$). Increased levels of blood urea and creatinine revealed that disturbances of renal function in *P. falciparum* infection were more frequent than in *P. vivax* infections in accordance with other studies [24,25]. Plewes *et al.* reported that parasite density was positively correlated ($r = 0.49$, $P < 0.001$) with serum creatinine in *P. falciparum* infection [26], as observed in this study. Malaria can cause disturbance of renal function through mechanisms of mechanical obstruction by infected erythrocytes, glomerular and tubular damage associated with immune pathology, hypovolaemia due to various reasons and decreased renal microcirculation [25]. Severe uraemia was found in one *P. falciparum* patient (275.7 mmol/L) causing death due to uraemic encephalopathy.

Liver enzymes were only slightly elevated in all groups, highest in the *P. vivax* group but differences between the groups were not significant. The findings were in accordance with a previous study by Pir *et al.* that demonstrated similar increased ALT levels in *P. falciparum* and *P. vivax* infections [27]. Hepatic dysfunction in malaria infection is common and varies from mild to severe, it is often associated with the onset of complications and mortality [28]. Increased AST and other inflammatory markers in severe *P. vivax* infection were also reported by Chaves *et al.* [5].

Jaundice is a very common manifestation in malaria and correlates usually with parasitaemia. Bilirubin levels and parasitaemia were positively correlated but the association was not significant ($P = 0.15$). Jaundice in malaria is the result of intravascular haemolysis of infected erythrocytes, stress-induced normal erythrocyte haemolysis and hepatic dysfunction [29]. All patients with *P. falciparum* mono-infection had elevated total and direct bilirubin, 55.6% each of Group 2 and Group 3 also showed elevated values, the difference between Groups 1 and 2 as well as 1 and 3 was significant by logistic regression models controlled for gender and by Mann–Whitney test. This is consistent with a study conducted by Pir *et al.* showing an increase in bilirubin more often in *P. falciparum* than *P. vivax* infection [27]. This fits the finding that parasitaemia was positively correlated with bilirubin levels.

Comparing Group 1 and Group 3, parasitaemia was significantly lower in Group 3 and cerebral symptoms, anaemia and leucocytosis less often as well. Creatinine, urea and bilirubin were significantly lower in mixed infection than in *P. falciparum* mono-infection. A protective effect of *P. vivax* seems to be the only plausible explanation. More studies to elucidate these findings and the mechanisms behind them should be conducted.

Severe malaria caused by different species of *Plasmodium* has some similarities and differences in terms of clinical features and laboratory results. Both *P. falciparum* and *P. vivax* infections can cause severe malaria although *P. vivax* mono-infection presented without cerebral symptoms. Parasitaemia was positively correlated with an increase in urea, creatinine, bilirubin and leucocytosis.

Interestingly, mixed infections were overall more benign than *P. falciparum* mono-infections with lower parasitaemia and less cerebral symptoms. This could indicate a protective effect of mixed infections compared to *P. falciparum* mono-infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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