An Autochthonous Case of Severe *Plasmodium knowlesi* Malaria in Thailand

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Abstract. A 58-year-old Thai man was infected with *Plasmodium knowlesi* in Chantaburi Province, eastern Thailand. In addition to pyrexia, the patient developed hypotension, renal failure, jaundice, and severe thrombocytopenia. The parasitemia at the time of admission was 16.67% or ~503,400 parasites/ μ L. With artesunate treatment and supportive care, the patient recovered uneventfully. The occurrence of complicated knowlesi malaria in a low-endemic area underscores the risk of high morbidity from this simian malaria.

INTRODUCTION

Plasmodium knowlesi, a simian malaria parasite with long-tailed (*Macaca fascicularis*) and pig-tailed macaques (*Macaca nemestrina*) as natural reservoirs, is endemic in Southeast Asian countries.¹ Knowlesi malaria in humans is often undiagnosed by routine microscopy as a result of shared morphological features of certain blood stages with *Plasmodium falciparum* and *Plasmodium malariae*, and responsiveness to diverse anti-malarial compounds *albeit* differences in parasite clearance property. Of importance, knowlesi malaria can cause severe complications and at times lead to mortality. Almost all complicated cases reportedly occurred in areas with a high prevalence of infection, especially in Malaysian Borneo, Sabah, and peninsular Malaysia.^{2,3} In Thailand, *P. knowlesi* contributes to < 1% of all malaria cases based on large epidemiological surveys using molecular detection methods.^{4,5} Of these, > 70% of knowlesi malaria patients were co-infected with *P. falciparum*, *Plasmodium vivax*, or both. It is noteworthy that all these knowlesi malaria patients, either having mono- or mixed species infections, had uncomplicated symptoms and were responsive to chloroquine and a combination of artesunate and mefloquine.^{4–6} Herein, we describe severe manifestations of knowlesi malaria in a newly recognized Thai patient.

CASE REPORT

In February 2013, a 58-year-old Thai male developed fever with chill for 4 days and oliguria for 1 day before admission. Thirteen days before initial febrile symptoms, the patient had spent 2 nights camping in Khao Khitchakut National Park in Chantaburi Province bordering Cambodia about 280 km southeast of Bangkok (12°86'55"N, 102°14'20"E). The patient reported that he was frequently bitten by mosquitoes while staying in the forest and he neither used mosquito repellent nor a net while sleeping. He also noticed several troops of wild long-tailed macaques roaming around his camping area. His illness began with a low-grade fever, malaise, myalgia, and loss of appetite. On the following days, high fever with a shaking chill occurred. One day before admission, his symptoms turned to be more pronounced with severe prostration and marked reduction in urine volume. At presentation, he

was conscious, lethargic with pale conjunctivae, and scleral icterus. Evidence of hepatosplenomegaly was not detected upon examination. His axillary temperature was 38.5°C, pulse rate 80 beats/min, respiratory rate 24 breaths/min, and blood pressure 80/50 mm of Hg. Complete blood count revealed the following results: hemoglobin 8.9 g/dL, hematocrit 24.3%, total leukocytes 8,080 cells/ μ L with neutrophils 49%, lymphocytes 26%, atypical lymphocytes 20%, monocytes 5%, and platelets 9,000/ μ L. Blood film examinations showed both asexual and sexual stages of malaria parasites in normochromic normocytic erythrocytes with an infected rate of 16.67% (or ~503,400 parasites/ μ L), comprising 23% young trophozoites, 58% amoeboid trophozoites, 6% band-shaped trophozoites, 9% double trophozoites, 0.75% schizont, 0.25% microgametocyte, and 3% macrogametocytes based on examination of 400 infected erythrocytes in Giemsa-stained thin blood films (Figure 1). Kidney function revealed blood urea nitrogen 85 mg/dL and creatinine 4.70 mg/dL. Serum electrolytes showed normal levels of sodium, potassium, and chloride but bicarbonate was 15.0 mmol/L. Liver function tests displayed increased levels of total bilirubin, direct bilirubin, aspartate transaminase, and alkaline phosphatase, although albumin was lower than its normal range. Prothrombin time and partial thromboplastin time were normal (Table 1). Urinalysis was unremarkable. Test results were negative for antibody against human immunodeficiency virus (HIV) and hepatitis B surface antigen. Chest radiograph and electrocardiogram were normal.

Because the initial blood smears of the patient contained various stages of malaria with unusually high parasite density, nested polymerase chain reaction analyses targeting the 18S rRNA and the mitochondrial cytochrome *b* genes of human malaria species were performed as previously described.^{4,5} Results of these assays confirmed that the patient was infected with *P. knowlesi*.

The patient was initially treated with intravenous fluids and dopamine with close monitoring of fluid balance to maintain the blood pressures within normal range. Anti-malarial treatment was initiated by three doses of 2.4 mg/Kg intravenous artesunate given at 12-hour interval and followed by oral artesunate 125 mg daily for 5 days. The parasite density rapidly declined and became negative from the third day onward. The serum creatinine levels returned to normal on the fourth day, whereas serum bilirubin and aspartate transaminase levels gradually declined. The platelet counts began increasing after the first day of artesunate administration and approaching normal level on the fifth day post-treatment. There was a

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FIGURE 1. *Plasmodium knowlesi* in Giemsa-stained thin (A) and thick (B) blood smears of the patient.

tendency toward an increment of monocyte counts, although the number of atypical lymphocytes showed an opposite trend. Complete defervescence was observed on the third day of hospitalization corresponding to the disappearance of malaria parasites in blood smears. Ten months later, the patient remained well without clinical recrudescence. Follow-up tests for liver function, renal function, and complete blood counts showed normal values, and malaria was negative by both microscopy and molecular detections (Table 1).

DISCUSSION

In Thailand, all *P. knowlesi*-infected native individuals identified so far had uncomplicated symptoms with low parasite density.^{4,5} Meanwhile, severe manifestations of knowlesi malaria largely overlap those of severe falciparum malaria *albeit* cerebral malaria has not been documented. The high parasitemia could lead to multiple organ dysfunctions.^{2,3} Hypotension in this patient could have arisen from a negative fluid balance, probably from inadequate fluid intake caused by illness, increased insensible loss from fever, or other causes.⁷

Although pathophysiology of *P. knowlesi*-associated acute renal failure in human remains unknown, the clinical course

of such severe complication in knowlesi malaria seems to be reminiscent to that in falciparum malaria in terms of a short duration of rising levels of serum creatinine and rapid return to normal with effective antimalarial treatment.⁷⁻⁹ Acute renal failure in falciparum malaria is usually associated with hypovolemia, hyperparasitemia, high blood viscosity, and renal hypoperfusion.⁸ Recent studies have shown that liberal fluid resuscitation does not improve acid-base balance and renal function in severe falciparum malaria, whereas inappropriate fluid replacement may exacerbate pulmonary edema.^{10,11} Of importance, acute renal injury in severe falciparum malaria seems to result from pathological processes associated with parasitized erythrocytes sequestered in microcirculation. Although it is unknown whether macrovascular fluid depletion or microvascular sequestration of parasitized erythrocytes primarily contribute to acute renal injury in knowlesi malaria, artesunate treatment but not fluid resuscitation lead to rapid recovery of renal function in severe falciparum malaria.^{10,11} Meanwhile, jaundice seems to be a common feature observed in renal failure in severe malaria and mainly stems from acute hemolysis that could further compromise renal function.^{8,12,13} Despite the rarity of overt liver failure in malaria, transient disturbance of hepatic function is not uncommon.^{13,14} An increased direct serum bilirubin level without bilirubinuria would suggest rapid intravascular hemolysis during high parasitemia in this patient. Increased levels of hepatic transaminases were not infrequently observed in both falciparum and vivax malaria, suggesting some hepatocyte injury during the course of infection.^{13,14} Although thrombocytopenia is a common finding in all non-zoonotic human malaria, severe thrombocytopenia (< 20,000/mL of blood) has been reported in severe knowlesi malaria.^{2,3,15} Despite remarkably low platelet levels in this patient (8,000 to 9000/ μ L), clinical bleeding was not observed during the course of infection. Meanwhile, atypical (or reactive) lymphocytosis and monocytosis in this patient that have also been frequently identified in acute falciparum and vivax malaria could have arisen from host immune responses culminating in destroying intraerythrocytic parasites as their levels return to normal during recovery.^{16,17} Taken together, complicated symptoms in the patient reported herein conform to those found in high endemic areas.^{2,3}

Pathogenesis of severe knowlesi malaria remains largely unknown. In falciparum malaria, severe manifestations could be mediated by both parasite-derived and human-derived factors.¹² However, the majority of severe knowlesi malaria patients tend to have high parasite burden akin to that observed in severe falciparum malaria.³ Meanwhile, retrospective clinical analyses of severe knowlesi malaria reveal that parenteral artesunate treatment conferred faster parasite clearance and low case-fatality relative to quinine, whereas early treatment with artesunate resulted in no mortality rate.^{3,18} Consistently, *in vitro* drug susceptibility study has shown that *P. knowlesi* exhibited high sensitivity to artemisinins, whereas sensitivities to chloroquine and mefloquine were lower.¹⁹ Therefore, parenteral artesunate has been recommended by the World Health Organization (WHO) as the treatment of choice for severe knowlesi malaria.²⁰

On the other hand, delay in diagnosis and treatment could lead to high parasite density that reportedly had a strong association with disease severity.^{2,3} Meanwhile, acute renal failure and hepatic dysfunction in knowlesi malaria akin to

TABLE 1
Hematological and biochemical profiles of the patient

Date and time	Normal value	Day 1			Day 2	Day 3	Day 4	Day 5	Day 6	Day 14	Day 300
		4.28	10.37	16.46	6.23	4.59	6.43	6.11	6.12	10.32	15.19
Blood Chemistry											
Glucose (mg/dL)	70–100		225		157	164	107				
BUN (mg/dL)	7–20	85			96	85					16
Creatinine (mg/dL)	0.7–1.3	4.7			3.6	2.3	1.4	1.1			0.7
Total protein (g/dL)	6.3–8.2	5.9									7.1
Albumin (g/dL)	3.5–5.0	2.8									4.1
Globulin (g/dL)	2.9–3.3	3.1									3
Total bilirubin (mg/dL)	0.3–1.9	6.15				4.77	4.04				1.1
Direct bilirubin (mg/dL)	0–0.3	4.8									0.8
SGOT (AST) (U/L)	5–35	132				81					38
SGPT (ALT) (U/L)	0–40	33									41
Alkaline phosphatase (U/L)	40–120	116									56
CBC											
RBC ($\times 10^6/\mu\text{L}$)	4.5–6.0	3.02		3.98	3.65	3.65	3.03	3.12	3.49	3.4	4.52
Hemoglobin (g/dL)	13.0–17.0	8.9		11.4	10.65	10.4	8.5	8.8	9.9	9.5	13.2
Hematocrit (%)	39–51	24.3	27.3	31.9	28.6	29	24.5	25.4	28.8	29.2	37.2
MCV (fL)	80–95	80.5		80.2	78.4	79.5	80.9	81.4	82.5	85.9	82.3
MCH (pg)	27–33	29.5		28.6	29	28.5	28.1	28.2	28.4	27.9	29.2
MCHC (g/dL)	33–37	36.6		35.7	37.1	35.9	34.7	34.6	34.4	32.5	35.5
RDW (%)	11.0–14.5	14.8		15.3	14.6	14.8	14.6	14.4	14.7	14.9	11.8
WBC ($\times 10^3/\mu\text{L}$)	4.5–11.0	8.08		7.97	10.61	11.32	8.54	8.21	7.66	7.35	8.92
Neutrophil (%)	40–71	46		53.4	50	40	45	46.2	44.6	53.7	57.9
Lymphocyte (%)	12–44	26		26.5	29	33	38	35.6	39.6	33.6	30.6
Atyp Lymph (%)	0	20		10	7	4	3	0	0	0	0
Monocyte (%)	0–7.3	5		9.4	13	23	14	16.7	14.4	9.1	8.1
Eosinophil (%)	0–4.1	0		0.3	1	0	0	1.3	1.3	2.2	2.8
Basophil (%)	0–1.8	0		0.4	0	0	0	0.2	0.1	1.4	0.6
Platelets ($\times 10^3/\mu\text{L}$)	150–450	9,000		8,000	49,000	48,000	56,000	92,000	139,000	388,000	148,000
Coagulation											
PT (sec)/INR	10–13			14/1.18							
PTT (sec)	20–33			27.6							
Electrolyte											
Na (mmol/L)	136–145	141	141	143	142	140	143				
K (mmol/L)	3.5–5.1	3.9	4.1	4.1	3.5	3.9	4.4				
Cl (mmol/L)	95–105	102	105	104	104						
Bicarbonate (mmol/L)	22–32	15	18	20	21						
Magnesium (mmol/L)	1.6–2.3				2.1						
Malaria											
Density (%)	0	16.67	9.2	8.5	0.05	0	0	0	0	0	0

BUN = serum urea nitrogen; SGOT (AST) = aspartate aminotransferase; SGPT (ALT) = alanine aminotransferase; CBC = complete blood cell; RBC = red blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; WBC = white blood cell; PT/INR = prothrombin time/international normalized ratio; PTT = partial thromboplastin time.

this patient have been observed in a German tourist who acquired the infection in southern Thailand.²¹ This report lends further evidence that severe knowlesi malaria can occur in low-endemic areas and the possibility of developing severe complications may be higher than previously appreciated. Therefore, awareness of proper diagnosis and close monitoring of patients infected with *P. knowlesi* should be underscored regardless of disease endemicity.

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