

Case series of naturally acquired *Plasmodium knowlesi* infection in a tertiary teaching hospital

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Abstract. *Plasmodium knowlesi* is a simian malaria parasite and is recently recognized as the fifth malaria parasite infecting humans. Manifestation of the infection may resemble other infection particularly dengue fever leading to inappropriate management and delay in treatment. We reported three cases of naturally acquired *P. knowlesi* in Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia. Clinical manifestations were quite similar in those cases. Microscopically, the diagnosis might be challenging. These cases were confirmed by polymerase chain reaction method which serves as a gold standard.

INTRODUCTION

Plasmodium knowlesi is a simian malaria parasite and is recently recognized as the fifth malaria parasite infecting humans (White, 2008). Human infections with *P. knowlesi* are more common than previously thought (Singh *et al.*, 2004). In Malaysia, the first case of naturally acquired *P. knowlesi* was reported by Chin *et al.* (1965) and in Sarawak the infection was associated with severe manifestation of multi organ failure (Cox-Singh *et al.*, 2008). Currently the disease is not only confined to that particular area but it is widely distributed in other places in Malaysia (Singh *et al.*, 2004; Cox Singh *et al.*, 2008; Vythilingam *et al.*, 2008; Singh & Daneshvar, 2010). There have been many reported *P. knowlesi* cases from neighbouring countries: Thailand (Jongwutiwes *et al.*, 2004, Sermwittayawong *et al.*, 2012), Vietnam (Van den Eede *et al.*, 2009), Indonesian Borneo (Figtree *et al.*, 2010) Singapore (Ng *et al.*, 2008; Ong *et al.*, 2009)

and Palawan, Philippines (Luchavez *et al.*, 2008). Numerous cases have been reported in travelers to these areas, which makes it not only a local disease occurring in the Southeast Asian countries but it has been exported to other countries (Kantele *et al.*, 2008; Bronner *et al.*, 2009; Ennis *et al.*, 2009; Ong *et al.*, 2009; Figtree *et al.*, 2010). *Plasmodium knowlesi* can cause severe malaria in 6.5% of human infection (Daneshvar *et al.*, 2009). In previous publications, death has been reported in some cases with hyperparasitemia (Cox-Singh *et al.*, 2008; Daneshvar *et al.*, 2009; Singh & Daneshvar, 2010) and the overall case fatality rate was 1.8% (Daneshvar *et al.*, 2009).

In terms of diagnosis, it is difficult to ascertain diagnosis by blood film microscopy as the parasite is often incorrectly identified. *Plasmodium knowlesi* is commonly, misidentified as *Plasmodium malariae* since the late blood stages are morphologically similar on microscopy, and molecular methods of detection are

necessary for accurate diagnosis (Singh *et al.*, 2004; Cox-Singh *et al.*, 2008; Wilairatana *et al.*, 2010). Polymerase chain reaction (PCR) remains the best method for accurate diagnosis of *P. knowlesi* malaria (Cox-Singh *et al.*, 2008; Wilairatana *et al.*, 2010).

In this case report we highlight the atypical presentation of thrombocytopenia in *P. knowlesi* infection which mimicked dengue fever. In our local setting where both dengue and malaria are still endemic, *P. knowlesi* infection should be included as one of the differential diagnosis in any patients presenting with thrombocytopenia.

Case Report 1

A 71-year-old Malay male, presented with fever for 8 days prior to admission associated with chills, rigors, poor oral intake, body ache, joint pain and minimal cough. However, in this patient, there was no history of retro-orbital pain, bleeding tendencies, abdominal

pain, vomiting, jaundice or headache. On further questioning, there was a history of jungle tracking one week prior to the onset of fever. Clinically, patient was pink, sweating and lethargic. He was febrile with on and off spiking temperature daily ranging from 38°C to 39.5°C (Figure 1). Systemic review was unremarkable.

On admission, his full blood count showed normal haemoglobin (12.3 g/dL), haematocrit level of 39.6% and total white cells (6.3×10^3 uL), but platelet count was low (97×10^9 /L). His platelet count had reduced to 45×10^9 /L on the next day.

Dengue serology revealed positive IgM and negative IgG. Coagulation profiles were normal except for partial thromboplastin time was prolonged. Liver function test revealed borderline high alkaline phosphatase (121 IU/L) and normal transaminases. Total bilirubin (56umol/L) (direct bilirubin: 27umol/L, indirect bilirubin: 29 umol/L) and

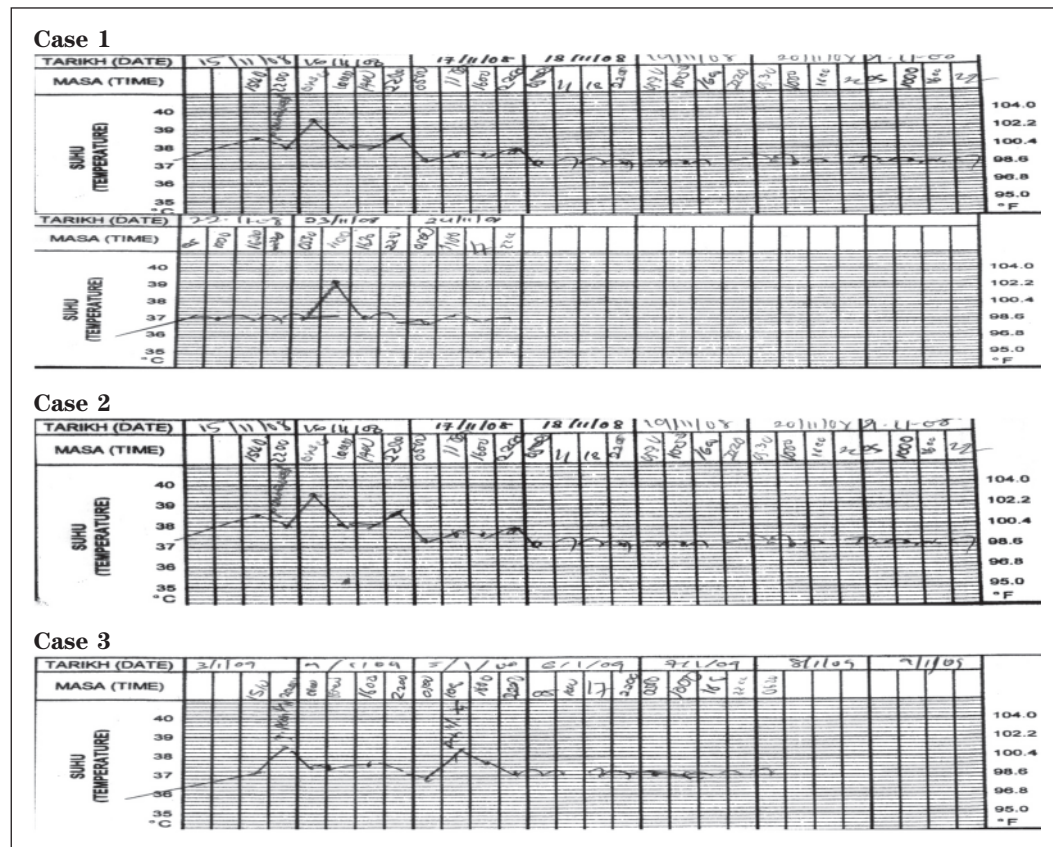


Figure 1. Temperature chart above showing fever spikes ranging from every 24h to 36h apart in three different cases

lactate dehydrogenase (997 IU/L) were raised. Blood urea and serum electrolytes levels were normal. In view of positive IgM for dengue serology, he was initially treated as dengue fever. However, fever persisted and the clinical picture was atypical for dengue fever. Serial blood films for malaria parasites (BFMP) were then ordered; they showed atypical *P. malariae* but features with level of parasitaemia of 10520/uL blood. Blood sample was sent to a referral laboratory for confirmation of *Plasmodium* species by PCR which yielded *P. knowlesi*. Other blood investigations such as Typhidot, blood and urine cultures were negative.

Antimalarial drugs were prescribed only on day 9 of admission. Patient was treated with tablet chloroquine 600mg stat and 300mg after 6 hours then 300mg once daily for 2 days, tablet fansidar 500/25mg (3 tablets) stat and tablet doxycycline 100mg once daily for a week. Patient responded well to above treatment. No more spiking temperature noted after 24 hours of treatment with oral chloroquine and was discharged well after BFMP were negative twice.

Case Report 2

A 36-year-old Malay male, presented with intermittent fever for three days prior to admission associated with sweating, chills, rigors, retro-orbital pain, jaundice, rashes over both lower limbs. However, there was no bleeding tendencies, tea coloured urine, pale stool or abdominal pain. There was history of visiting the jungle about 2 to 3 weeks before the onset of fever.

Clinically, patient had jaundice, daily spiking temperature ranging from 38°C to 40.6°C (Figure 1), slightly tachycardia and presence of petechial rashes over both lower limb. His blood pressure was stable. On abdominal examination, there was hepatomegaly about 2 fingers breath palpable and others systemic review was unremarkable. Full blood count taken on admission showed thrombocytopenia (platelet of $39 \times 10^9/L$) and high haematocrit level of 44.6%. Other blood indices were normal. Dengue serology was negative. Partial thromboplastin time was prolonged

(44.9 seconds). Full blood picture revealed *P. malariae* infection. However, blood film for malarial parasite showed presence of *Plasmodium falciparum* with level of parasitaemia 40/uL blood. Owing to the discrepancy in these results, the blood sample was sent to a referral laboratory for confirmation of *Plasmodium* species by PCR which yielded *P. knowlesi*. Liver function tests revealed high alanine transaminase (201 IU/L) and aspartate transaminase (94IU/L), borderline high alkaline phosphatase (122 IU/L) and raised total bilirubin (33umol/L). Blood potassium level was low (2.8mmol/L). Sodium, urea and creatinine were normal. Other microbiological investigations such as Typhidot, blood and urine cultures were negative.

Initially, in view of high haematocrit, he was given fluid therapy. Excessive fluid therapy was given and unfortunately, he developed iatrogenic fluid overload with pleural effusion. He was treated with tablet chloroquine 600mg stat, then 300mg once daily, tablet Fansidar 3 tablets stat and tablet primaquine 40mg stat then once daily for two days. After two days of treatment, he responded well and afebrile. He was discharged well after negative BFMP twice.

Case Report 3

A previously healthy 47-year-old Malay male with no known medical illness claimed having intermittent fever for nearly two weeks duration associated with chills, sweating, myalgia, joint pain and night sweat. Four days prior to admission, he had vomiting, loose stools three times per day and mild epigastric pain. There was no history of bleeding tendencies, blackish stool or urinary tract infection symptoms. There was history of jungle tracking prior to onset of fever.

On examination, he had on and off spiking temperature ranging from 39°C to 38.8°C (Figure 1), was alert and conscious, dehydrated, had tinged jaundice, was not tachypnoeic and had no petechial rash. Vital signs were stable. On abdominal examination, his liver and spleen were enlarged. Other systemic review was unremarkable. On admission, his full blood count showed

mild to moderate anaemia with 10.5 g/dL haemoglobin, low 2.5×10^3 /uL total white cells count, markedly low platelet count of 14×10^9 /L and normal haematocrit level of 33%. Despite of low platelet count, no bleeding tendencies or bruises observed.

Full blood picture showed normocytic normochromic anaemia, left shifted cells and thrombocytopenia most probably due to underlying infection. Dengue serology and typhidot were negative while blood culture did not grow any organism. Coagulation profiles were normal. Blood Urea and creatinine levels were raised with 13.9 mmol/l and 148 mmol/L respectively. Serum sodium and potassium were normal. His aspartate transaminases (59 IU/L) and total bilirubin (29 umol/L) were raised. Other liver function indices were unremarkable. Subsequent liver function test showed increased aspartate transaminases and bilirubin level. In view of impaired liver function test, he underwent hepatitis screening and was found to be hepatitis C positive. The BFMP examination showed presence *P. malariae*/*P. knowlesi* with parasitaemia level of 40,200/uL. Blood sample was sent to a referral laboratory for confirmation of *Plasmodium* species by PCR which yielded *P. knowlesi*.

He was treated as malaria on day 1 of admission based on BFMP result and was given tablet chloroquine 500mg stat, 250mg 6 hours later, and then 250mg twice daily for two days. He responded well to treatment and was afebrile on day 3 of admission.

DISCUSSION

Plasmodium knowlesi is commonly misidentified as *P. malariae* since the blood stages are morphologically similar on microscopy, and molecular methods of detection are necessary for accurate diagnosis (Singh *et al.*, 2004; Cox-Singh *et al.*, 2008). The daily fever spike seen is due to the *P. knowlesi* 24-hour asexual life cycle, the shortest of all primate malarias. It frequently manifests as asymptomatic infection and chronic with low level parasitemia (Singh *et al.*, 2004).

Pertaining to case 1, patient was initially treated as dengue fever in view of positive dengue IgM. Later, the dengue IgG was also positive in this patient. However, as he presented with prolonged fever and the haematocrit level was normal, these features did not really correlate with dengue fever. The most likely diagnosis was malaria following a positive BFMP result. A positive dengue IgM in this patient may suggest a double infection with *P. knowlesi* and dengue virus.

In case 2, patient was misdiagnosed as dengue fever in view of high haematocrit level. As a result, he was treated excessively with fluid therapy and developed iatrogenic fluid overload. His liver function test was impaired which contributed to hepatitis C infection. Generally, inappropriate treatment following wrong diagnosis in dengue and existing comorbidity might possibly happened in patient with *P. knowlesi* infection.

Generally, manifestations in these case series mimiced dengue fever. Common manifestations were fever, chills, joint pain and jaundice. Liver function tests particularly raised AST, ALT and bilirubin levels were most probably as a result of liver destruction and haemolysis caused by *P. knowlesi*. Inappropriate treatments may possibly happen in *P. knowlesi* infection which may lead to iatrogenic complication. All the above patients had history of visiting the jungle that might be the source of infection. *Plasmodium knowlesi* is known to reside in its natural host; the long-tailed macaque monkeys (*Macaca fascicularis*), pig-tailed macaques (*Macaca nemestrina*) and banded leaf monkeys (*Presbytis melalophos*) and it is prevalent in Malaysian forest (Vythilingam *et al.*, 2006; Singh & Daneshvar, 2010; Vythilingam, 2010). Some of the mosquito species are attracted both to humans and its host, for example, *Anopheles latens* and *Anopheles cracens* were predominantly found in Kapit, Sarawak and Kuala Lipis, peninsular Malaysia respectively (Vythilingam *et al.*, 2006, 2008; Vythilingam, 2010). As thrombocytopenia is also a common manifestation of malaria infection (Patel *et*

al., 2004), speciation of the *Plasmodium* species is crucial to differentiate *P. knowlesi* from others such as *P. malariae* which definitely has an impact in patients' management.

As seen in these cases, *P. knowlesi* is commonly mistaken for *P. malariae* by microscopy due to similarity of it's the blood stages. *Plasmodium knowlesi* can be misidentified as *P. falciparum* if only ring forms are identified during microscopy examination (Ong *et al.*, 2009; Wilairatana *et al.*, 2010). *Plasmodium knowlesi* malaria was initially thought to be *P. falciparum* and then *P. malariae*, this was confirmed as *P. knowlesi* after inoculation of the infected human blood into rhesus monkeys (Chin *et al.*, 1965). The common misidentification of *P. knowlesi* is that, early trophozoites of *P. knowlesi* may appear as ring forms, similar to *P. falciparum*. Late and mature trophozoites with "band forms", schizonts and gametocytes of *P. knowlesi* in human infections were generally indistinguishable from those of *P. malariae* (Singh *et al.*, 2004).

It is essential to differentiate between *P. knowlesi* and *P. malariae* malaria as the outcome might be fatal in the former. There is no standard guideline for the treatment of *P. knowlesi* malaria and the optimal treatment remains to be determined (Singh & Daneshvar, 2010; William *et al.*, 2011). Recent studies indicated that uncomplicated cases are being successfully treated with chloroquine alone or in combination with primaquine (Singh *et al.*, 2004, Daneshvar *et al.*, 2010; Singh & Daneshvar, 2010). However, there is a contradicting report on the use of primaquine since *P. knowlesi* has no hypnozoite (Wilairatana *et al.*, 2010). Quinine and artemisinin derivatives have also been shown to be effective and can be used for treatment in severe cases (William *et al.*, 2011). However, in a recent study, the use of parenteral artemisinin derivatives for treatment of severe cases showed advantages over quinine in terms of shorter parasite clearance (2 vs. 4 days), death outcome (1 vs. 5 patients) and case fatality

rate of severe cases (16.6% vs. 31%) (William *et al.*, 2011). Relapse after treatment and drug resistance have not yet been reported. The optimal malaria prophylaxis for this species is still unknown. In this report, all patients had responded rapidly to oral chloroquine. However, these patients should be closely monitored during the course of treatment since previous report had demonstrated fatal outcome in some cases (Daneshvar *et al.*, 2009; William *et al.*, 2011).

Although most human infections with *P. knowlesi* manifest as mild symptoms, severe infections are likely to occur and could be fatal. *Plasmodium knowlesi* infection should be suspected as an etiologic agent of malaria particularly in cases with daily fever spikes and blood smears resembling *P. malariae*. All these cases had a history of visiting jungle areas which is consistent with all the previous *P. knowlesi* reports. This is an important fact since there is no final evidence on human-mosquito-human transmission as long as all cases have a history of visiting jungle areas (thus allowing the possibility of monkey-mosquito-human transmission). Epidemiologic studies into the parasite's reservoir and mosquito vector will be important in the prevention of this emerging zoonotic disease as stated by Vythilingam (2010).

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