Complication of Corticosteroid Treatment by Acute *Plasmodium malariae* Infection Confirmed by Small-Subunit rRNA Sequencing[▽]

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We report a case of acute *Plasmodium malariae* infection complicating corticosteroid treatment for membranoproliferative glomerulonephritis in a patient from an area where *P. malariae* infection is not endemic. A peripheral blood smear showed typical band-form trophozoites compatible with *P. malariae* or *Plasmodium knowlesi*. SSU rRNA sequencing confirmed the identity to be *P. malariae*.

CASE REPORT

A 72-year-old man with membranoproliferative glomerulonephritis, hypertension, and α -thalassemia trait was admitted to our hospital because of chills and rigor for 2 days and a fever on the day of admission. He had been diagnosed with membranoproliferative glomerulonephritis 3 years prior to admission, when he presented with nephrotic syndrome. Autoimmune marker testing showed a raised level of antinuclear antibody ($>1/720$), with a normal level of anti-double-stranded-DNA antibody (<5 IU/ml) and a negative result for antineutrophil cytoplasmic antibody (ANCA). Testing for hepatitis B virus surface antigen showed a negative result. His initial renal biopsy specimen at diagnosis showed immune deposits of IgA, IgG, IgM, C3, and C1q. He was treated with high-dose corticosteroid with partial response. Two months prior to admission, he had recurrent lower limb edema and was confirmed to have a relapse of the nephrotic syndrome, with urine protein excretion of 2.78 g/day. Prednisolone at 50 mg once daily was started 50 days before admission, with a gradual tapering dose. On the day of admission, his prednisolone dosage was 35 mg once daily. The patient was a resident of Hong Kong. He occasionally traveled to southern China, but he has never traveled outside this area in his lifetime. He has never received a blood transfusion.

On admission, he had a temperature of 39.3°C and a heart rate of 138 beats per minute. His blood pressure was 144/68 mm Hg. Physical examination revealed bilateral lower limb edema. The spleen was not palpable. A blood test showed thrombocytopenia with a platelet count of 69×10^9 /liter. The platelet count 6 weeks before admission was within the normal range (253×10^9 /liter). The total white cell count was 4.4×10^9 /liter, with a neutrophil count of 4.09×10^9 /liter and a lymphocyte count of 0.26×10^9 /liter. The hemoglobin level was 7.4 g/dl. Biochemical tests revealed a creatinine level of 2.68 mg/dl and an albumin level of 31 g/liter. The levels for bilirubin and liver parenchymal enzymes were within the

normal range. A peripheral blood smear showed normal-sized erythrocytes, thick rings (Fig. 1a), band-form trophozoites (Fig. 1b), and schizonts containing dark brown pigments (Fig. 1c), compatible with *Plasmodium malariae* or *P. knowlesi*. The level of parasitemia was 0.1%. There was no growth in one set of blood culture after 5 days of incubation. Urine culture was not performed, in view of normal urinalysis except proteinuria. The nasopharyngeal aspirate was negative for influenza viruses A and B, for parainfluenza virus types 1, 2, and 3, for adenovirus, and for respiratory syncytial virus by direct immunofluorescence (19) and for pandemic (H1N1) influenza virus by reverse transcriptase PCR (16). A chest radiograph showed left apical pleural thickening, but lung fields were clear. Oral chloroquine (600 mg, then 300 mg once 6 h later, and then 300 mg once daily for 2 days) was started on the second day of admission. Intravenous ceftriaxone at 1 g once daily was also given in view of possible bacterial coinfection. His condition gradually improved, his platelet count returned to 155×10^9 /liter on day 9 of hospitalization, and he was discharged on the same day. Urine protein excretion was reduced to 0.57 g/day 19 days after admission. He remained asymptomatic at the time of writing, 6 months after discharge.

SSU rRNA sequencing. DNA extraction, nested PCR amplification, and DNA sequencing of the small-subunit (SSU) rRNA gene of the *Plasmodium* species from our patient were performed according to a previous publication (12, 13), using LPW12271 (5'-TCAAAGATTAAGCCATGCAAGTGA-3') and LPW12272 (5'-CCTGTTGTTGCCTTAAACTCC-3') for the primary PCR amplification and LPW12273 (5'-TTTTTATAAGGATAACTACGAAAAGCTGT-3') and LPW12274 (5'-TACCCGTCATAGCCATGTTAGGCCAATACC-3') (Sigma-Proligo, Singapore) for the nested PCR amplification and DNA sequencing. The sequences of the PCR products were compared with sequences of closely related *Plasmodium* species in GenBank by multiple sequence alignment using ClustalX 1.83 (15). Phylogenetic relationships were determined using the neighbor-joining method. Sequencing of the SSU rRNA gene of the *Plasmodium* species from our patient showed that there were 0- to 2-base (0 to 1%) differences between the SSU rRNA gene sequence of the *Plasmodium* species from our patient and those of *P. malariae* (GenBank accession no. AB489196.1, AF487999.1, and AF488000.1) but >19 -base

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FIG. 1. Peripheral blood smear of our patient, taken on the day of admission. (a) Thick ring; (b) band-form trophozoite, typical of *P. malariae* or *P. knowlesi*; (c) schizonts containing dark brown pigments.

(9%) differences between the SSU rRNA gene sequence of the *Plasmodium* species from our patient and those of other *Plasmodium* species, indicating that the *Plasmodium* species from our patient was *P. malariae* (Fig. 2).

Traditionally, human malaria can be caused by one of four *Plasmodium* species, *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Recently, a simian *Plasmodium* species, *P. knowlesi*, was described to be a cause of severe acute human infection resembling *P. falciparum* infection (3, 13). Morphologically, *P. knowlesi* is indistinguishable from *P. malariae*, and these two species can be distinguished only by molecular technologies, such as SSU rRNA sequencing. Among these five

Plasmodium species, *P. malariae* is considered to be the most benign species causing malaria in terms of acute illness (18). Severe and acute presentations of *P. malariae* infections have rarely been reported in the literature (Table 1). In this article, we describe a case of SSU rRNA sequencing-confirmed acute *Plasmodium malariae* infection complicating corticosteroid treatment.

Our patient might have contracted the initial *P. malariae* infection many years ago, causing the membranoproliferative glomerulonephritis, with exacerbation of the acute *P. malariae* disease due to corticosteroid therapy; or alternatively, he might have acquired the parasite recently and had a severe acute infection due to corticosteroid therapy. For both scenarios, the patient would most likely have acquired the infection in south-

FIG. 2. Phylogenetic tree showing the relationships of the patient's *Plasmodium* species to other, closely related *Plasmodium* species. The tree was inferred from SSU rRNA data by the neighbor-joining method using Kimura's two-parameter correction and was rooted using *Cryptosporidium baileyi* (EU814425.1). Two hundred one nucleotide positions were included in the analysis. The scale bar indicates the estimated number of substitutions per 50 bases. All names and accession numbers are given as cited in the GenBank database.

TABLE 1. Severe cases of *Plasmodium malariae* infections reported in the literature^a

Reference or source	Age (yr)	Sex	Underlying disease(s)	No. of platelets (10 ⁹)/liter	Complication(s)	Treatment	Confirmation of <i>P. malariae</i> by PCR sequencing (target gene)
5	28	M	None	30	ARDS, acute renal failure, shock, lactic acidosis, disseminated intravascular coagulopathy	Intravenous quinine	Yes (SSU rRNA gene)
10	32	M	None	93	Generalized convulsion	Intravenous quinine	Yes (18S rRNA gene)
14	42	F	Metastatic carcinoma of the colon, splenectomy	Not available	Confusion	Oral chloroquine	Not performed
Present case	72	M	Membranoproliferative glomerulonephritis, hypertension, alpha thalassemia trait	69	Sepsis, thrombocytopenia	Oral chloroquine	Yes (SSU rRNA gene)

^a M, male; F, female; ARDS, acute respiratory distress syndrome.

ern China, an area where malaria is still prevalent, due to frequent travel (23). *P. malariae* is well known to cause chronic malarial nephropathy (4), but acute renal failure has also been reported (9). *P. malariae* infection usually leads to immune complex-mediated glomerular disease, resulting in nephrotic syndrome. Although it is highly likely that the patient would have acquired the malaria which led to membranoproliferative glomerulonephritis, *P. malariae* infection was not seen on a blood film previously. One possibility is that *P. malariae* usually exhibits low-level parasitemia (2). *P. malariae* infections are often diagnosed after corticosteroid therapy in the treatment for glomerulonephritis (9), and it is possible that corticosteroid suppresses the host immunity and allows the proliferation of *P. malariae*. The effect of steroid on *Plasmodium* infections has been studied in animal models, with contradictory results. In a mouse model infected with *Plasmodium yoelii*, the dexamethasone group had higher numbers of rings, trophozoites, and schizonts than the control group (11). However, in another study, using squirrel monkeys, dexamethasone was shown to suppress *P. falciparum* in a dose-dependent manner (7). *Plasmodium* infections are frequently missed, especially in patients who have not traveled to areas of endemicity. However, *P. malariae* may manifest in patients in areas where *P. malariae* infection is not endemic, because of recrudescence (1, 17) or importation via animal species (6). The acute illness with thrombocytopenia in our patient may be confused with severe bacterial sepsis. However, there was no evidence of other infections from microbiological investigations of blood, sputum, urine, and nasopharyngeal aspirate samples. The acute onset and prompt recovery of thrombocytopenia suggests that the low platelet count was related to the severe infection (8).

SSU rRNA sequencing is a useful tool for accurate identification of *Plasmodium* species. Only three cases of severe acute infections due to *P. malariae* have been reported (Table 1). The first case was that of a 28-year-old immunocompetent man who presented with a fever and multiple organ dysfunction syndrome 5 weeks after returning to France from Côte d'Ivoire (5), whereas the other two cases were cerebral malaria in a 32-year-old man from Bangladesh (10) and a 42-year-old woman who had metastatic carcinoma of the colon and a history of splenectomy (14). It has been found that *Plasmodium* species, even those that are theoretically distinguishable by morphology, are frequently misidentified, even in areas of endemicity where the blood films are examined by experienced

microscopists (3). For our patient, we were unable to distinguish between *P. malariae* and *P. knowlesi* by blood film examination. Since *P. malariae* is rarely associated with acute sepsis, and septic workup did not reveal any coinfection, *P. knowlesi* was initially suspected and SSU rRNA sequencing was performed to confirm the identification of the *Plasmodium* isolate to the species level, which unexpectedly turned out to be a case of *P. malariae*. In fact, one of the three reported cases of severe *P. malariae* infections was confirmed by SSU rRNA sequencing (5). Similar to what was found for bacterial and fungal infections (20–22), a polyphasic approach using a combination of phenotypic identification and rRNA sequencing is required for identification and novel species discovery in parasitic infections in the long run.

Nucleotide sequence accession number. The SSU rRNA gene sequence of the *Plasmodium* species has been lodged within the GenBank sequence database under accession number GU950655.

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