Genetic Confirmation of Quinine-Resistant *Plasmodium falciparum* Malaria Followed by Postmalaria Neurological Syndrome in a Traveler from Mozambique

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A case of quinine-resistant *Plasmodium falciparum* malaria, followed by a postmalaria neurological syndrome and a recurrence episode, is described. Genetic characterization of the *P. falciparum* isolate obtained by analysis of *msp1* and *msp2* amplicons revealed the coexistence of two genotypes causing the first malaria episode and the presence of a unique isolate responsible for the recurrence.

CASE REPORT

Fifteen days after returning from Bilene (Maputo Province, Mozambique), a previously healthy 42-year-old man was admitted to the National Institute for Infectious Diseases L. Spallanzani, Rome, Italy, with a 4-day history of headache and febrile illness. *Plasmodium falciparum* malaria was diagnosed on the basis of blood film examination; the initial level of parasitemia was >100,000 parasites/μl. The patient had not taken antimalarial prophylaxis during a business trip to Mozambique. On examination, the patient was fully conscious and had a temperature of 38°C. Hepatomegaly was detected. Acute complications included hemolysis and severe thrombocytopenia. The patient was treated with intravenous (i.v.) quinine (QN; 20 mg of the hydrochloride salt per kg initially and then 10 mg/kg three times a day) plus oral doxycycline (100 mg two times a day). The parasitemia was cleared by day 4, whereupon the patient was treated with oral QN (8 mg of base/kg three times daily) plus doxycycline until day 8. He was discharged from the hospital on day 9.

Nine days later, however, the patient developed a low-grade fever with acute confusion (inappropriate speech and markedly disturbed behavior), postural tremor, and nominal aphasia. He was readmitted to our hospital on day 20. On examination, the patient had a temperature of 38.2°C with no clinically detectable focus of infection and without meningeal irritation. He was in an acute confusional state with nominal aphasia and showed a fine postural tremor of the arms that worsened when he tried to move his arms. The lowest Glasgow coma score was 12. No abnormalities were found in the cardiovascular and respiratory systems. No previous history of neurological or psychiatric illness was ascertained. No medication had been taken by the patient at home. Simultaneous thick and thin blood film tests on 3 different days were negative for malarial parasites. Computerized tomography and gadolinium-enhanced T1- and T2-weighted magnetic resonance imaging scans of the brain were also normal except for the presence of maxillary sinus exudate. On day 20, treatment with i.v. ceftriaxone (2,000 mg once a day) was begun. Analysis of a cerebrospinal fluid sample obtained by lumbar puncture revealed mild lymphocytic pleocytosis (45 lymphocytes/μl), a normal glucose concentration, and an elevated protein concentration of 1.29 g/liter (normal range, 0.2 to 0.4 g/liter). Pending herpes simplex virus testing, i.v. acyclovir (10 mg/kg three times a day) was empirically added to the treatment regimen on day 24 and stopped on day 28. Subsequent tests of cerebrospinal fluid for viral, bacterial, and fungal infections were all negative, including PCR analysis for herpesvirus types 1 and 2, human herpesvirus 6, cytomegalovirus, poliovirus, echoviruses, coxsackieviruses, and *Mycobacterium tuberculosis*. A PCR assay for *P. falciparum* in cerebrospinal fluid was also negative. A complete blood cell count with differential and a biochemical screening were also normal, including analyses of blood electrolyte and serum glucose, urea, and creatinine concentrations, liver function tests, and acid-base status. Serologic tests for herpes simplex virus types 1 and 2, cytomegalovirus, varicella-zoster virus, echoviruses, coxsackieviruses, *Trypanosoma brucei*, *TREPONEMA pallidum*, and dengue virus were negative. Cultures of blood, urine, and stool samples were negative. The acute confusional state steadily improved over the course of a week, and the patient had no neurological symptoms on day 26. He remained afebrile from that day until discharge from the hospital on day 29.

On day 33, the patient complained again of fever and was admitted again to our hospital on day 35. *P. falciparum* malaria was diagnosed with an initial level of parasitemia of >100,000 parasites/μl; a complete blood cell count and a total bilirubin test were normal. He was treated with i.v. QN (20 mg of the hydrochloride salt per kg initially and then 10 mg/kg three times a day) plus clindamycin (600 mg three times daily) until day 42; on day 37, a single oral dose of pyrimethamine (PY; 75
FIG. 1. Two percent agarose gel showing the products obtained by amplification with primers specific for the msp1 (lanes 1 to 5) and msp2 (lanes 6 to 10) genes. Lanes: M, Eurobioladder-L (Eurobio); 1 and 6, day 1; 2 and 7, day 2; 3 and 8, day 5; 4 and 9, day 35; 5 and 10, day 37. Lanes 1 to 3 and 6 to 8 (first hospital admittance) show two PCR bands, indicating the occurrence of at least two P. falciparum genotypes in the patient. The presence of a unique band, the upper one, in lanes 4 and 5 and lanes 9 and 10 (third hospital admittance) demonstrates the selection of one genotype. This genotype carries a mutant-type codon profile, as shown in Table 1.

The spread of resistance to the available antimalarials among malaria parasites represents a major worldwide health problem that seriously hampers efforts to control the disease. At present, clinical resistance to QN monotherapy occurs sporadically in Southeast Asia and western Oceania. From in vitro assays there is evidence of very little QN resistance in South America and Africa (3, 12).

We report here the first genetically characterized case of QN-resistant P. falciparum malaria acquired by a nonimmune traveler to Mozambique with neuropsychiatric manifestations of postmalaria neurological syndrome (PMNS).

PMNS is a self-limiting postinfective encephalopathy that occurs within 2 months after recovery from P. falciparum malaria whose neuropsychiatric manifestations are wide-ranging, including an acute confusional state or acute psychosis, cerebellar ataxia, generalized convulsions, motor aphasia, or fine tremor (2, 5, 8, 10, 11).

In a prospective study conducted in Vietnam, the overall incidence of PMNS after P. falciparum malaria was 1.2 per 1,000 cases and PMNS was associated with mefloquine treatment and with the severity of the preceding malaria infection (8). This syndrome has also been reported in nonimmune individuals (2, 5); this suggests that immunological mechanisms are implicated in PMNS. Indeed, the pathogenesis of PMNS is possibly mediated immunologically, caused by a cross-reaction of antibodies to antigens expressed by certain other agents causing encephalitis; moreover, the patient’s

Finally, in vitro pLDH tests to evaluate the level of resistance of the recurrent isolate to QN and CQ were performed as described by Makler and Hinrichs (6). The results of pLDH tests (50% inhibitory concentration of QN, 0.110 μg/ml; 50% inhibitory concentration of QN, 0.120 μg/ml) confirmed the full resistance of the isolate to CQ and QN, in accordance with the molecular marker analysis results.

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symptoms began 10 days after the completion of antimalarial treatment, rendering unlikely a toxic effect due to the previous therapy.

Genetic characterization of the *P. falciparum* isolates obtained revealed the coexistence in the patient of two genotypes causing the first malaria episode and the presence of a unique isolate responsible for the recurrence episode (Fig. 1).

The PCR analysis we performed demonstrated the selective pressure exerted by QN in favor of the strain with the resistant genotype that was later responsible for the recrudescence episode. In vivo resistance to QN was confirmed by an in vitro test and by molecular identification of an *mdr1* Tyr-86 point mutation. The direct correlation between the presence of that mutation and QN resistance has been reported by Duraisingh et al. (1), although it is not consistently found (7, 9). As expected, in vitro testing showed CQ resistance, in line with the presence of mutated alleles at codons 76 and 220 of the *pfcr* gene. The complete parasite clearance and recovery from disease obtained after sulfadoxine-PY treatment suggested that the recurrent isolate was fully sensitive to the drug. Kublin et al. reported that a dihydrofolate reductase triple mutation is strongly associated with sulfadoxine-PY treatment failure; therefore, the absence in our case of the mutations at codons 51, 59, and 108 fully supports the finding of sulfadoxine-PY efficacy (4).

This study confirms the importance of a drug resistance surveillance system based on nonimmune travelers. The exclusion of a possible new infection gives the chance to make the best use of molecular approaches aimed at the genetic analysis of malaria parasites and permits correct interpretation of the outcome of antimalarial therapy.

We observed the coexistence of a PMNS and *P. falciparum* QN-resistant isolates, but we cannot speculate about any possible correlation in our case. If similar findings occur in the future, the possible link may deserve further investigation.

Finally, these results further support the inclusion of PMNS in the differential diagnosis of patients with any neurological abnormality after recovery from *P. falciparum* malaria.

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**REFERENCES**


