Plasmodium vivax Malaria Infection Diagnosed by Indirect Fluorescent Antibody Test

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We present the diagnostically challenging case of a 51-year-old Japanese male who visited Papua New Guinea for one month. Approximately a month after returning to Japan, he experienced a high fever. Malaria was suspected and he was admitted to Tsukuba University Hospital. Although the blood smear did not reveal the malarial parasite, a diagnosis of malaria was made using an indirect fluorescent antibody test (IFAT). The patient was treated and discharged but symptoms returned three months later. This time, the blood smear was positive for malarial parasites. IFAT was useful in this case for the early diagnosis of *Plasmodium vivax* and for ruling out infection by *Plasmodium falciparum*.

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Introduction

The World Health Organization estimates that, worldwide, 270 million cases of acute malaria infection and 110 million clinical cases of malaria develop each year (1). Although malaria was eradicated from Japan in the early 1960's, each year several severe cases of this disease are reported (2). Most Japanese do not possess immunity to malaria; consequently, those who contract malaria tend to develop severe disease unless rapid treatment is given. Early detection is vital to prevent progression of the disease. Diagnosis is dependent upon the detection of the malarial parasite in a blood smear. However, malaria may be present even when the parasite cannot be confirmed using this method. It has been reported that the indirect fluorescent antibody test (IFAT) is useful for detecting malaria in cases where the clinical signs indicate malaria but where the blood smear fails to show evidence of the parasite (3). This report illustrates the use of IFAT in detecting malaria in such a case.

Case Report

A 51-year-old Japanese man, a botanist, conducted research in Papua New Guinea between August 20 and September 27, 1992. As a precaution, he took 300 mg of chloroquine phos-

phate once a week to prevent the development of malaria. He returned to Japan without symptoms of malaria. However, about a month after his return, he developed a high fever ($>38^{\circ}\text{C}$) that recurred every other day, beginning on October 28, 1992. On his own accord, he took 300 mg of chloroquine phosphate on November 3, 1992. He was admitted to Tsukuba University Hospital on November 5, 1992. The patient's history was unremarkable except for the presence of mild fatty liver first detected at the age of 50. His visit to Papua New Guinea was his first stay in an area endemic for malaria. On admission his physical appearance was good. His temperature was 36.7°C . There was no hepatosplenomegaly, jaundice, or lymphadenopathy. His skin showed no evidence of bites or eruption. Hematologic study showed slight anemia, but peripheral blood smears with Giemsa stain showed no evidence of malarial parasite. While blood chemistry findings all were within the range of normal. The level of C-reactive protein was very high (Table 1). An abdominal echogram confirmed the presence of a fatty liver, but showed no sign of splenomegaly. Malaria infection was strongly suspected, even though parasites could not be detected by standard examinations of peripheral blood smears. Further serological testing was performed using IFAT. The results were sent to the Department of Parasitology, Gunma University School of Medicine, for interpretation.

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IFAT was carried out according to the modified method of Voller and O'Neill (4). Four-fold dilutions of sera, (1:4–1:4,096), were applied to spots of *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) on glass slides. Diluted fluorescein (×50) conjugated rabbit anti-human IgG (DAKO, Denmark) was applied as a secondary antibody. Results were read using an incident light illuminating type of fluorescent microscope (Model BH-RFC, Olympus, Japan) (5). The IFAT was positive for *P. vivax* (Fig. 1), but was nonspecific for *P. falciparum*.

Because malaria was highly suspected, the patient was treated with chloroquine phosphate on admission. His body temperature remained normal throughout his hospital stay. C-reactive protein levels normalized on the sixth hospital day, and the patient was discharged the 26th hospital day. The treatment regimen is shown in Table 2. No side effects were associated

with the treatment.

Despite his apparent recovery, the patient was readmitted on February 18, 1993 with a temperature of 39.7°C. His serum total bilirubin and C-reactive protein levels were elevated (Table 3). Blood smears taken on this second admission revealed the ring forms of malarial parasites in about 1% of the erythrocytes (Fig. 2). We administered the same treatment regimen as before

Table 2. Treatment of This Case

1st day	Chloroquine phosphate	600 mg
6 hours later	Chloroquine phosphate	300 mg
2nd–3rd day	Chloroquine phosphate	300 mg/day
4th–17th day	Primaquine	15 mg/day
Total	Chloroquine phosphate	1,500 mg
Total	Primaquine	210 mg

Table 1. Laboratory Findings on First Admission

1. Hematology		4. Immunoserological testing	
Erythrocytes	406×10 ⁴ /mm ³	C-reactive protein	11.1 mg/dl
Hemoglobin	12.6 g/dl	IgG	1,148 mg/dl
White blood cell	4,800/mm ³	IgA	230 mg/dl
Neutrophils	43%	IgM	572 mg/dl
Lymphocytes	26%	Indirect Fluorescent Antibody Test	
Monocytes	13%		
Eosinophils	4%		
Basophils	1%		
Platelets	17.9×10 ⁴ /mm ³	Titer against <i>P. vivax</i> antigen	
2. Blood chemistry		Positive control	1:256
Total protein	6.6 g/dl	Negative control	<1:4
Aspartate aminotransferase	14 IU/l	Patient	1:256
Alanine aminotransferase	32 IU/l		
Lactate dehydrogenase	447 IU/l	Titer against <i>P. falciparum</i> antigen	
Total bilirubin	0.6 mg/dl	Positive control	1:1,024
Urea nitrogen	18.4 mg/dl	Negative control	<1:4
Creatinine	0.9 mg/dl	Patient	1:16
3. Blood smears with Giemsa stain			
No malarial parasite			

Table 3. Laboratory Findings on Second Admission

1. Hematology		4. Immunoserological testing	
Erythrocytes	498×10 ⁴ /mm ³	C-reactive protein	4.3 mg/dl
Hemoglobin	14.7 g/dl	IgG	929 mg/dl
White blood cell	5,600/mm ³	IgA	160 mg/dl
Neutrophils	88%	IgM	245 mg/dl
Lymphocytes	6%	Indirect Fluorescent Antibody Test	
Monocytes	4%		
Eosinophils	1%		
Atypical cells	1%		
Platelet	13.4×10 ⁴ /mm ³	Titer against <i>P. vivax</i> antigen	
2. Blood chemistry		Positive control	1:256
Total protein	6.8 g/dl	Negative control	<1:4
Aspartate aminotransferase	23 IU/l	Patient	1:256
Alanine aminotransferase	31 IU/l	Titer against <i>P. falciparum</i> antigen	
Lactate dehydrogenase	461 IU/l	Positive control	1:1,024
Total bilirubin	1.2 mg/dl	Negative control	<1:4
Urea nitrogen	13.8 mg/dl	Patient	1:4
Creatinine	0.9 mg/dl		
3. Blood smears with Giemsa stain			
Ring forms of unidentified malarial parasites

Fig. 1. Results of IFAT are positive for *Plasmodium vivax* antigen (×400).

Fig. 2. Smear of peripheral blood reveals ring forms of unidentified malaria parasites (arrow) on patient's second admission to the hospital. About 1% of the erythrocytes were infected (Giemsa stain, ×1,000).

Fig. 3. Clinical course.

(Table 2) and also included 15 mg/day of primaquine for a period of 14 days. The parasites were absent from blood smears taken on day 2 of the second hospital visit. The patient became afebrile on hospital day 3. The C-reactive protein level was normal on hospital day 7 (Fig. 3). The patient recovered uneventfully, and had no further relapse.

Discussion

Direct microscopic observation of malarial parasites in erythrocytes traditionally has been believed to be most useful method for diagnosing malaria. Laveran first detected malarial parasites using this method in 1880 (6). Since that time, other seroimmunological methods, such as IFAT (4), radioimmunoassay (RIA), and enzyme linked immunosorbent assay (ELISA) (7) have been developed for large-scale diagnoses of malaria in endemic areas (8). However interpretation of the obtained titers by IFAT is still a topic of discussion.

In the case reported here, on first admission we could not identify malarial parasites microscopically. However, we strongly suspected a diagnosis of malaria based on the patient's clinical symptoms and recent history; microscopic evidence of the disease may have been masked because of the administration of an antimalarial drug (chloroquine phosphate) just prior to the patient's first admission to our hospital. The diagnosis of malaria by IFAT is possible in cases in which malarial parasites cannot be identified microscopically. Malarial parasites were observed microscopically during the patient's second admission, but were evident in ring forms which make it difficult to differentiate the species of malaria. Despite the lack of microscopic identification, chloroquine phosphate was administered based on the earlier positive diagnosis of *P. vivax* using IFAT. This diagnosis was then confirmed by IFAT. Thus, in this case, the diagnosis of malaria was made possible using IFAT, even

though the parasite could not be identified microscopically, probably because of self-medication with chloroquine.

Other cases in which IFAT is useful include: 1) patients who have been diagnosed with malaria in an endemic area and who require additional treatment in Japan, 2) patients who have a past history of malaria and who have a fever, which suggests a recurrence or a relapse of malaria, and 3) for the detection of malaria in blood donated from a person who has visited an endemic area (9).

The spread of multidrug-resistant *Plasmodium falciparum* (10, 11) and the insecticide-tolerant *Anopheles* (12) has increased significantly. The number of Japanese traveling to these areas also has increased, to about 10 million a year. In addition, an increasing number of individuals from endemic areas (such as Southeast Asia) now work and reside in Japan. Most of the outbreaks of malaria in Japan are believed to be caused by such imported malaria. A total of 51 cases of malaria were reported to the Ministry of Health and Welfare of Japan in 1992 (13). The incidence of imported malaria, and malaria induced by blood transfusion or injection may increase as the number of cases of imported malaria increases (14). Thus, clinicians must watch for outbreaks of malaria in Japan, even though malaria was eradicated from this country in 1961. Seroimmunological diagnosis, in particular by IFAT is an important tool for the detection of malaria, especially when microscopic evidence of the parasites is not available.

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