

First Case of Imported Plasmodium Ovale from Iran

Dear Editor,

Malaria is one of the most important vector-borne parasitic diseases in the world, particularly in tropical and subtropical areas.^{1,2} It is caused by one, or rarely more than one, of four *plasmodia* species namely, *Plasmodium vivax*, *P. falciparum*, *P. malariae* and *P. ovale*. *Plasmodium ovale* has a pattern of fever and relapse similar to that of *P. vivax*, but generally milder clinical symptoms,³ *Plasmodium ovale* is mostly limited to tropical Africa and Southeast Asia,^{4,6} with a prevalence of 10-15% and 2.0-9.4%, respectively.⁷ Iran is an endemic region for malaria in the Middle East with *P. vivax*, *P. falciparum*, and rarely *P. malariae* infections. Herein the first authentic imported case of *Plasmodium oval* in Iran is reported.

A twenty years old Nigerian soccer player was referred to a Health Centre in Bandar Abbas, Iran with fever (38.5°C) chills, anorexia and bone pain. The patient had arrived in Iran, and resided in Bandar Abbas one month before the onset of clinical symptoms. The vital signs were within normal range and the laboratory analysis of his blood showed, a platelet count of 136000. His abdomen was soft without organomegaly and his urine analysis was also normal. The patient stated that he had a history of malaria almost the year before in Nigeria, and had been treated. However, he could not remember the type of malaria and the treatment he received.

Standard Giemsa-stained thick and thin blood smears from finger-pricked blood samples were made, and microscopically examined. Typical trophozoites of *P. ovale* were detected. In thin films, many erythrocytes were slightly enlarged assuming an oval shape with ragged margins and heavy stippling. To further characterize the parasite genus, a molecular genomic sequencing technique was employed. Blood samples scratched from non-stained smears were used to extract the total genomic DNA using Qiagen kit (QIAamp DAN Blood).⁸ The fragments of 18 ssrRNA with a band of 787 bp was amplified using PLF (Forward; 5': agtgatatcaatcgagttt 3') and UNR (Reverse; gacggatatctgatggtttc) primers,⁹ (figure 1). The amplified product in unit 700 bp was sequenced at SEQLAB, Germany (Germany; <http://www.seqsondemand.de>) to confirm the species of *Plasmodium ovale*. Nucleotide sequence data were registered in the GenBank database with the accession no. GQ397481.

The patient received standard treatment with 1 g chloroquine phosphate (600 mg base) orally for two days followed by 500 mg chloroquine phosphate (300 mg base) on the third day. (Chloroquine tablets were 250 mg with 150 mg base). Administered dosage was 10 mg/kg on first and second day of treatment and 5 mg/kg on the third day. Primaquine was administered to prevent relapse with a dosage of 45 mg weekly (0.75 mg/kg/week) for 8 weeks (Primaquine tablets were 26.3 mg with 15 mg base). All people in near contact with the patient were examined for likely malaria infection. The pa-



Figure 1: Agarose gel electrophoresis, showing PCR results with PLF and UNR primers. Lanes 1: Positive control, 2: Negative control, 3: Plasmodium (patient), 4: M: Molecular weight marker (100bp)

patient's peripheral blood smears were negative 6 weeks after the end of the treatment.

Appropriate treatment of malaria depends on the accurate diagnosis of human *Plasmodium* species. The microscopic examination of Giemsa-stained thick and thin blood films is the method of choice for the diagnosis of malaria.¹⁰ Nevertheless, many similar morphological features between *P. vivax* and *P. ovale* make their differentiation in cases of low parasitemia or mixed infections difficult. Such a difficulty can be overcome using molecular techniques. The sequence of 700 bp unit of 18sr RNA gene for the isolate was conducted to compare with GenBank information. The results confirmed identity of the isolate as *Plasmodium ovale*.

Although the presence of *P. ovale* is usually low, and can easily be missed,⁵ smears obtained from the patient contained relatively high number of parasites. In deed, some investigators believe that variant-type *P. ovale* is associated with higher parasitemia than classic-type.¹¹ Moreover, a suggestion has been presented that at least two different types of *P. ovale* are circulated in human hosts in Southeast Asian and African countries.¹¹

The history stated by the patient indicates that most likely he had been infected outside Iran, probably in his home country. Therefore, travel-related malaria, which is transmitted by travelers, tourists, businessmen and refugees from malaria endemic areas to non-endemic regions, would be a cause of concern. Such a concern is prominent when reports indicating new cases of autochthonous malaria are considered.^{12,13}

Earlier in 1938 a case of *P. ovale* malaria was reported from Iran.¹⁴ However, owing to the absence of any description, illustration, and epidemiological documents, it was categorized as a very doubtful case based on the criteria set by Lysenk and Beljaev.¹⁴ Therefore, it seems legitimate to suggest that the case described herein is the first imported *P. oval* malaria in Iran based on clinical, microscopic and molecular evidence.

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