J Vector Borne Dis 51, June 2014, pp. 149–151

Plasmodium malariae infection: A case of missed diagnosis

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Key words Malaria; *Plasmodium malariae;* polymerase chain reaction; schizonts

The presence of *Plasmodium falciparum* schizonts in peripheral blood smear is a rare occurrence and usually a cause for concern. However, some studies correlate schizontaemia to current or impending symptoms and/or hyperparasitaemia[1], while other studies do not indicate severity and same was observed in uncomplicated cases[2]. A potential confounder for the clinical consideration of the presence of schizonts may be the missed diagnosis of other *Plasmodium* sp coinfections. The diagnosis of other species by microscopy is often missed due to low parasite densities or the damaged morphology of the parasites. In addition, the skill of the microscopist is an important factor as well as the total time spent in examining the slide, the type of smear examined, and the choice and method of staining. Here, we report such a case of diagnostic difficulty encountered in an endemic area of India.

Case report

A 6-yr old female tribal child from Ranchi, Jharkhand presented with a seven day history of fever at a primary health centre during an ongoing study. The study was approved by the Ethics Committee of NIMR. She had no other complaints. There was no significant past medical history, known drug allergies, or antimalarials intake in the past week. The vital signs were stable and there were no signs or symptoms of severe malaria. Axillary temperature recorded at presentation was 97.6°F. Her hemoglobin was 8.7 g/dl. Immunochromatographic test using a *PfHRPII* antigen-based rapid kit (ParaHit, Span, India) was positive for *P. falciparum*. Thick and thin blood smears were obtained and stained with Giemsa. Routine oil-immersion microscopic examination of the thick and thin blood smears showed *P. falciparum* rings and schizonts (Figs. 1 and 2). The asexual parasite count was 5920 parasites/µl (ring forms 101/200 WBCs and schizonts 47/200 WBCs). A qualitative G6PD test was conducted and the patient was not found G6PD deficient. The patient was treated under supervision for uncomplicated malaria with oral artesunate plus sulphadoxine-pyrimethamine (AS 100 mg/day for 3 days; and SP 750/37.5 mg single dose) and primaquine (15 mg single dose) as per national treatment guidelines. The patient was followed-up during treatment on Day 7 post-presentation, and thereafter weekly until Day 28. Parasite clearance time was less than 24 h and adequate clinical and parasitological response, i.e. the absence of symptoms and parasitaemia, was maintained until the end of follow-up. Due to the high density of peripheral schizonts, the smear

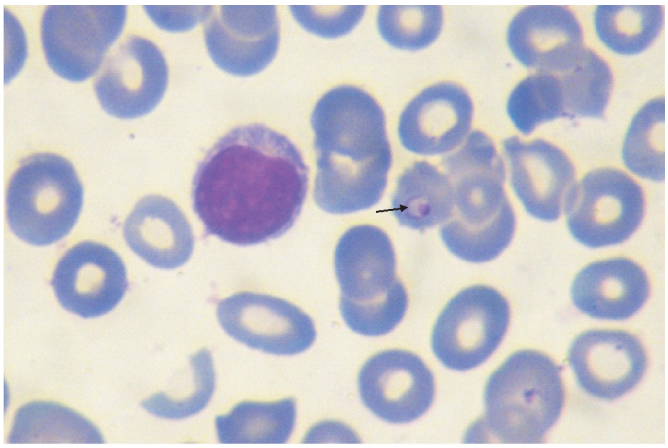

Fig. 1: Ring form of *P. malariae* misdiagnosed as *P. falciparum* in Giemsa-stained peripheral blood smear.

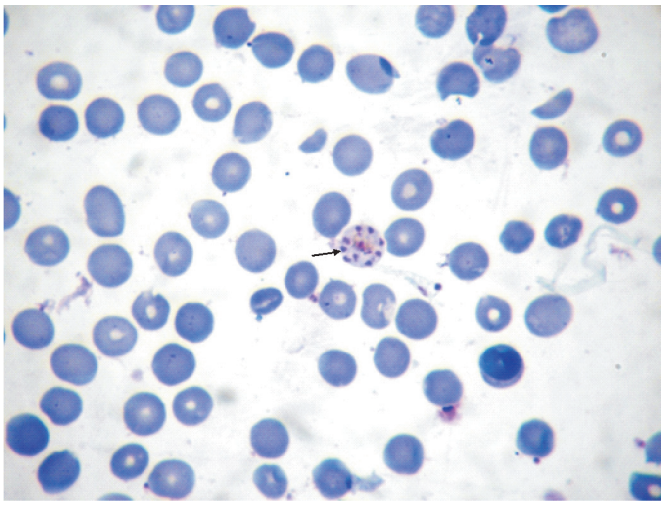

Fig. 2: Schizont (with merozoites arranged in rosette form) of *P. malariae* misdiagnosed as *P. falciparum* in Giemsa-stained peripheral blood smear.

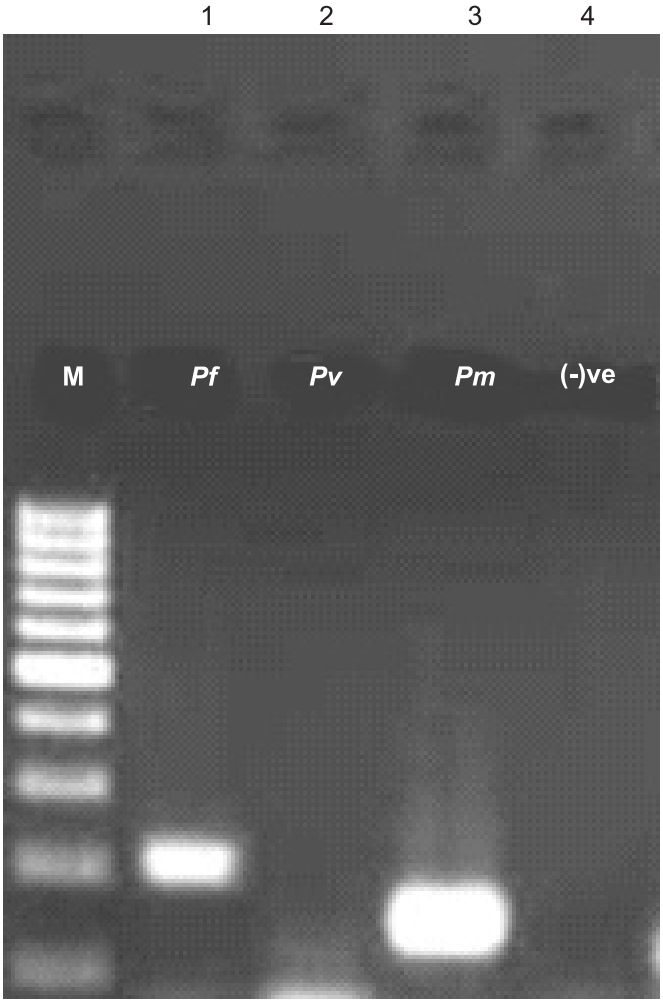

Fig. 3: Agarose gel electrophoresis of amplification products of the nested PCR. Lane M: 100 bp ladder markers; Lanes 1, 2 and 3: Patient sample with different species-specific primers, i.e. *P. falciparum, P. vivax* and *P. malariae*, respectively; and Lane 4: Negative control (sterile distilled water).

was re-examined by the expert microscopist who confirmed the same diagnosis but were cautious about the morphology of the schizonts, specifically their pigmentation and the number of merozoites. Molecular diagnosis was carried out by nested polymerase chain reaction (PCR) with primers described by Snounou *et al*[3] and modified cycling parameters as described by Johnston *et al*[4] which detected coinfection with *P. falciparum* and *P. malariae* (Fig. 3). However, the characteristic band form trophozoites, or gametocytes of the later parasite species were still not observed.

DISCUSSION

In the present report, microscopy showed *P. falciparum* rings and schizonts. The patient was stable and responded to treatment which led to suspicion of mixed infection since *P. falciparum* schizontaemia has been reported to be associated with severe malaria[1]. The diagnosis of mixed infection is important for deciding appropriate treatment and also for appropriate control measures.

Plasmodium malariae is uncommon in India; however, cases are occasionally reported from certain states particularly those with a high incidence of *P. falciparum*[5–8]. *Plasmodium malariae* infections are often missed by microscopy which may be due to either low level of infection or damaged morphology of parasite[6, 9] and can be diagnosed by using PCR[3]. *Plasmodium malariae* can recrudescence over a long period[10], has the potential to develop nephrotic syndrome[11] and can infect mosquitoes over a long-period which sustains its transmission[9] posing challenge for its control. However, the parasite rapidly responds to routinely used antimalarial drugs, and thus, is of little relevance clinically as long as some form of malaria is diagnosed and treated.

Peripheral smear in uncomplicated *P. falciparum* malaria usually shows ring forms. The presence of schizonts is likely to be associated with severe malaria[1, 12] and also helps in identifying patients who need intense monitoring[1].

There are few old reports of uncomplicated *P. falciparum* malaria with schizontaemia[2, 13], however, diagnosis of mixed infections in these cases may not have been supported due to unavailability of PCR. The possible explanation given for this was the presence of genetically older strains of *P. falciparum* developing habits of asexual reproduction like other *Plasmodium* sp[2].

The patient, reported did not have any complication and recovered with oral artesunate plus sulphadoxine-pyrimethamine. This led to the suspicion of mixed infection with *P. malariae,* which was diagnosed with microscopy and PCR. The report highlights the importance of identifying mixed infections by careful microscopic examination of blood smears and also the utility of PCR as a sensitive diagnostic tool.

ACKNOWLEDGEMENTS

Authors are thankful to the National Institute of Malaria Research (ICMR) for intramural fund support. We would like to thank Shri Vijay Om, Laboratory Technician and the NIMR field team for their technical support.

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Received: 22 December 2013 *Accepted in revised form:* 19 February 2014