

Letter to Editor

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Exflagellated microgametocytes of *Plasmodium vivax* in peripheral blood: A diagnostic artifact mimicking co−infection

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The World Health Organization South-East Asia Regional Office estimates a significantly higher burden—around 15 million cases and 20 000 deaths per year. India alone accounts for approximately 77% of the total malaria cases reported in this region[1,2]. Among the five *Plasmodium* species infecting humans, *Plasmodium* (*P.*) *vivax* is the most prevalent species in India. Its asexual blood stages—ring forms, trophozoites, schizonts—and gametocytes are routinely identified in peripheral blood smears. However, the observation of exflagellated microgametes in human blood is exceedingly rare and typically occurs under certain *in vitro* conditions[3].

Exflagellation, the process where male gametocytes (microgametocytes) release motile microgametes, generally occurs in the gut of the mosquito. *In vitro*, this phenomenon can be triggered by a high blood pH (>7.7), lower temperatures, and prolonged exposure to anticoagulants such as ethylenediaminetetraacetic acid (EDTA). These factors may facilitate exflagellation artifactually in peripheral blood smears[3,4]. Though clinically insignificant, the morphological resemblance of exflagellated microgametes to other blood-borne pathogens—especially *Borrelia* spp. or *Trypanosoma cruzi*—can pose a diagnostic challenge and may lead to misinterpretation.

To date, fewer than 15 cases of exflagellated *Plasmodium* microgametes being observed in human blood have been documented, making this a rare yet noteworthy diagnostic artifact[3].

This letter describes a case of a 29-year-old male presented with intermittent fever, associated with chills and rigors for 8 days. A complete blood count and peripheral smear a low hemoglobin level (9 g/dL), indicated microcytic hypochromic anemia. The total leukocyte and platelet counts were within normal limits.

Examination of the Giemsa-stained peripheral smear revealed multiple intraerythrocytic forms of *P. vivax*, including ring forms, schizonts, and gametocytes. Interestingly, several extracellular, flagella-like structures resembling spirochetes were also observed. This unusual finding raised the suspicion of *Borrelia* spp. co-infection.

To verify this, additional fresh EDTA blood samples were examined; however, these did not reveal any spirochetal organisms. Furthermore, serological testing for *Borrelia* antibodies was performed and was negative. Based on the morphological features and the absence of *Borrelia* infection, the extracellular structures were identified as exflagellated microgametes of *P. vivax*—a rare, *in vitro* artifact rather than a true co-infection.

Exflagellation of *Plasmodium* microgametocytes is considered an exceptionally rare event in human peripheral blood[3]. Hitherto, only isolated cases of exflagellated malarial parasites have been documented[1,2,5]. Gametocytes are the sexual forms of the parasite that initiate transmission to mosquitoes. During the early sexual phase, microgametocytes undergo exflagellation—a process by which up to eight long, slender flagella (microgametes) emerge from a single male gametocyte. This process is completed within approximately 20 minutes after ingestion by the mosquito[4].

Solarte *et al*. noted that specific anticoagulants like heparin can promote exflagellation by altering blood pH, whereas EDTA tends to inhibit the process[6]. Interestingly, most published cases,

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including the present one, have reported exflagellation in EDTA-anticoagulated samples, suggesting that additional variables may influence the process.

Due to its rarity, exflagellated *Plasmodium* forms can be misidentified as other hemoparasites. Morphologically, they appear as thin filamentous structures approximately 10-15 μm in length with an oval, dark-blue nucleus. In contrast, *Borrelia* spp. are spiral-shaped, measure 5-20 μm in length, and lack a visible nucleus, while *Trypanosoma* species exhibit an undulating membrane, a kinetoplast, and a centrally located nucleus[3]. Accurate identification is essential to prevent diagnostic errors and inappropriate treatment.

Exflagellated microgametes of *P. vivax* in human peripheral blood are a rare, *in vitro* artifact that can mimic other blood-borne pathogens such as *Borrelia*, *Trypanosoma*, or microfilariae. Awareness of this morphological mimicry is crucial for pathologists and laboratory personnel to avoid misdiagnosis. Recognizing this uncommon manifestation reinforces the importance of correlating peripheral smear findings with clinical data and supporting laboratory tests.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Patient's consent

Written informed consent was obtained from the patient for the publication.

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Authors' contributions

JJ and CSBRP developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both JJ and CSBRP contributed to the final version of the manuscript. JJ supervised the project.

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