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First Report of Neutrophil Involvement of Exflagellated *Plasmodium vivax* Microgametes

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Dear editor

Malarial infections pass through multiple stages, beginning in the hepatic parenchymal cells where invading sporozoites mature into schizonts. On maturation, these schizonts rupture and release merozoites into the bloodstream, which, in turn, target erythrocytes, leading to the clinical manifestations of the disease [1]. Exflagellation is characterized by thin, flagella-shaped microgametes emerging from male malaria gametocytes; occurs in the midgut of the *Anopheles* mosquito; and has rarely been observed in peripheral blood specimens.

Here, we present a case of *Plasmodium vivax* infection characterized by exflagellation of microgametes and accompanied by the unusual presence of exflagellated microgametes within the cytoplasm of peripheral leukocytes. To the best of our knowledge, this is the first report of neutrophil involvement of exflagellated *Plasmodium*.

A pregnant 26-yr-old Pakistani woman presented with a fever >40°C lasting for 7 days. The patient, who arrived from Pakistan 10 days previously, had no medical history of malarial infection. She had been treated with non-steroidal anti-inflammatory drugs (NSAIDs), without improvement of symptoms, 4 days prior to her arrival at a local hospital.

At presentation, the patient appeared acutely ill. Her blood pressure measured 90/50 mmHg, and body temperature was 37.2°C. Complete blood cell and differential counts revealed anemia with thrombocytopenia (white blood cell [WBC] 6.61×10^9/L, hemoglobin 9.7 g/dL, platelets 64×10^9/L) and leukoerythroblastic changes (nucleated red blood cell [nRBC] 1/100 WBC). Peripheral blood smears were performed within 40 min of the blood draw and revealed *P. vivax*-infected erythrocytes (Fig. 1). Parasite density was determined to be 20887.6/μL. A malaria Pf/Pv strip (Asan Pharmaceutical, Whasung, Korea) yielded a positive result for only *P. vivax* antibodies. Malaria nested PCRs (Eone Laboratory, Incheon, Korea) revealed *P. vivax* only. In addition, 15-20 μm filamentous microgametes containing round-to-oval-shaped chromatin structures were observed. Some microgametes were distributed outside of the RBCs, while others appeared exflagellated from the gametocyte. Some microgametes were observed within the cytoplasm of neutrophils. Neutrophils containing microgametes exhibited phagosomes within the cytoplasm and/or nuclear condensation and ruffled plasma membranes (Fig. 2).

Quinine sulfate and clindamycin treatment were commenced, instead of primaquine, owing to the pregnancy. Two days later, another blood smear was performed immediately after collection. Schizonts and gametocytes remained in the blood; however, no microgametes were observed. Follow-up peripheral blood morphologic examinations were performed 9 days after initial diagnosis and showed no malarial parasites. Four days later, the patient underwent an abortion due to the death of the

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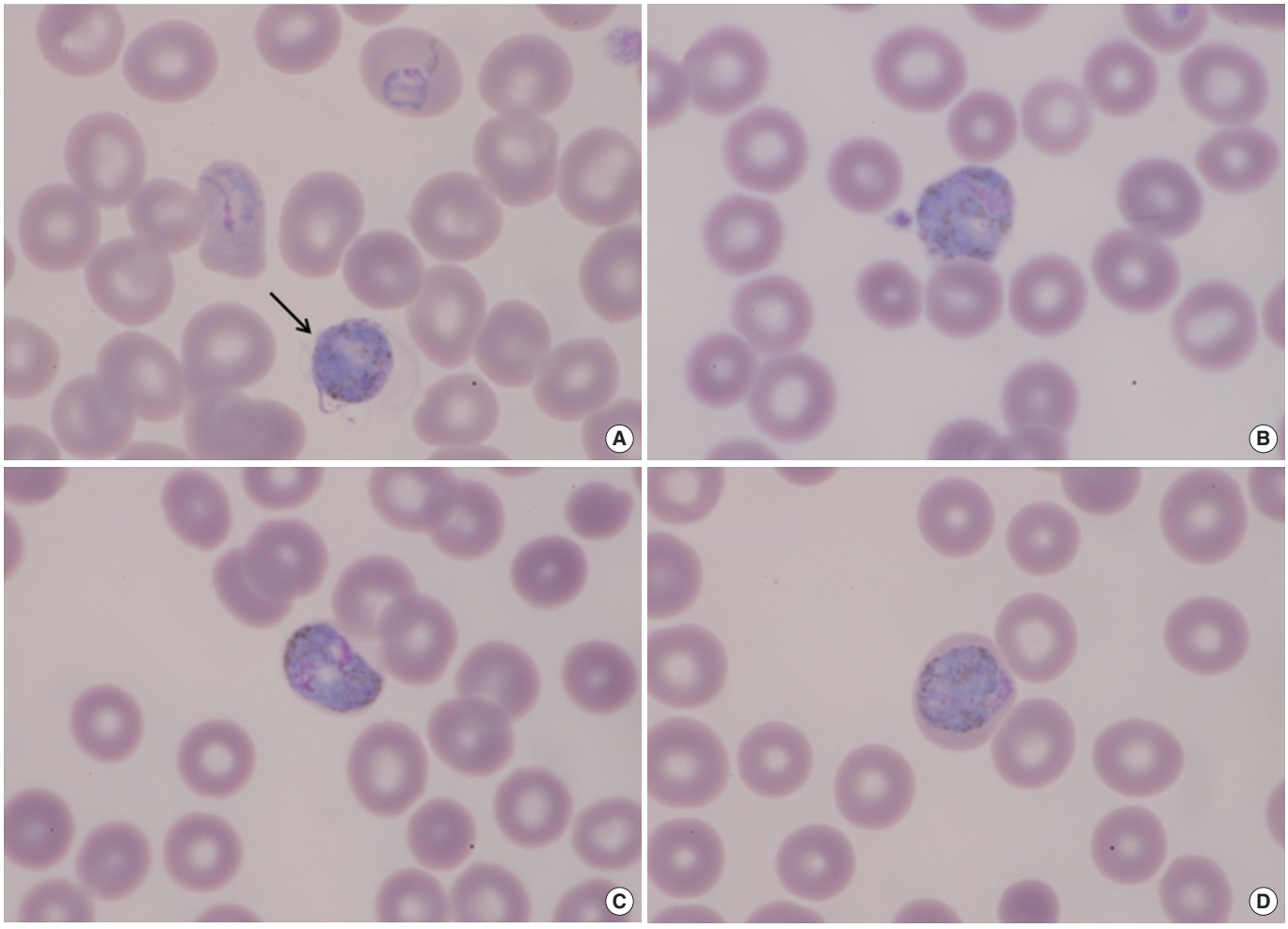

Fig. 1. *Plasmodium vivax*-infected erythrocytes. (A) Amoeboid trophozoite. The arrow indicates a microgametocyte showing exflagellation of microgametes. (B) Mature trophozoite. (C) Schizont in division. (D) Mature macrogametocyte (Wright-Giemsa stain, ×1,000).

fetus. One month after the initial diagnosis, she was readmitted with a fever and diagnosed with a relapse of *P. vivax*. The patient was treated with primaquine, and *Plasmodium* was no longer observed 5 days after treatment.

Although reported by MacCallum in 1897 [2], the presence of exflagellated microgametes in blood is rare; only one case has been reported, with only RBC involvement, in Korea [3]. *In vitro* studies have revealed important roles of both temperature and pH in exflagellation [4, 5]. Exflagellation of malarial parasites generally occurs at pH >7.6 and temperatures <30°C. Delays in sample processing can result in *in vitro* exflagellation owing to increased blood pH that results from decreased carbon dioxide levels and temperature. In this case, although blood pH and temperature were not available, the processing time was <40 minutes, suggesting *in vivo* exflagellation.

Clinically, exflagellated microgametes can suggest coinfection with other parasites, as their shape is similar to those of *Borrelia*, spirochetes, microfilaria, and *Trypanosoma*. *Borrelia* is a helical organism containing 7-22 periplasmic flagella but no chromatin. *Trypanosoma* is curved with a single nucleus and small kinetoplast; the microfilaria is large (between 100 and 200 μm) [6] and has multiple nuclei. In contrast, exflagellated microgametes are characterized by a sinuous body containing ovoid chromatin structures. Phagocytosis by neutrophils is a major host immune response to pathogens. There are reports that WBC can survive for several hours in the midgut of the mosquito and phagocytize parasites, thereby reducing the spread of malaria [7]. However, in the case of *Leishmania*, neutrophils are not activated and serve as a vector for silent entry of the pathogen into macrophages and are eventually killed by apoptosis [8]. In the present case, neutrophils might have been involved in phagocytosis of the parasite. However, the nuclear condensation and zeiosis of the neutrophil

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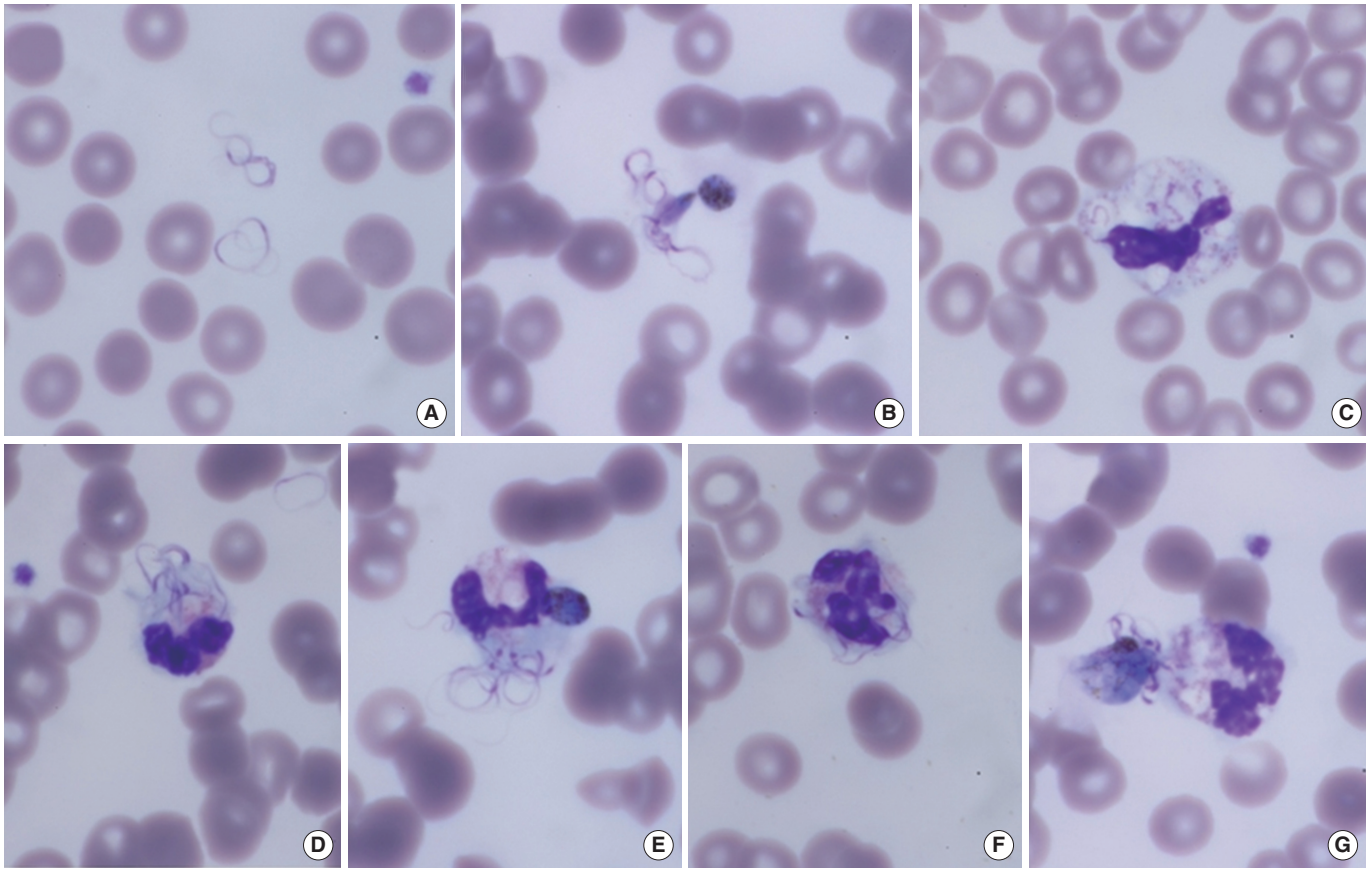

Fig. 2. Microgametes of *Plasmodium* vivax and neutrophils containing microgametes. (A) Microgamete of *P. vivax*. (B) Exflagellation of microgametes from a microgametocyte. (C) Neutrophils containing microgametes within the phagosome. (D-G) Microgametes observed within the cytoplasm of neutrophils. Neutrophils show nuclear condensation and a ruffled plasma membrane (Wright-Giemsa stain, ×1,000).

plasma membrane suggested apoptosis due to infection with the malaria parasite [9]. This is the first report of neutrophil involvement of exflagellated *Plasmodium*. More studies are necessary to determine whether *Plasmodium* can infect neutrophils and use them as a vector. In conclusion, we report a rare case of *P. vivax* infection showing exflagellation with neutrophil involvement that could be misidentified for other bloodborne parasites.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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