DOI: 10.7860/NJLM/2021/48737:2527

Case Report

Pathology Section

Exflagellated Microgametes of *Plasmodium vivax* in Human Peripheral Blood: An Unusual Finding in Malaria

ANJU SUSSANNA THOMAS[1], MEETA THOMAS[2], MO ANNAMMA[3]

ABSTRACT

Among the various diagnostic tests for the detection of malarial parasites, peripheral blood smear examination is the most common test performed for species identification by assessing the morphology of various developmental stages. The stages usually seen on the peripheral blood smear of a patient with *Plasmodium vivax* infection include schizonts, trophozoite forms and gametocytes. In the lifecycle of malarial parasites, exflagellation of microgametes usually occurs within the mosquito and is an extremely rare finding in human blood. Here, the author report a case of a 24-year-old female patient, who presented with *Plasmodium vivax* infection and exflagellated microgametes in the peripheral blood smear. To date, there have been only three cases reported from India that demonstrated similar findings. Due to it's rarity, it is crucial to be aware of such unusual exflagellated forms in the context of diagnostics to differentiate between malaria and coinfection with haemoparasites such as spirochetes and trypanosomes.

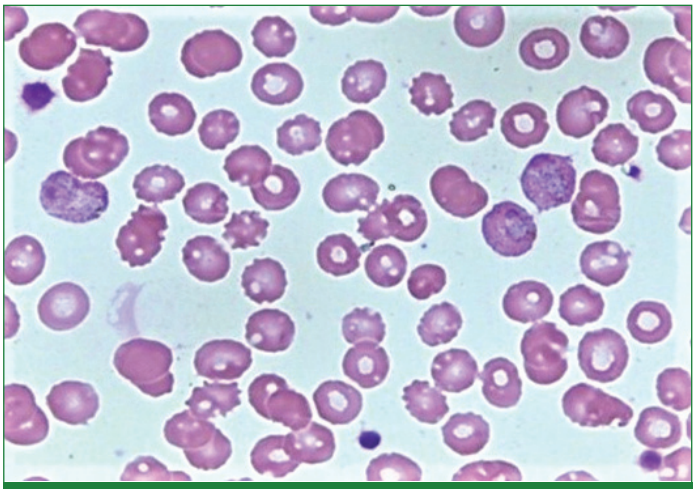
Keywords: Blood pH, Exflagellation factor, Haemoparasites, Haemozoin

CASE REPORT

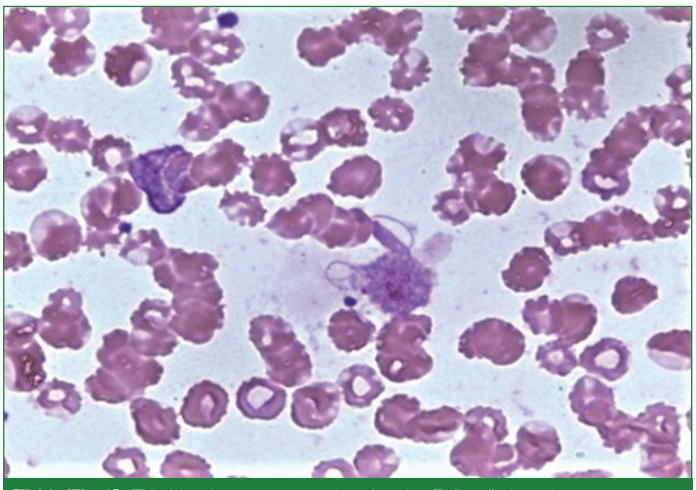
A 24-year-old female, reported to the Emergency department, with a history of high-grade fever with alternate day spikes associated with chills and rigor, myalgia and headache since five days duration. She had an episode of transient loss of consciousness. There was no history of abdominal pain, diarrhea, dysuria, weakness of limbs or bleeding manifestations. Physical examination revealed mild pallor and hypotension with intermittent febrile episodes. There was no organomegaly or lymphadenopathy and all other systems were within normal limits. With a recent travel history to Uttar Pradesh, a northern state in India with a higher incidence of malaria than Kerala, a possibility of malarial infection was considered.

Routine haemogram on the patient's peripheral blood, received in the laboratory in an Ethylene Diamine Tetra-Acetic Acid (EDTA) vacutainer tube, showed haemoglobin 10.8 g/dL with a haematocrit of 32%; total White Blood Cells (WBC) count of 7600 cells/mm^3 composed of 89% neutrophils, 9% lymphocytes, 1% each of eosinophils and monocytes; and platelet count of 130,000 cells/mm^3. Routine peripheral blood film examination stained with Leishman stain [Table/Fig-1] revealed trophozoites (amoeboid and few ringforms) of the malarial parasite, consistent with *Plasmodium vivax* species. However, additional peripheral blood thin smears and buffy coat preparation showed few filamentous structures scattered and in clusters outside the erythrocytes, morphology of which were consistent with exflagellated microgametes [Table/Fig-2-4]. The microgametes were seen as thin undulating structures with a dark, oval to rod-like nucleus in the centre, while some showed similar nuclei towards one end. Few neutrophils containing phagocytosed haemozoin pigment were also noted. These exflagellated forms were not seen in the freshly prepared fingerprick smears. Rapid diagnostic test for malaria antigen parasite lactate dehydrogenase (pLDH) was positive, while serology tests for Leptospira and dengue were negative. Liver function tests showed mild hyperbilirubinemia with normal liver enzymes.

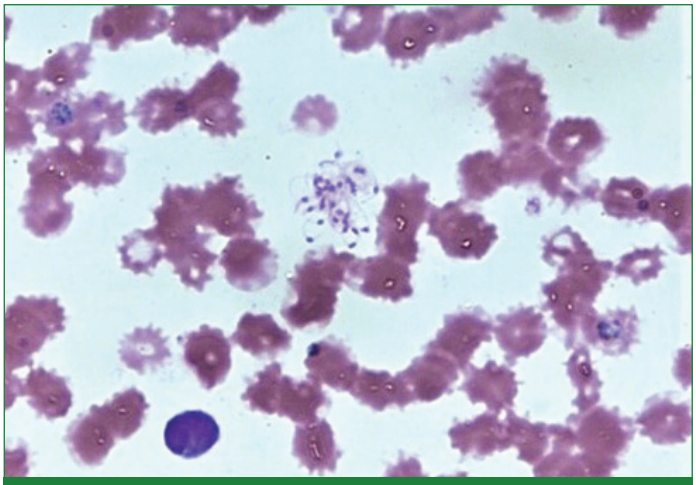
The patient was treated with Intravenous (IV) and oral artesunate-based combination therapy as well as primaquine therapy. A follow-up blood examination performed one week later, showed a complete treatment response with the absence of parasitemia.



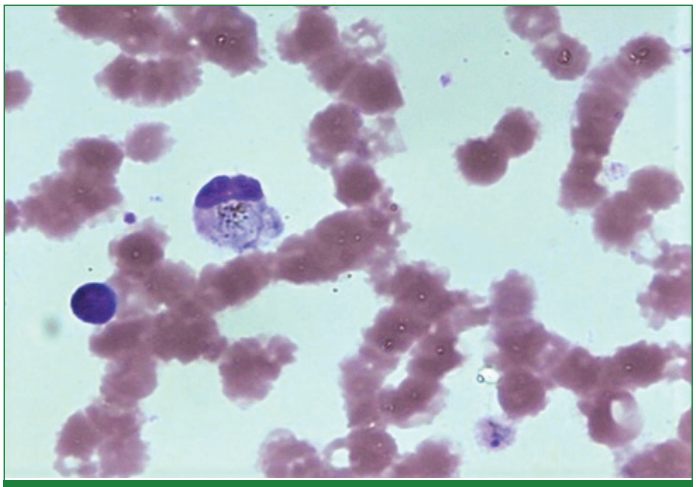
[Table/Fig-1]: Thin blood smear preparation showing *Plasmodium vivax* infected erythrocytes- trophozoite forms (Leishman stain; X1000).



[Table/Fig-2]: Thin blood smear preparation showing Exflagellating microgametocyte (Leishman stain; X1000).



[Table/Fig-3]: Buffy coat smear preparation showing clusters of filamentous microgametes with ovoid chromatin material (Leishman stain; X1000).



[Table/Fig-4]: Buffy coat smear preparation showing neutrophil with ingested haemozoin pigment (arrow) (Leishman stain; X1000).

DISCUSSION

According to the World Malaria Report in 2019, prepared by the World Health Organisation (WHO), India has the highest total malaria cases (58%) among the Southeast Asian countries, and 47% of the global burden of *Plasmodium vivax (P.vivax)* infection [1]. In its life cycle, *Plasmodium vivax* has two stages of reproduction which take place in two hosts. The asexual cycle of the parasite (exo-erythrocytic and erythrocytic schizogony) takes place in humans, where schizonts mature to gametocytes through intermediate stages of ring and amoeboid trophozoites. These stages are readily identifiable on peripheral blood smear examination. The sexual cycle occurs within the mosquito gut following ingestion of mature gametocytes in its blood meal. During this stage of sporogony, the female macrogametocyte transforms into a macrogamete, while the male microgametocyte undergoes exflagellation to produce several highly motile and very slender microgametes. The microgametes penetrate the macrogamete, generating zygotes that transform into motile ookinetes, which invade the gut wall and develop into oocyst containing sporozoites. However, the occurrence of exflagellated microgametes is found only within the mosquito and is extremely rare to find in the human peripheral blood [2].

Although gametocyte activation recognised by exflagellation has been described primarily to occur in the mosquito gut, there have been few cases reported in the literature where these changes were observed in human blood. It was MacCallum, in 1897, who first reported the presence of flagellated gametocytes during microscopic examination of infected human blood [3]. Since then, there have only been around 15 cases published in the literature to the author's knowledge [4]. However, there are only three cases reported from India that demonstrated exflagellated microgametes of *Plasmodium vivax* in human blood.

The process of exflagellation to form microgametes is initiated by signals like a drop in temperature by 5°C, and an increase in pH, which usually occurs in the gut of the mosquito following the dissipation of carbon dioxide from the blood meal [3,5]. Additionally, the presence of Mosquito-derived Exflagellation Factor (MEF), later found as Xanthurenic Acid (XA) triggers microgamete formation, which in turn depends on the pH and bicarbonate levels [4,6].

However, it is uncommon to find the high blood pH (7.7) that is thought necessary to permit exflagellation in usual clinical laboratory conditions. But, if blood samples collected into EDTA-containing tubes are left unstoppered and get exposed to ambient air or if processed with delay, it can cause a decrease in pCO_2 consequently rising the pH from the normal 7.4 to at least 7.7 [7]. These factors mimic the mosquito gut environment activating exflagellation. Some unidentified factors could also be accountable for exflagellation in human blood. Few reports suggest the role of phosphodiesterase inhibitors and caffeine in inducing this phenomenon, but the exact mechanism is unclear [7]. Although a study suggested that heparin can induce pH changes and EDTA inhibits exflagellation by preventing pH change, most of the published cases, including the present case, observed exflagellation in EDTA preserved blood [4]. In the present case also, exflagellated microgametes were demonstrated in the smears prepared from the EDTA-anticoagulated blood sample stored within the laboratory for about one hour at a room temperature of 20-22°C.

Hence, to reduce the development of in vitro changes, it is suggested to prepare fresh blood smears either through a direct finger prick or preferably within 30 minutes of collecting blood into EDTA tubes [3]. This was corroborated in the present case, as the exflagellated forms were not seen in the freshly prepared blood smears from direct finger prick, supporting an in vitro change.

Lack of awareness of this rare finding can result in a diagnostic dilemma with haemoparasites such as borrelia and trypanosomes or lead to misinterpretation as an artifact. The similarity in the symptoms and signs of malaria and relapsing fever (Borreliosis) and the resemblance of *P.vivax* microgametes to spirochetes can pose diagnostic confusion. However, there are morphological features that can help distinguish between them [8,9]. Exflagellated microgametes are seen as thin, filamentous, undulating structures measuring approximately 12-15 µm long, with a characteristic dark rod-shaped nucleus on Romanowsky stains, outside the erythrocytes. On the contrary, spirochetes like Borrelia are about 5-20 µm long, spiral-shaped and lack chromatin material. The absence of a nucleus is, thus, a major differentiating feature from the exflagellated microgamete. Trypanosomes can often be identified by their curved body, single nucleus and a small kinetoplast.

CONCLUSION(S)

Exflagellation of malarial parasite is an extremely rare phenomenon in human blood, however it may occur in vitro due to the various factors discussed in this report. Lack of awareness of these unusual exflagellating microgametes on peripheral smears may pose diagnostic problem by suggesting coinfection with other haemoparasites. Hence, meticulous microscopic examination of the specific morphological features and preparation of fresh blood smears by direct finger prick is crucial to eliminate misdiagnosis and to ensure correct treatment.

REFERENCES

[1] World malaria report 2019. Geneva: World Health Organization; 2019. License: CC BY-NC-SA 3.0 IGO.
[2] Global Health, Division of Parasitic Diseases and Malaria. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2020.

[3] Hummert BA. *Plasmodium vivax* ookinetes in human peripheral blood. J Clin Microbiol. 1994;32(10):2578-80.
[4] Sable MN, Chhabra G, Mishra S. Exflagellation of *Plasmodium vivax* in peripheral blood: An uncommon finding and its significance. J Lab Physicians. 2019;11(2):161-63.
[5] Kuehn A, Pradel G. The coming-out of malaria gametocytes. J Biomed Biotechnol. 2010;2010:976827.
[6] Arneth B, Keller C, Schaefer S. Malaria exflagellation in a human peripheral blood smear. ID Cases. 2017;10:51-52.
[7] Gerber JE, Ukena TE, Cote L, Wyllie JM, Winn WC Jr. Exflagellation of malarial parasites in human peripheral blood. J Clin Microbiol. 1981;13(1):236-37.
[8] Tembhare P, Shirke S, Subramanian PG, Sehgal K, Gujral S. Exflagellated microgametes of *Plasmodium vivax* in human peripheral blood: A case report and review of the literature. Indian J Pathol Microbiol. 2009;52(2):252-54.
[9] Prasad CS, Aparna N, Harendra Kumar ML. Exflagellated microgametes of *plasmodium vivax* in human peripheral blood: An uncommon feature of malaria. Indian Journal of Hematology & Blood Transfusion. 2011;27(2):104-06.

PARTICULARS OF CONTRIBUTORS:
1. Former Assistant Professor, Department of Pathology, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala, India.
2. Associate Professor, Department of Pathology, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala, India.
3. Former Professor and Head, Department of Pathology, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Meeta Thomas,
Associate Professor, Department of Pathology, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla-689101, Kerala, India.
E-mail: dr_mthomas@yahoo.co.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 30, 2021
- Manual Googling: Apr 10, 2021
- iThenticate Software: Jun 02, 2021 (13%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jan 29, 2021**
Date of Peer Review: **Mar 16, 2021**
Date of Acceptance: **Apr 14, 2021**
Date of Publishing: **Oct 01, 2021**