

Case report

EXFLAGELLATION IN PLASMODIUM VIVAX MALARIA: DIAGNOSTIC DILEMMAS AND SOLUTIONS

Abstract

Trophozoites gametocytes and Schizonts are the most common developmental stages of *plasmodium vivax* found in human blood. Exflagellation of microgametes occurs in mosquitos but not in humans during the life cycle malaria parasite. Exflagellation can occur in collected human blood and may cause diagnostic confusion with organisms such as spirochetes and trypanosomes. We present an unusual case of exflagellation in a six-year-old male patient with exflagellated microgametes, an unusual form of plasmodium vivax in a human peripheral blood smear that may create diagnostic confusion with organisms such as spirochetes and trypanosomes.

Keywords: Exflagellation, *Plasmodium Vivax*, Malaria, Microgametes

Introduction:

Malaria, caused by parasites of the genus Plasmodium and transmitted to humans by the anopheles mosquito, is still the leading cause of death worldwide, and early diagnosis and rapid treatment are required to prevent adverse outcomes [1]. In 2021, the WHO estimated 247 million malaria infections and 619,000 deaths worldwide [2]. The sexual phase of *plasmodium* happens in mosquitoes and the asexual phase happens in man the intermediate host. All asexual stages of Plasmodium vivax, including ring forms, late trophozoites, schizonts, and gametocytes, are commonly seen in human blood. Sexual stages of plasmodium vivax including exflagellation of microgametocytes happen in mosquitoes.

Exflagellation of microgametocytes in mosquito

Exflagellation is the process by which activated male microgametocytes develop motile flagellar microgametes that detach from the residual body by binding to erythrocytes in mosquitos [3]. Successful exflagellation was considered a reasonable proxy for the viability of mature male gametocytes [4]. Plasmodium gametocytes can recognize the intestinal

environment after being swallowed during blood feeding in order to commence sexual reproduction and infect the midgut of the mosquito. Temperature changes, pH changes, and the presence of the insect-specific chemical xanthurenic acid have all been proven to be key stimuli detected by gametocytes in order for them to activate and begin sexual reproduction [5][6]. One male gametocyte forms approximately around 6-8 microgametes [3] Ex flagellated forms of microgametocytes and microgametes are reported rarely in humans.

In this case report, we discuss an unusual case of a patient-reported with an exflagellation of microgametes in peripheral blood. Under the microscope, exflagellated microgametes form resembles both *Trypanosoma* species and spirochetes, creating a diagnostic quandary.

Case report

The case was reported at SRL Diagnostics; Dubai Health Care City.

A 6-year-old male patient presented with a fever who had a recent traveling history to India from the United Arab Emirates. An EDTA sample collected from an outside clinic was transported at ambient temperature to our reference laboratory for examination. Blood smear was prepared from the EDTA blood and stained with Leishman stain. A blood smear was examined under a microscope identified ring forms, trophozoite, and schizont forms of *plasmodium vivax*. In addition to intraerythrocytic parasites, multiple extraerythrocytic, thin, long, filamentous extra erythrocytic flagella-like structures were observed, leading to the initial assumption that this was a case of malaria with a coinfection by *Trypanosoma* species or spirochetes such as *Borrelia*. However, after a detailed examination of the extraerythrocytic structures, they were identified as exflagellated microgametocytes of *plasmodium vivax*.

Discussion

Exflagellation of microgametes in human peripheral blood are reported rarely. Ford J *et al* [2003] reported a similar case of exflagellation of *plasmodium vivax*. Mukund N. Sable *et al*. [2019] in their study well described the exflagellation of microgametes in human peripheral blood and its diagnostic dilemma due to the resemblance with *Trypanosoma* species and *Borrelia* – a spirochete.

The reason behind the rare phenomenon explained is when blood containing *plasmodium* microgametocytes collected in an anticoagulant test tube when subjected to environmental factors, including change in pH and temperature;(drop in temperature with increase in PH results) in increased CO₂ which results in exflagellation[7]. When blood collected in anticoagulant tubes comes into contact with the air environment, resulting in a drop in CO₂,

the pH rises, mimicking the change that gametocytes are subjected to when they reach the mosquito gut and triggering exflagellation [5].

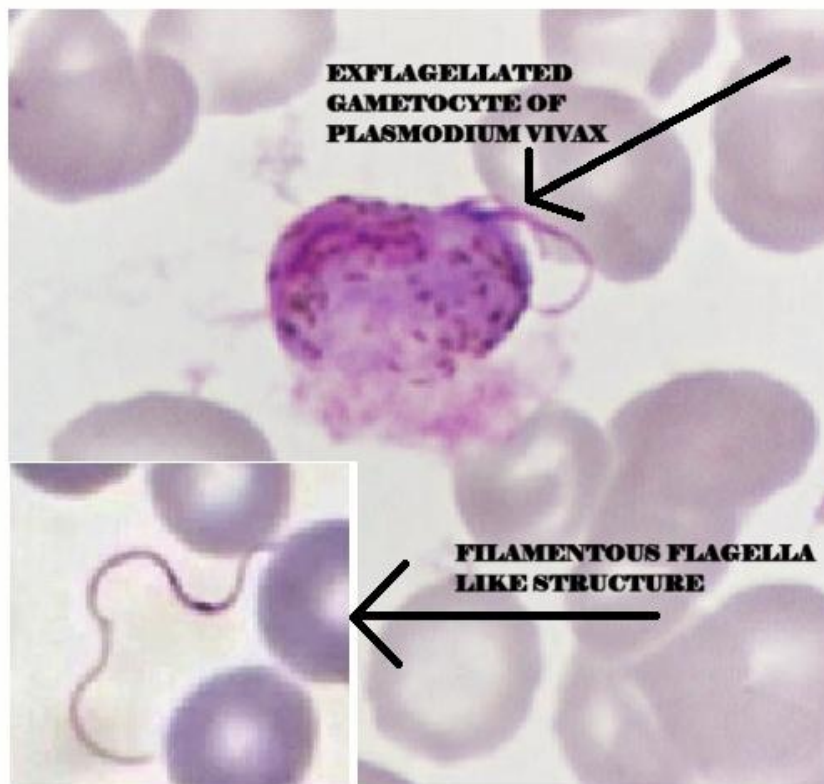


Fig: 1 Patient blood smear showing Ex flagellation of plasmodium vivax

A careful morphological examination with improved knowledge is required in differentiating the exflagellated form of Plasmodium, which appears as a thin filamentous structure of around 10-15 in length with an oval-shaped dark blue nucleus. Borrelia, on the other hand, is 5-20 in length with a spiral structure and no nuclei, and Trypanosoma has an undulating membrane, kinetoplast, and a nucleus [8].

Conclusion

Exflagellation can occur in human blood and may cause diagnostic confusion with organisms such as spirochetes and trypanosomes. With rising globalisation and frequent travel requirements in today's world, it is important for clinical laboratories and physicians globally to be aware of all common and unusual morphological forms of malarial parasites found in human blood to avoid errors in diagnosis.

Consent

As per international standards or university standards, parental(s) written consent has been collected and preserved by the author(s).

Ethical Approval

As per international standards or university standards, written ethical approval has been collected and preserved by the author(s).

References

1. Talapko, J., Škrlec, I., Alebić, T., Jukić, M., & Včev, A. (2019). Malaria: the past and the present. *Microorganisms*, 7(6), 179.
2. World malaria report 2022 [Internet]. [cited May 6, 2023]. Available from: <https://www.who.int/publications-detail-redirect/9789240064898>
3. Kuehn, A., & Pradel, G. (2010). The coming-out of malaria gametocytes. *BioMed Research International*, 2010.
4. Delves, M. J., Straschil, U., Ruecker, A., Miguel-Blanco, C., Marques, S., Dufour, A. C., ... & Sinden, R. E. (2016). Routine in vitro culture of *P. falciparum* gametocytes to evaluate novel transmission-blocking interventions. *Nature protocols*, 11(9), 1668-1680.
5. Arneth, B., Keller, C., & Schaefer, S. (2017). Malaria exflagellation in a human peripheral blood smear. *IDCases*, 10, 51.
6. Billker, O., Lindo, V., Panico, M., Etienne, A. E., Paxton, T., Dell, A., ... & Morris, H. R. (1998). Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. *Nature*, 392(6673), 289-292.
7. Tembhare, P., Shirke, S., Subramanian, P. G., Sehgal, K., & Gujral, S. (2009). Exflagellated microgametes of *Plasmodium vivax* in human peripheral blood: a case report and review of the literature. *Indian Journal of Pathology and Microbiology*, 52(2), 252.
8. Prasad, C. S. B. R., Aparna, N., & Harendra Kumar, M. L. (2011). Exflagellated microgametes of *Plasmodium vivax* in human peripheral blood: an uncommon feature of malaria. *Indian Journal of Hematology and Blood Transfusion*, 27, 104-106.

