Exflagellation of Microgametocytes in *Plasmodium vivax* Malaria: A Diagnostic Conundrum

Asle Enger^a Øystein Anders Strand^{a,b} Trond Ranheim^a
Kjell Block Hellum^a

^aDepartment of Medicine, Akershus University Hospital, Nordbyhagen, ^bCentre for Tropical Medicine, Haukeland University Hospital, Bergen, Norway

Key Words

Malaria · Microgametocyte · Exflagellation

Abstract

Objective: To present a clinical diagnostic conundrum of unidentified structures in a blood smear from a patient with *Plasmodium vivax* malaria. **Clinical Presentation and Intervention:** A 37-year-old Ethiopian male presented with a 4-month history of chills, chronic diarrhea and weight loss. He was diagnosed with *P. vivax* malaria, advanced HIV infection and *Isospora belli* enteritis. Unidentified structures initially confusing to the diagnosticians were seen in blood smears taken on admission. The structures were initially considered to represent atypical spirochetes, but were later identified as microgametes and other exflagellation forms of *P. vivax*. The patient recovered after receiving adequate treatment for his infections. **Conclusion:** This case illustrates that exflagellation may be observed in blood smears from patients with *P. vivax* malaria. Size and morphological characteristics differentiate malaria microgametes and other exflagellation forms from microfilaria, spirochetes and trypanosomes.

Introduction

In the life cycle of the malaria parasites exflagellation of male malaria gametocytes (microgametocytes) occurs in the stomach of the *Anopheles* mosquito. Exflagellation of microgametocytes in human blood in malaria is, however, a very rare event first observed by MacCallum [1] in 1897 in smears from a patient with *Plasmodium falciparum* infection. During exflagellation the microgametocyte shows violent internal activity where up to 8 long, thin processes (flagella) extend from the membrane and nuclear material travels down each flagellum [2, 3]. The release of up to 8 flagellate microgametes, which are actively motile spermatozoa seeking to fertilize a female macrogametocyte, occurs within less than 2 min [3]. Albeit being a well-known experimental in vitro phenomenon [4–9], exflagellation is only rarely described in the clinical setting of malaria [1, 10, 11]. Increased awareness of the exflagellation process and of the different exflagellation forms may prevent confusion when examining blood smears for plasmodia. Therefore, we report different stages of exflagellation as seen in blood drawn from a patient with *Plasmodium vivax* infection.

Copyright © 2004 S. Karger AG, Basel

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2004 S. Karger AG, Basel
1011–7571/04/0135–0298\$21.00/0

Accessible online at:
www.karger.com/mpp

Øystein Anders Strand, MD, PhD, Associate Professor
Centre for Tropical Medicine, Department of Medicine
Haukeland University Hospital, NO–5021 Bergen (Norway)
Tel. +47 67 92 88 00, +47 22 44 26 20, +47 97 15 26 66
Fax +47 67 92 90 04, E-Mail oyststrand@hotmail.com

Fig. 1. Red blood cells infected with several ring forms of *P. vivax*. Magnification $\times 2,500$.

Fig. 3. Exflagellation of numerous microgametes from microgametocytes. Magnification $\times 3,000$.

Fig. 2. Early process of flagella formation from microgametocyte and 2 microgametes. Magnification $\times 2,750$.

Fig. 4. Microgamete apparently adhering to red blood cell. Magnification $\times 3,000$.

Case Report

A 37-year-old Ethiopian male was admitted 6 months after having left his homeland with a 4-month history of chills every second day, chronic diarrhea and weight loss. He had a previous history of malaria and severe allergy to sulfonamides. He was last treated for malaria 2 years prior to admission. During the present illness he had consulted several physicians without obtaining a definite diagnosis. On admission a moderate enlargement of the liver and spleen was noted. Blood smears prepared from EDTA blood after transport to the laboratory at ambient temperature showed ring forms, trophozoites and gametocytes of *P. vivax*, including an unusually large proportion of red blood cells containing 2 and 3 ring forms (fig. 1). The parasitemia was 2%. In the smears taken on admission, structures initially suspected to represent spirochetes, atypical trypanosomes,

atypical microfilariae or artifacts were noticed (fig. 2–4). The patient was left untreated overnight, and the following morning control smears of EDTA blood and blood obtained by finger pricking were prepared at the bedside without delay. Structures like those observed in smears taken on admission were not observed in these smears. The patient was thereafter treated with standard chloroquine and given 15 mg primaquine daily for 14 days. Although a source of initial confusion, the structures in figures 2–4 were eventually identified as exflagellating microgametocytes and microgametes of *P. vivax*. Serology for trypanosomiasis, leishmaniasis and filarioses, direct microscopy of blood for microfilaria, and cultures from blood and bone marrow in NNN media and standard media were all negative. The patient tested positive for antibodies to human immunodeficiency virus-1 (HIV-1). The plasma concentration of HIV RNA was 1.6×10^6 copies·ml⁻¹ and the CD4 count 0.03×10^9 liter⁻¹. A large num-

ber of *Isospora belli* cysts were found in the stool and the diarrhea was controlled by pyrimethamine. Antiretroviral therapy was started with good immune restoration and resolution of the symptoms.

Discussion

This case of *P. vivax* malaria represented a diagnostic conundrum because structures eventually identified as exflagellating forms of *P. vivax* microgametocytes were initially a source of confusion. Exflagellating forms of malaria parasites are only rarely seen in diagnostic samples, and then most often in *P. falciparum* infections. This case report serves as a reminder of the fact that exflagellation may be observed in smears from patients with *P. vivax* infections, and that the presence of exflagellation forms may be confusing to diagnosticians. In vitro studies indicate that exflagellation is strongly temperature and pH dependent, mainly occurring at temperatures below 30°C and at pH >7.6 [4–10]. In this patient, there was a usual interval of 30 min from venous sampling of EDTA blood to slide preparation by the laboratory technician. The sample was transported in-house at room temperature and was not exposed to particularly low temperatures. However, exflagellation was not observed in the set of pretreatment control smears prepared at the bedside immediately after sampling the following morning. We

thus suggest that the exflagellation of microgametocytes which was observed in the smears prepared from the EDTA blood obtained at admission represented an ex vivo phenomenon occurring in the 30-min interval from blood sampling to smear preparation.

Conclusion

The morphological forms present in blood smears from this patient provided rare visual evidence of the exflagellation process. This case further shows that the confusion that may arise during examination of blood smears from malaria patients can be reduced by paying attention to the fact that exflagellation may occur in *P. vivax* infections and by becoming familiar with the microscopic findings. Size and morphological characteristics differentiate malaria microgametes from microfilaria, spirochetes and trypanosomes.

Acknowledgments

We sincerely thank Ms Elin Gulbrandsen, Department of Clinical Chemistry, Akershus University Hospital for her help in the preparation and reading of malaria slides and for phototechnical assistance.

References

- 1 MacCallum WG: On the flagellated form of malaria parasite. *Lancet* 1897;ii:1240.
- 2 Garnham PCC: *Malaria Parasites and Other Hemosporidia*. Oxford, Blackwell Scientific, 1966, p 1114.
- 3 Knell AJ: Malaria: The problem; in Knell AJ (ed): *Malaria*. Oxford, Oxford University Press, 1991, pp 12–15.
- 4 Hawking F, Wilson ME, Gammage K: Evidence for cyclic development and short-lived maturity in the gametocytes of *Plasmodium falciparum*. *Trans R Soc Trop Med Hyg* 1971; 65:549–559.
- 5 Carter R, Nijhout MM: Control of gamete formation (exflagellation) in malaria parasites. *Science* 1977;195:407–409.
- 6 Smalley ME, Sinden RE: *Plasmodium falciparum* gametocytes: Their longevity and infectivity. *Parasitology* 1977;74:1–8.
- 7 Ogwan'g RA, Mwangi JK, Githure J, Were JBO, Roberts CR, Martin SK: Factors affecting exflagellation of in vitro cultivated *Plasmodium falciparum* gametocytes. *Am J Trop Med Hyg* 1993;49:25–29.
- 8 Billker O, Shaw MK, Margos G, Sinden RE: The roles of temperature, pH and mosquito factors as triggers of male and female gametogenesis of *Plasmodium berghei* in vitro. *Parasitology* 1997;114:1–7.
- 9 Templeton TJ, Keister DB, Muratova O, Procter JL, Kaslow DC: Adherence of erythrocytes during exflagellation of *Plasmodium falciparum* microgametes is dependent on erythrocyte surface sialic acid and glycoproteins. *J Exp Med* 1998;187:1599–1609.
- 10 Gerber JE, Ukena TE, Cote L, Wyllie JM, Winn WC: Exflagellation of malaria parasites in human peripheral blood. *J Clin Microbiol* 1981;13:236–237.
- 11 Weinstein MP, Alcidi DV: Occurrence of exflagellation and microgametes in peripheral blood of a patient with malaria. *J Infect Dis* 1982; 154:448.