

1 ref. EIMC-D-18-00370R1 REVISADO

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2

3 Diagnosis at first sight

4

5 **Patient from India with fever and uncommon findings**

6

7 **Paciente de la India con fiebre y hallazgos inusuales**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

8 A 28-years-old male patient from India that had not travelled to his
9 country in the last nine months presented to the emergency room with general
10 discomfort, dysthermia feeling, sweating, chills, and shivering. In the physical
11 examination, temperature was quantified in 40.5 °C. No neurological deficit or
12 peripheral lymphadenopathy was found. Blood was taken and sent to the local
13 lab for examination and then sent to our hospital in order to confirm the
14 diagnosis. This sample was stored for almost 3 days at room temperature.

15 Giemsa stain showed some strange forms, but the morphology of
16 parasite was compatible with *Plasmodium vivax*. These forms were described
17 like a structure smaller than a blood cell with peripheral flagella, a dark nucleus
18 and mobile filament forms compatible with exflagellating microgametocytes
19 (Figure 1). The patient was treated with primaquine for two weeks and the
20 infection was finally confirmed with PCR.

21
22 In the natural life cycle of the malarial parasites, exflagellation of the
23 microgametocyte, fertilization, and ookinetes production occur in the mosquito
24 gut. The usual plasmodium forms seen in human peripheral blood smears
25 include trophozoites, schizonts, macro, and microgametocytes. However, it has
26 been demonstrated that exflagellation can occur *ex vivo*. Here a case of
27 exflagellation in human blood is described. Exflagellation *ex vivo* is very
28 uncommon but since 1880, Laveran had already noticed, while examining
29 microscopically the blood of a patient suffering from malaria, “a body with
30 mobile peripheral filaments” and “mobile filament freed for the body”.¹
31 Exflagellation process was first described in 1884.² Five years later, Golgi *et al.*

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32 saw few flagellate forms corresponding to those described by Laveran.³ In
33 1891, Romanowsky contributed with a staining method based on eosin and
34 methylene blue that allowed seeing better these structures.⁴ Marchiafava *et al.*
35 in 1892 also described “a form with flagella”.⁵ However, it was not until 1898
36 that Manson and Ross published that malaria organism had a second live,
37 outside and independent of the human body.⁶ Nowadays, it is known that
38 exflagellation of the microgametocyte occurs in the gut of mosquitoes, but if
39 malaria blood is observed some time after it has been withdrawn from the blood
40 vessels another form is seen, “the flagellated body”. By 1897, MacCallum
41 discovered exflagellation and recognized its significance in the life cycle of
42 malaria parasite. He described the fertilization process and recognized the
43 flagellum as the male gamete.⁷ After that, there are several references to this
44 phenomenon.⁸

45
46 According to some authors, exflagellation can be induced *in vitro* by
47 dropping the temperature of cultured gametes from 37°C to 28°C, thus
48 simulating the change from host to vector temperatures, by either increasing the
49 pH from 7.5 to 8.2 or by the addition of a gametocyte-activating factor (GAF),
50 the oxanthurenic acid. This compound has been isolated from the mosquito gut
51 and is supposed to be implicated in the maturation of the microgametocytes.⁹ In
52 this case, probably a delay in sample processing and a dropping of the
53 temperature could be the cause of exflagellation process. Fertilization has been
54 shown to take place, at least *in vitro* conditions, almost immediately following
55 exflagellation within 10 to 20 minutes after the initiation of gametogenesis.¹⁰

56

1
2 57 The experimental *in vitro* phenomenon of exflagellation is very common,
3
4
5 58 but it is rarely described in the clinical setting of malaria. Nevertheless, it is
6
7 59 important to consider it when atypical structures are found in the blood from a
8
9
10 60 patient with suspected malaria to avoid mistake in the diagnosis.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

61 **References**

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
1. Laveran CLA. A Newly Discovered Parasite in the Blood of Patients Suffering from Malaria. Parasitic Etiology of Attacks of Malaria. Rev Infect Dis 1982;4(4):908-11. <https://doi.org/10.1093/4.4.908>
 2. Hewitt R. Bird malaria [Monograph]. Am J Hyg Monogr 1940;15.
 3. Golgi, C. On the cycle of development of malarial parasites in tertian fever: Differential diagnosis between the intracellular malarial parasites of tertian and quartan fever. In: Kean BH, Mott KE, and Russell AJ, editors. Tropical Medicine and Parasitology: Classic Investigations. New York: Cornell University Press; 1978, p. 26-35.
 4. Garnham PCC. Malaria Parasites and other *Haemosporidia*. Oxford: Blackwell Scientific Publications; 1966.
 5. Marchiafava E, Bignami A. On Summer-Autumn Malarial Fevers. London: New Sydenham Society; 1894.
 6. Manson P. Surgeon-Major Ronald Ross's recent investigations on the mosquito-malaria theory. Brit Med J 1898;1:1575–7. <https://doi.org/10.1136/bmj.1.1955.1575>
 7. MacCallum WG. On the haematozoan infections of birds. J Exp Med 1898;3(1):117-36.
 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):252-4.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

84 9. Billker O, Shaw MK, Margos G, Sinden RE. The roles temperature, pH
85 and mosquito factors as triggers of male and female gametogenesis of
86 *Plasmodium berghei in vitro*. Parasitology 1997;114:1–7.

87 10. Carter R, Nijhout MM. Control of gamete formation (exflagellation) in
88 malaria parasites. Science 1977;195:407-9.

