Retinal Changes in Uncomplicated and Severe Plasmodium knowlesi Malaria

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Background. Plasmodium knowlesi causes severe malaria, but its pathogenesis is poorly understood. Retinal changes provide insights into falciparum malaria pathogenesis but have not been studied in knowlesi malaria.

Methods. An observational study was conducted in Malaysian adults hospitalized with severe (n = 20) and nonsevere (n = 24) knowlesi malaria using indirect ophthalmoscopy (n = 44) and fundus photography (n = 29).

Results. The patients’ median age was 44 years (range, 18–74 years). No coma or deaths occurred. Photography detected retinal changes in 11 of 12 patients (92%) with severe and 14 of 17 (82%) with nonsevere knowlesi malaria. Nonspecific retinal whitening occurred in 3 (35%) and 5 (29%) patients with severe and nonsevere disease, respectively; hemorrhages in 2 (17%) and 3 (18%); loss of retinal pigment epithelium in 1 (8%) and 4 (24%); and drusen in 9 (71%) and 12 (75%). All changes were mild, with no significant differences between severe and nonsevere disease. Patients with retinal hemorrhages had lower platelet counts than those without (median, 22 vs 43 × 109/L; P = .04).

Conclusions. The paucity of specific retinal findings associated with disease severity in knowlesi malaria contrasts with the retinopathy of severe adult falciparum malaria with and without coma, suggesting that falciparum-like microvascular sequestration in the brain is not a major component in severe knowlesi malaria pathogenesis.

Keywords. malaria; Plasmodium knowlesi; retinopathy.
Hospital, an adult tertiary referral hospital in Kota Kinabalu, Sabah, Malaysia [4]. Consecutive patients with polymerase chain reaction (PCR)–confirmed *P. knowlesi* monoinfection underwent ocular examination if they were nonpregnant, ≥12 years old, had no major comorbid conditions or concurrent illness, had not been previously enrolled in the study, were willing and able to cooperate with eye examination, had no contraindications to tropicamide or phenylephrine eye drops, and did not have severe bilateral corneal scarring or cataracts precluding fundoscopy. Patients were included if they were within 96 hours of commencing antimalarial treatment, based on previous studies demonstrating persistence of retinal changes within this time period [16, 17].

Clinical details of these patients have been reported elsewhere [4]. Severe malaria was defined as the presence of ≥1 of the following: unrousable coma (Glasgow Coma Scale score <11), multiple (>2) convulsions, respiratory distress (respirations >30/min and oxygen saturation <94%), hypotension (systolic blood pressure ≤80 mm Hg), jaundice (bilirubin >43 µmol/L plus parasitemia >20 000 /µL and/or creatinine >132 µmol/L), significant abnormal bleeding, hypoglycemia (blood glucose <2.2 mmol/L), metabolic acidosis (bicarbonate <15 mmol/L or lactate >4 mmol/L), severe anemia (hemoglobin <7.0 g/dL), acute kidney injury (creatinine >265 µmol/L), and hyperparasitemia (>100 000 parasites/µL). Informed consent was provided by study participants or their guardian. The study was approved by the ethics committees of the Malaysian Ministry of Health and Menzies School of Health Research.

**Study Procedures**

Standardized history and physical examination were documented. Hematological and biochemical values, acid-base parameters, and lactate levels (determined by bedside blood analysis; iSTAT system) were obtained on admission. Parasite counts were determined with microscopy, and parasite species were identified with PCR [18, 19]. Patients were treated according to hospital guidelines, as described elsewhere [4].

Assessment of visual function and detailed eye examination were performed by one of 5 experienced ophthalmologists. Visual function assessment included testing of visual acuity using a 6-meter Snellen chart, color vision using a D15 chart, and visual fields (Humphrey Field Analyzer; Carl Zeiss Meditec). Detailed eye examination included pupillary light reaction, anterior segment slit-lamp examination, intraocular pressure measurement (slit-lamp–mounted Goldmann applanation tonometer; Carl Zeiss model SL115 Classic with AT 020), and fundus examination using indirect ophthalmoscopy 30 minutes after administration of tropicamide and/or phenylephrine eye drops for mydriasis. Fundus photography was performed using a 9-field protocol on patients able to be transported to the fundus camera (TopCon Medical Systems). Fundus fluorescein angiography (Heidelberg Retina Angiograph 2; Heidelberg Engineering) was performed in 1 patient with abnormal retinal findings. Fundus photographs were reread by 3 blinded observers (S. B., B.D., and R. J. M.), and interpreted by consensus (G. G., S. B., B. D., and R. J. M.). When appropriate, retinal findings were classified as mild, moderate, or severe according to a previously published classification for *P. falciparum* malarial retinopathy [20].

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism software (version 6.01; GraphPad Software). Intergroup differences were compared using the Mann–Whitney test for continuous variables or χ²/Fisher exact test for categorical variables.

**RESULTS**

**General Findings**

A total of 44 patients were enrolled, including 20 with severe and 24 with nonsevere knowlesi malaria. Baseline demographic features are shown in Table 1. Patients with severe knowlesi malaria were older than those with nonsevere knowlesi malaria (median age [interquartile range (IQR)], 51.5 [40–55] vs 40.5 [24–48] years; *P* = .02). Eight patients had a history of hypertension (3 with severe knowlesi malaria and 5 with nonsevere knowlesi malaria). No patient had diabetes, and no patient reported having had malaria in the previous 2 months. Among the 20 patients with severe knowlesi malaria, severity criteria included jaundice (n = 11; 55%), hyperparasitemia (n = 11; 55%), respiratory distress (n = 8; 40%), hypotension (n = 6; 30%), acute kidney injury (n = 3; 15%), metabolic acidosis (n = 2; 10%), and abnormal bleeding (n = 1; 5%). Seven patients (35%) had 1 severity criterion, 7 (35%) had 2 criteria, 3 (15%) had 3, and 3 (15%) had 4. No patient had coma, and no deaths occurred. The epidemiological and clinical features of these patients have been reported elsewhere [4].

**Visual Assessment and Eye Examination**

Visual assessment and eye examination results are shown in Table 2. There were no differences in visual acuity, visual fields, or color vision between patients with severe and those with nonsevere knowlesi malaria. Cataracts were common, occurring in 12 of 20 patients (60%) with severe knowlesi malaria and 9 of 24 patients (38%) with nonsevere knowlesi malaria. Fundoscopy was performed in 1 patient with abnormal retinal findings. Fundus photographs were reread by 3 blinded observers (S. B., B.D., and R. J. M.), and interpreted by consensus (G. G., S. B., B. D., and R. J. M.). When appropriate, retinal findings were classified as mild, moderate, or severe according to a previously published classification for *P. falciparum* malarial retinopathy [20].

**Table 1. Baseline Features of Patients With Knowlesi Malaria**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Severe Malaria (n = 20)</th>
<th>Nonsevere Malaria (n = 24)</th>
<th><em>P</em> Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>51.5 (20–74)</td>
<td>40.5 (18–71)</td>
<td>.02</td>
</tr>
<tr>
<td>Male sex</td>
<td>15 (75)</td>
<td>17 (71)</td>
<td>.76</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3 (15)</td>
<td>5 (21)</td>
<td>.71</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Previous malaria (self-reported)</td>
<td>7 (35)</td>
<td>8 (33)</td>
<td>.58</td>
</tr>
<tr>
<td>Previous malaria in past 2 mo (self-reported)</td>
<td>0 (0)</td>
<td>(0)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Abbreviations: IQR, interquartile range; NA, not assessed.

* Data represent No. (%) of patients unless otherwise indicated.
Comparison Between Indirect Ophthalmoscopy and Retinal Photography

Almost all lesions seen with indirect ophthalmoscopy were confirmed using fundus photography. In 1 patient a single retinal hemorrhage was seen with indirect ophthalmoscopy but not on the photographs, and in another, a small area of retinal whitening on photography was thought to be drusen at indirect ophthalmoscopy. Photography was more sensitive than indirect ophthalmoscopy at detecting retinal changes, with changes noted in 25 of 29 patients (86%) with photography versus 13 of 44 (30%) with indirect ophthalmoscopy (P < .001). In 8 of 29 patients (28%) who underwent fundus photography, retinal changes were seen on photographs but not with indirect ophthalmoscopy; these changes included nonspecific retinal whitening in 6, drusen in 6, and hemorrhage in 1.

Retinal Hemorrhages

Five patients were noted at photography to have retinal hemorrhages, including 2 of 12 (17%) with severe and 3 of 17 (18%) with nonsevere disease. All were classified as mild (1–5 hemorrhages per eye) according to criteria developed for falciparum malarial retinopathy [20]. Three patients had single dot or blot hemorrhages in a single eye. Two patients had multiple hemorrhages: 1 with nonsevere knowlesi malaria who had a white-centered hemorrhage in one eye and a white-centered hemorrhage and a dot hemorrhage in the other and another patient with severe knowlesi malaria who had 3 dots and 1 blot in one eye and 2 blots in the other. Both of these patients had severe thrombocytopenia (platelet count, 19 × 10^3/µL and 8 × 10^3/µL, respectively). The median (IQR) admission platelet count among the 5 patients with retinal hemorrhages seen at photography was lower than that of patients without retinal hemorrhages (22 [13.5–38.5] × 10^3/µL vs 43 [27.5–72] × 10^3/µL; P = .04), although there were no differences in prothrombin time, activated partial thromboplastin time, or hemoglobin.

Retinal Whitening and Vessel Discoloration

Eight of 29 patients (28%) with knowlesi malaria had nonspecific retinal whitening on photography, including 3 of 12 (25%) with severe and 5 of 17 (29%) with nonsevere disease. In all of these patients, the retinal whitening appeared as occasional scattered spots in the peripheral or macular retina. In 3 patients, there was also loss of retinal pigment epithelium (RPE) (Figure 1B). No patient had the moderate-severe whitening characteristic of severe falciparum malaria.

One case of vessel whitening was seen, in a patient with nonsevere knowlesi malaria (Figure 2). The whitening, located at the superotemporal region of the right eye, involved an arteriolar, with sheathing of the vessel wall and a thin patent lumen observed on

### Table 3. Retinal Findings Among Patients With Knowlesi Malaria

<table>
<thead>
<tr>
<th>Finding</th>
<th>Severe Malaria (n = 20)</th>
<th>Nonsevere Malaria (n = 24)</th>
<th>P Value</th>
<th>Severe Malaria (n = 20)</th>
<th>Nonsevere Malaria (n = 24)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any retinal lesion</td>
<td>11 (92)</td>
<td>14 (62)</td>
<td>.50</td>
<td>6 (30)</td>
<td>7 (29)</td>
<td>.95</td>
</tr>
<tr>
<td>Hemorrhages</td>
<td>2 (17)</td>
<td>3 (18)</td>
<td>&gt; .99</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>.49</td>
</tr>
<tr>
<td>Nonspecific retinal whitening</td>
<td>3 (25)</td>
<td>5 (29)</td>
<td>&gt; .99</td>
<td>2 (10)</td>
<td>1 (4)</td>
<td>.58</td>
</tr>
<tr>
<td>Cotton wool spot</td>
<td>1 (8)</td>
<td>1 (6)</td>
<td>&gt; .99</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Vessel whitening</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>&gt; .99</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>&gt; .99</td>
</tr>
<tr>
<td>RPE depigmentation</td>
<td>1 (8)</td>
<td>5 (29)</td>
<td>.35</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>&gt; .99</td>
</tr>
<tr>
<td>Drusen</td>
<td>9 (75)</td>
<td>12 (71)</td>
<td>&gt; .99</td>
<td>4 (20)</td>
<td>4 (17)</td>
<td>&gt; .99</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not assessed; RPE, retinal pigment epithelium.

Data represent No. (%) of patients. No patient had papilloedema.
fundus photography. No inflammatory changes were noted, and the overlying vitreous was clear. A fluorescein angiogram, obtained at the same time, indicated a patent vessel with no evidence of vasculitis or surrounding retinal ischemia (Figure 2). Also in this eye, there was an area of focal RPE hypopigmentation along the inferotemporal arcade, with a corresponding window defect on fluorescein angiography suggesting an abnormality in the choriocapillaries rather than the retinal vessels. The patient also had some nonspecific macular whitening in both eyes. The angiogram was normal at the sites of the macular whitening, indicating normal retinal perfusion. Repeat fundus photography 1 month after admission demonstrated no change in the nonspecific macular whitening or in the area of vessel whitening.

Drusen and Other Incidental Findings
About half of patients (13 of 29) with retinal lesions at fundus photography had small numbers of hard drusen but no other lesions. Drusen are commonly regarded as a normal variant [21], and they increase in prevalence and number with age. At indirect ophthalmoscopy, the median (IQR) age of patients with drusen was higher than that of those without drusen (52 [48.5–67] vs 38 [25.5–50.5] years; P = .005), although this difference was not significant among those who underwent retinal photography (43 [30–51] vs 39 [20–55] years; P = .78).

One 71-year-old patient with nonsevere malaria had a Weiss ring in one eye, a common finding in persons aged >65 years, caused by vitreous shrinkage and subsequent posterior vitreous detachment. Other incidental findings included retinitis pigmentosa and a myelinated nerve fiber layer, in 1 patient each. Two patients with severe malaria and 1 with nonsevere malaria had an increased cup-to-optic-disc ratio.

DISCUSSION

Retinal changes in knowlesi malaria in this study were common but were mild and occurred equally in severe and nonsevere disease, suggesting that the lesions observed are not directly related to the pathological processes leading to severe knowlesi malaria. This
is different from severe falciparum malaria, which is associated with more specific retinal changes. In adults, these can include moderate-severe retinal whitening, multiple white-centered hemorrhages, and, rarely, papilloedema [8, 9]. In falciparum malaria these changes are of use diagnostically and in elucidating pathogenesis of disease [11]. They are most prominent in fatal or cerebral falciparum malaria. Among adults in a large observational study of Bangladeshi and Indian patients with falciparum malaria, malarial retinopathy of at least moderate severity was seen in 62% of fatal cases (31 of 50) and 60% (61 of 101) of those with cerebral malaria [9]. Importantly, however, malarial retinopathy of at least moderate severity was also seen in 35% of adult patients (22 of 63) with severe but noncerebral falciparum malaria [9]. The lack of at least moderately severe retinal changes among patients with severe knowlesi malaria suggests differences in disease pathogenesis between the 2 Plasmodium species.

Nonspecific retinal whitening, although common among the patients with knowlesi malaria in this study, was scant and mild, with no patients having the moderate or severe retinal whitening characteristic of severe falciparum malaria. In the study of severe adult falciparum malaria, moderate-severe retinal whitening was seen in 55% of adults with cerebral falciparum malaria (56 of 101) and 29% (18 of 63) with severe noncerebral falciparum malaria [9]. In falciparum malaria, retinal whitening is thought to be due to retinal ischemia from obstruction of blood vessels by endothelial cytoadherence and sequestration of parasitized red blood cells within the retinal microvasculature. These changes mirror the sequestration and microvascular obstruction that occur in the brain and lead to coma [11]. In adult falciparum malaria, sequestration in the central nervous system is due primarily to the binding of parasitized erythrocytes to up-regulated endothelial intercellular adhesion molecule 1 (ICAM-1) [22]. In a single autopsy report of severe knowlesi malaria without coma, accumulation of parasitized erythrocytes within cerebral vessels was described [12]. However, ICAM-1 was not detected [12], consistent with findings of an in vitro study demonstrating variable binding of P. knowlesi to ICAM-1 [23]. Furthermore, in contrast to P. falciparum, in

Figure 2. Fundus photography and fluorescein angiogram in a patient with nonsevere knowlesi malaria. A, Fundus photography demonstrates whitening of an arteriole, with sheathing of the vessel wall. B–D, Fluorescein angiography, performed immediately after the fundus photography and with images obtained at 31 seconds (B), 43 seconds (C), and 4 minutes and 30 seconds (D), demonstrates dye flow throughout, including distal to the section of whitening, suggesting a patent arteriole. This flow, however, is notably attenuated. There was no leakage of dye to suggest active vasculitis or areas of capillary fallout to suggest retinal ischemia. There were no angiographic features suggestive of inflammation at this area or elsewhere in the retina.
P. knowlesi infection late-stage parasites are observed in peripheral blood [24], suggesting that sequestration does not occur to the same degree or through the same mechanisms as in falciparum malaria.

A paucity of endothelial cytoadhesion of P. knowlesi-infected red blood cells in central nervous system microvasculature might explain the lack of severe retinal whitening and absence of coma reported to date in severe knowlesi malaria. Although microvascular accumulation of parasitized red blood cells does occur in fatal knowlesi malaria, it is possible that clumping of P. knowlesi–infected and uninfected red blood cells and microvascular sludging, as reported in severe and fatal knowlesi malaria in rhesus monkeys [13–15], may be a more important mechanism of impaired microvascular perfusion in severe knowlesi malaria [25]. The high parasitemia associated with P. knowlesi may contribute to this microvascular sludging.

In the present study, some of the retinal changes that did occur among patients with knowlesi malaria seemed to relate to defects in the RPE. Hard drusen were present in most patients with knowlesi malaria, as found in previous population studies using fundus photography [26]. Commonly regarded as a normal variant increasing with age, drusen are accumulations of waste products in the RPE, which, if sufficiently large, can cause death of the RPE cells and overlying retina, resulting in age-related macular degeneration. It is likely that the frequency of drusen and RPE defects among the patients with knowlesi malaria in our study relates to their older age; however, because neither healthy controls nor patients with falciparum or vivax malaria were included, an association with knowlesi malaria cannot be excluded.

Retinal hemorrhages were relatively common in patients with severe and nonsevere knowlesi malaria; however, they were all classified as mild (1–5 hemorrhages in ≥1 eye) according to criteria developed to describe falciparum malarial retinopathy [20] and were white-centered in only 1 patient with nonsevere knowlesi malaria. In adults with severe falciparum malaria, moderate-severe retinal hemorrhages were seen in 9 of 101 (9%) with cerebral malaria and 6 of 63 (10%) with noncerebral malaria, with white-centered hemorrhages thought to result from intraluminal fibrin deposition, common in both groups (32% and 20%, respectively). Thrombocytopenia is near-universal in knowlesi malaria [4, 27–29], and a lower platelet count was associated with the presence of hemorrhages. The 2 patients with multiple hemorrhages in the present study had severe thrombocytopenia, which may have been contributory.

Retinal vessel whitening was seen in only 1 patient in our study, a patient with nonsevere knowlesi malaria, and the normal findings at fluorescein angiography and lack of acute inflammatory changes suggested that it was probably related to an old event. In falciparum malaria, retinal vessel whitening is prominent in African children [6] but absent in Bangladeshi adults [8, 9]. It is thought to be due to obstruction of the vessel segment by dehemoglobinized parasitized erythrocytes [7].

One limitation of the current study was that it included neither healthy controls nor patients with falciparum or vivax malaria. This limited our ability to assess the specificity of findings for knowlesi malaria, and it was also not possible to determine the background rate of retinal lesions in the population.

In conclusion, retinal lesions in patients with knowlesi malaria are common but are mild, nonspecific, and unrelated to disease severity. This contrasts with the greater severity and specificity of retinopathy reported in severe falciparum malaria, including in adults without coma. In conjunction with the absence of any report to date of coma definitively associated with severe knowlesi malaria, this suggests that pathophysiological mechanisms differ between these 2 species. Although central nervous system microvascular accumulation of parasitized red blood cells has been reported in fatal knowlesi malaria, the mechanisms and/or consequences probably differ from the endothelial cytoadherence-mediated sequestration characteristic of P. falciparum.

Notes

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