Clinical-Histopathological Correlation of the Abnormal Retinal Vessels in Cerebral Malaria

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Background: Clinically abnormal retinal vessels unique to cerebral malaria have previously been shown to be associated with a poor outcome in African children. There have been no studies of the histopathological correlates of these vessels.

Design: This is a descriptive study of the clinical-histopathological correlates of the retinal vessels of 11 children who died with cerebral malaria.

Results: The retinal vessels in children with cerebral malaria contained many parasitized red blood cells; these cells tended to cluster at the periphery of vessels or, in the case of capillaries, to fill the vessel. Those with late-stage parasites had markedly reduced amounts of hemoglobin. The pattern of dehemoglobinization corresponds to the pattern of clinically abnormal vessels.

Conclusions: The sequestration of late-stage parasitized red blood cells with reduced amounts of hemoglobin accounts for the unique white and pale orange retinal vessels seen in cerebral malaria. Clinical examination of these “marked” vessels offers a method to monitor a basic pathophysiological process of cerebral malaria in vivo.

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PATIENTS AND METHODS

This work took place within the context of a larger study of the clinical-histopathological correlations of cerebral malaria in children. In 1996 to 1998, children who were brought to the Queen Elizabeth Central Hospital in Blantyre, Malawi, with coma or severe malaria were admitted to the Malaria Research Project and Wellcome Trust Laboratories for further diagnosis and treatment. One of 7 ophthalmologists (S.L., J.G., B.H., J.L., J.L., A.M., or K.W.) examined the child on admission by indirect ophthalmoscopy, classified the retinal vessels as normal or abnormal, and took photographs if the clinical status of the patient permitted. In the event of death and autopsy, the eyes were fixed in 10% formalin and transported to Vancouver, British Columbia, for study. These studies were approved by the Health Science Research Committee in Malawi and performed only with the permission of the parent or guardian of the child.

After fixation, the eyes were processed into paraffin, sectioned at 3 µm, and stained with hematoxylin-eosin to assess the stage of the parasite and with diaminobenzidine to stain for hemoglobin peroxidase. The diaminobenzidine stain was prepared by diluting 30 mg of diaminobenzidine (Sigma-Aldrich Corp, St Louis, Mo) in 25 mL of Tris-buffered saline, 0.05 mol/L, pH 7.6, and adding 27 µL of 50% hydrogen peroxide just before use. The slides were deparaffinized, stained in this solution for 30 minutes at room temperature, dehydrated in graded alcohols, and coverslipped. The amount of hemoglobin in the parasitized red blood cells was assessed semiquantitatively from 1+ to 3+ in comparison with unparasitized red blood cells, and without knowledge of the findings on clinical examination.

Flat sections of the retina from a quadrant of the globe were dissected away from the retinal pigment epithelium, washed in water, and stained in the diaminobenzidine solution for 30 minutes, then picked up on a glass slide and allowed to air-dry overnight before coverslipping.

Flat sections showed an uneven linear distribution of parasitized red blood cells (Figure 7). Segments containing late-stage parasitized red blood cells could be found adjacent to segments containing very few parasitized red blood cells and more visible hemoglobin. There was a tendency for the dehemoglobinized cells to sequester at branch points.

COMMENT

When normal retinal vessels are examined by ophthalmoscopy, it is the column of blood that is viewed; the vessel wall is transparent. Although some inflammatory conditions cause white vessels because of sheathing, this has a different clinical appearance than the white vessels in cerebral malaria and it does not affect capillaries. We reasoned that the abnormal appearance of the vessels in cerebral malaria could result from either loss of transparency of the vessel wall or a change in color of the vessel contents. Our studies indicate that the latter phenomenon is likely to be the cause.

The distinct abnormalities in the hemoglobin content of some red blood cells in our cases is consistent with the fact that malaria parasites metabolize hemoglobin during their life cycle. The overall distribution of dehemoglobinization in vessels is consistent with the pattern of abnormal vessels we see clinically; both the flat sections and the cross sections show the patchy nature of the distribution. The large arcade vessels are not abnormal either clinically or histologically. We were not able to pinpoint individual abnormal vessels on clinical examination.
for histopathological examination; however, the Table shows that there is a reasonable association between the histopathological findings of decreased hemoglobinization and the clinical finding of abnormal vessels. Histopathological examination shows abnormal hemoglobin content in some cases that were judged normal on clinical examination, but this might be explained in 2 ways: first, dehemoglobinization occurs in various degrees of severity and may be recognized clinically only if it passes some histopathological threshold of severity. Second, we now recognize that abnormal vessels are present more often than reported, but their clinical detection depends on the degree of magnification available to the observer, the experience of the observer in recognizing these unusual vessels, and the ability of the examiner to examine all quadrants of the retina out to the periphery. (The latter can be a problem because of deviation of the eyes and limitation of head position caused by indwelling central venous catheters.) We have been surprised to find abnormal vessels, especially at the capillary level, in photographs of retinas that had been judged by experienced observers to be normal on ophthalmoscopy. Individual variations in fundus pigment might also influence the ease with which the abnormal vessels may be recognized during life.

Besides the absence of hemoglobin in the parasitized cells, there is the presence of hemozoin pigment and the body of the parasite, both of which appear dark on

<table>
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<tr>
<th>Patient No.</th>
<th>Postmortem Interval, h</th>
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<th>Hemoglobinization*</th>
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</table>

* 1 indicates marked dehemoglobinization; 2, some dehemoglobinization, and 3, normal hemoglobinization.

Figure 4. Case 5. Top, Photomicrograph of a larger retinal vessel shows a central core of well-hemoglobinized red blood cells surrounded by parasitized red blood cells containing schizont stages and little hemoglobin. This would give a vessel an orange appearance clinically (hematoxylin-eosin, original magnification ×200). Bottom, Photomicrograph shows the central core of well-hemoglobinized red blood cells surrounded by parasitized red blood cells containing little hemoglobin (diaminobenzidine, original magnification ×200).

Figure 5. Case 21. Photomicrograph of retinal vessel shows peripheral localization of parasitized red blood cells with normal hemoglobin similar to the unparasitized red blood cells centrally. The parasites are young-mid trophozoite stage (hematoxylin-eosin, original magnification ×200).
This occurs when late-stage parasitized red blood cells sequester in the deep vascular beds of many organs, particularly the brain. There is no inflammatory sheathing of any vessels. Although these vessel abnormalities were referred to as “obstructions” when first described, they are not accompanied by the features of obstructed blood flow that characterize retinal branch vein and artery occlusions.

Many children with malaria are severely anemic, but the retinal findings described in anemia are limited to hemorrhages, cotton-wool spots, and vessel tortuosity; abnormal color of vessels is not a typical feature. Changes in retinal vessels caused by anemia should be generalized, rather than patchy, and we do not think anemia is an explanation for the abnormal vessels in cerebral malaria. We have studied (clinically and histologically) the retinas of children who died with malarial anemia, and they do not resemble those of children with cerebral malaria.

Our light microscopic studies show no abnormalities of the endothelial cells or of the surrounding glial elements that might explain opacification of the vessel wall. There is no inflammatory sheathing of any vessels.

A consistent pathophysiological feature of cerebral malaria is the sequestration of parasitized red blood cells in the deep vascular beds of many organs, particularly the brain. This occurs when late-stage parasitized red blood cells adhere to the endothelium via “knobs” demonstrable by electron microscopy. Such knobs were demonstrated on the parasitized red blood cells in retinal vessels in a previous case report, but this contained no clinical information about the vessel appearance. We have observed that abnormal vessels remain for many hours to days before reverting to normal, which indicates that the abnormal red blood cells are “stuck” in place; we believe that we are viewing sequestration of parasitized red blood cells in the retinal vessels caused by cytoadherence.

The dynamics of blood flow through the abnormal vessels and the oxygenation of surrounding tissue is not clear. Although these vessel abnormalities were referred to as “obstructions” when first described, they are not accompanied by the features of obstructed blood flow that characterize retinal branch vein and artery occlusions. Investigators performing fluorescein angiography for the main purpose of studying another abnormal retinal feature in cerebral malaria noted the abnormally colored vessels (referred to as “obstructions” and “sheathing”), but their studies did not specifically include angiographic correlations of these (Simon P. Harding, FRCOphth, written communication, December 10, 1999). They did report, however, that the blood-retinal barrier and retinal vascular flow remained substantially normal in 11 children with cerebral malaria. Despite the apparent dehemoglobinization of many red blood cells, we do not find the usual clinical evidence (cotton-wool spots, edema, retinal necrosis) or histological evidence (cytoid bodies, necrosis) of ischemic or hypoxic damage in the retina surrounding the vessels. The role of hypoxia in the pathophysiological process of cerebral malaria is controversial.

In summary, we have shown that the retinal vessels of children who died with cerebral malaria contain many parasitized red blood cells, that the cells with late-stage parasites have markedly reduced amounts of hemoglobin, and that the pattern of dehemoglobinization is consistent with the clinical findings. We believe that sequestration of dehemoglobinized red blood cells is the histological correlate of the unique white and orange abnormal retinal vessels in cerebral malaria. Observations of these vessels could be useful in improving our understanding of the pathophysiological mechanisms of cerebral malaria and perhaps in monitoring new drugs that target the parasites’ ability to metabolize hemoglobin or capacity to cytoadhere to vascular endothelium.

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REFERENCES


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**Ophthalmological Numismatics**

**A look at the past . . .**

Joseph George Beer, 1763-1821, became the first professor of ophthalmology at the University of Vienna, Vienna, Austria, in 1816, as well as the first chair of an independent academic ophthalmology department anywhere in the world. His department was the most famous ophthalmological school of the 19th century. Beer devised the iridectomy and was the first to describe acute glaucoma.

This medal was struck in his memory after his death in 1821. It was engraved by J. Theuring and issued in tin. The obverse (Figure 1) depicts Beer’s bust facing ahead and slightly right; the reverse (Figure 2), a 10-line inscription.

Courtesy of: Jay M. Galst, MD, 30 E 60th St, New York, NY 10022.