Histologic Artifacts of Autolytic Müller Cell Foot Process Swelling in Postmortem Examination of Infant Eyes Potential Pitfall in the Evaluation of Traumatic Retinal Hemorrhages

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Head trauma is often associated with retinal hemorrhages. Clinical analysis of retinal hemorrhages is important in evaluating the differential diagnosis of accidental and nonaccidental pediatric head trauma. In fatal cases, pathologic diagnoses of the age, location, and severity of retinal hemorrhages are important factors in determining the manner of death. Histologic artifacts that mimic retinal hemorrhage could present pitfalls with profound legal implications. We have noted an artifact of Müller cell foot process swelling in the postmortem examination of infant eyes that can strikingly mimic nerve fiber layer (NFL) hemorrhages. We evaluated the postmortem examinations of infant eyes at our institution from the past 2½ years, from April 24, 2012, through November 11, 2014, to glean greater insight into this artifact. This study received a waiver from institutional review board review from the Stanford Research Compliance Office.

IMPORTANCE Retinal hemorrhages are an important sequela of fatal head trauma. The accurate pathologic diagnosis of retinal hemorrhages has critical implications for determination of the manner of death.

OBSERVATIONS We describe an autolytic postmortem histologic artifact of eosinophilic Müller cell foot process swelling that mimics a nerve fiber layer hemorrhage. From April 24, 2012, through November 11, 2014, we conducted postmortem examination of the eyes of 23 infants and children who were referred to our institution for possible nonaccidental head trauma. A focal artifact of Müller cell foot process swelling was identified in most patients (16 of 23) up to 4 years of age. Three infants, all of whom were younger than 3 months, demonstrated diffusely swollen Müller cell foot processes with intensely eosinophilic cytoplasm that mimicked erythrocytes of nerve fiber layer hemorrhages. The difference in the mean age between patients with diffuse eosinophilic artifacts (1.7 months) and patients with only a multifocal, focal, or absent artifact (13.3 months) was 11.6 months (95% CI, 6.5-16.7 months). Glycophorin C immunohistochemical analysis was useful to differentiate this artifact from nerve fiber layer hemorrhage.

CONCLUSIONS AND RELEVANCE Our case review demonstrates an artifact of eosinophilic Müller cell foot processes swelling in postmortem examination of young infant eyes, a potential pitfall in the diagnosis of retinal hemorrhages. Our findings have important implications for the diagnosis of retinal hemorrhages in potential cases of nonaccidental head injury.
Figure 1. Müller Cell Foot Process Swelling in the Normal Eyes of a 7-Week-Old Infant

(Figure 1A). The swollen Müller cell foot processes were intensely eosinophilic and round, with an approximate diameter of 6 μm, similar to erythrocytes. The artifact was more prominent in the peripheral retina than in the posterior retina. Findings from a periodic acid-Schiff stain were negative for both intravascular erythrocytes and swollen Müller cell foot processes (Figure 1B). Findings from a human glycophorin C immunostain, using a mouse monoclonal antibody (clone Ret40f; Dako No., M0820), differentiated between the intravascular erythrocytes and the swollen Müller cell foot processes (Figure 1C). Findings from immunostains for glial intermediate filaments (glial fibrillary acidic protein and vimentin) showed no immunoreactivity in the swollen Müller cell foot processes (Figure 1D and E).

Case 2

The retinas of a 7-week-old girl with multifocal retinal hemorrhages demonstrated areas of intact internal limiting membrane and Müller cell foot processes (Figure 2A) and a focal area with NFL hemorrhage adjacent to the swollen Müller cell foot processes (Figure 2B). Findings from glycophorin C immunohistochemical analysis similarly differentiated the erythrocytes of the NFL hemorrhages from the swollen Müller cell foot processes (Figure 2C). This case demonstrates the potential pitfall of misinterpreting the number and/or distribution of retinal hemorrhages when the Müller cell foot process artifact mimics NFL hemorrhages.

Review of Archived Cases

To better understand the prevalence of histologic artifacts of Müller cell foot process swelling, we examined 23 consecutive pediatric forensic ophthalmic pathology cases referred to our institution during the past 32 months, from April 24, 2012, through November 11, 2014. The Table demonstrates that in our case series, artifacts of Müller cell foot process swelling were most prevalent and severe in the youngest infants, especially those younger than 3 months. The artifact is common in older
infants and children but tends to be more focal in these individuals. The difference in the mean age between patients with diffuse artifacts (1.7 months) and patients with only a multifocal, focal, or absent artifact (13.3 months) was 11.6 months (95% CI, 6.5-16.7 months). Furthermore, swollen Müller cell foot processes in very young infants are more intensely eosinophilic and resemble the color of erythrocytes, whereas in older children, the artifactual dilatations are paler and do not resemble the hue of erythrocytes. Diffuse or multifocal artifacts of Müller cell foot process swelling were found in cases from 4 of 5 consulting institutions. Müller cell foot process swelling artifacts were seen in patients with and without retinal hemorrhages. However, in our patients with retinal hemorrhages, blood was always noted in the outer layers of the retina in addition to the NFL.

Discussion

Retinal hemorrhages are a key sequela of pediatric head trauma. Severe bilateral hemorrhages that involve the peripheral retina are much more commonly seen in nonaccidental head trauma and are rarely seen in accidental trauma, birth trauma, or as a result of preexisting disease. The accurate postmortem pathologic diagnosis of retinal hemorrhages is therefore an important factor in building legal cases against child abusers and for preventing inaccurate charges against innocent caregivers.

Here, we describe an artifact of Müller cell foot process swelling that can closely mimic NFL hemorrhages and thus represents a potential pitfall in the postmortem diagnosis of retinal hemorrhages. The cause of the artifact is uncertain but probably relates to postmortem detachment of the internal limiting membrane and secondary injury to Müller cell footplates. Consistent with this possibility, Müller cell foot process swelling is not seen in regions where the internal limiting membrane is intact. The lack of glial fibrillary acidic protein and vimentin immunoreactivity in one case with swollen Müller cell foot processes suggests that intermediate filament deficiency might underlie greater plasma membrane compliance and a propensity for postmortem swelling in infants younger than 3 months. The observation that the artifact is more severe in the peripheral retina is probably related to its greater density of attachment plaques than the posterior retina.4 The potential misinterpretation of peripheral NFL hemorrhages in cases with true retinal hemorrhages only in the posterior retina could skew the interpretation of the probability of accidental or nonaccidental injury. The artifact was not unique to the postmortem handling method of 1 particular consulting institution. The artifact was seen in patients with and without true retinal hemorrhages, presenting the possibility that the artifact could confound the interpretation of the presence, number, or spatial distribution of retinal hemorrhages.

Retinal hemorrhages in cases of nonaccidental head injury most commonly involve the NFL but usually also involve outer layers of the retina.5 Thus, the absence of true hemorrhages in the outer retinal layers can potentially aid in the recognition of artifacts of Müller cell foot process swelling. Other features that can help differentiate artifacts from true NFL hemorrhages are the absence of grossly identifiable retinal hemorrhages and the lack of apparent biconcave shape in swollen Müller cell foot processes. True NFL hemorrhages and artifacts can appear similar on hematoxylin-eosin staining, and
the appearance of extravasated erythrocytes in the NFL and artifact of Müller cell foot process swelling is similar in histologic sections stained with periodic acid–Schiff. We found that glycophorin C immunohistochemical analysis readily differentiates between Müller cell foot process swelling and NFL hemorrhages and can serve as a valuable tool in difficult cases.

Conclusions

Here we describe artifacts of postmortem Müller cell foot process swelling that morphologically mimicked peripheral NFL hemorrhages in the peripheral retinas of infant eyes. In our case series, diffuse Müller cell foot process swelling artifacts with prominent eosinophilic cytoplasm that most closely mimicked NFL hemorrhages were seen only in infants younger than 3 months. However, owing to the small number of patients in our study, the precise age at which the extensive and potentially deceptive artifact ceases to appear is difficult to determine. When encountered, glycophorin C immunohistochemical analysis can aid in differentiating artifactual Müller cell foot process swelling from true NFL hemorrhage. Recognition of this artifact is important to prevent misdiagnosis of retinal hemorrhages in pediatric autopsies.

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REFERENCES