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Clinical histopathology of intrachoroidal splitting in open-globe injury: A retrospective case series of four patients

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Abstract:

PURPOSE: The purpose of this study is to observe the characteristics of intrachoroidal splitting (ICS) associated with choroidal detachment due to open-globe injury.

METHODS: A retrospective, observational case series study of four eyes diagnosed with choroidal detachment exhibited ICS. The *in vivo* pathologic changes of the ICS were recorded during surgery. Four specimens were obtained from the inner part of the ICS region. One specimen was stained with hematoxylin and eosin; one specimen was examined under scanning electron microscopy, and the other two specimens were examined under transmission electron microscopy.

RESULTS: All four patients presented with vortex vein rupture associated with large-scaled mid-peripheral ICS. The histopathologic observations indicated that ICS occurred between the medium-sized and large-sized choroidal vessel layers. Large vascular indentations and medium-sized choroidal vessels were observed on the inner part of the split interface. Postoperative outcomes of the four patients were poor. Vision in all four patients was no light perception before or after surgery. Three eyes became atrophic with silicon oil tamponade and band keratopathy, one eye was eventually enucleated for cosmetic reason.

CONCLUSION: Intrachoroidal tissue has the potential to split during trauma. The outcome of ICS with choroidal detachment in open-globe injury was poor.

Keywords:

Histopathology, intrachoroidal splitting, ocular trauma

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Introduction

Many morphologic studies of the choroid using enhanced depth imaging-optical coherence tomography (EDI-OCT) have been published in the past 5 years.[1-5] Intra-choroidal splitting (ICS) has been reported in some eye diseases based on EDI-OCT examination.[6-8] Natural ICS is also observed in avian, Corvus corone, and Asio otus eyes.[9]

Choroidal detachment is commonly observed in severe open-globe injury with Zone II and Zone III trauma (involving the ciliary body and choroid), and it usually leads to poor prognosis with low visual acuity.[10] Currently, there are no effective therapies established that cure traumatic choroidal detachment in open-globe injury. While surgically treating traumatic retinal detachment with choroidal detachment, we observed ICS and vortex vein rupture under the surgical microscope [Figure 1a and b, Video 1]. Part of the inner layer of the ICS region could not be reattached to the underlying tissue during surgery due to massive intrachoroidal hemorrhage and contraction of the choroidal tissue components. Hence, we excised the inner

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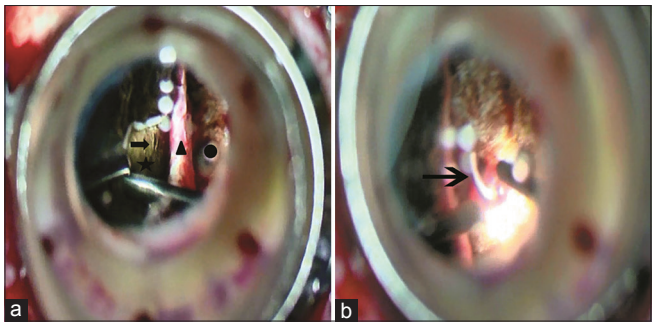

Figure 1: (a) Temporal keratoprosthesis was applied in this patient. The brown sheet on the left (★) was the inner part of the choroidal splitting, which includes the retinal pigment epithelium layer, Bruch's membrane, the choriocapillaris, and medium-sized choroidal vessels. Two parallel medium-sized choroidal vessels crawling along the outer splitting interface are indicated by the arrow (▲). The sheet in red (→) was the outer part of the splitting choroid with hemorrhage, and this layer detached from the sclera. The right side of the peripheral region with spotted dark brown color (●) was the lamina fusca (b) The arrow indicates the ruptured vortex vein floating in the vitreous cavity while its root was firmly fixed on the sclera. The brownish interface around the vortex vein is the lamina fusca. The left side of the ruptured vortex vein is the outer layer of the split choroid detached from the lamina fusca while the inner layer of the split choroid is not shown in the picture

Figure 2: The inner part of the split choroidal sheet was obtained from the retinal pigment epithelium-denuded region in Case 3, and the specimen was placed on a cellulose acetate membrane. The parallel lines on the specimen are indentations of the large-sized choroidal vessel. The brown color of the specimen is due to choroidal melanocytes within the specimen rather than retinal pigment epithelium cells on top

parts of the ICS sheet to reattach the remediable choroid and retina. We performed histopathologic analysis of the excised ICS specimens to further understand the pathogenesis of ICS.

Methods

We enrolled 165 patients with open-globe injury associated with choroidal detachment in the eye injury vitrectomy study.[11] All patients received a closure suture within 12 h of the injury. Among the 165 cases, 4 were diagnosed with ICS during vitrectomy surgery by microscopic observation. A second vitrectomy surgery was performed 12.9 ± 4.3 days after the primary injury. The data of the 4 patients are listed in Table 1. All four patients received 20-G vitrectomy, and a temporary keratoprosthesis was applied to three patients during the vitrectomy surgery due to corneal blood staining. The specimens of the inner part of the ICS were removed using micro-forceps through a 20 G incision for histopathologic analysis. All specimens were placed on a cellulose acetate membrane immediately after removal from the eye [Figure 2].

Light microscopy analysis

Specimen 1 was fixed in 10% neutrally buffered formalin and embedded in paraffin for light microscopy. The specimen was serially sectioned in 5-um thick slices and stained with hematoxylin and eosin (HE) for light microscopy observation.

Transmission electron microscopy

Specimens two and four were fixed in 3% glutaraldehyde and postfixed with 1% osmium tetroxide. They were processed for ultrathin sectioning. The sections were stained with uranium acetate and lead citrate, and ultrastructural properties of the ICS were analyzed by transmission electron microscopy (JEM-1230; JEOL).

Scanning electron microscopy

Specimen three was fixed in half-strength modified Karnovsky fixative. After rinsing in phosphate-buffered saline, the specimen was dehydrated in a graded series of ethanol, critical point dried, and sputter-coated with 20 nm gold and palladium. The sample then was examined with a scanning electron microscope (JSM-5600 LV; JEOL, Tokyo, Japan).

All patients and their family members provided written informed, special-designed consent regarding the use of the tissue in a research study, and they were informed as to the potential risks and possible poor outcomes of the surgery. This study was approved by the institutional review board (IRB) of the Peking University Health and Science Center (reference no. IRB 00001052–09070) as well as the IRB of Peking University Third Hospital (reference no. IRB 2014034), and conducted in accordance with the tenets of the declaration of Helsinki.

Results

Pre- and post-operative clinical features of the four intrachoroidal splitting cases

The visual acuity of all four patients was no light perception before or after the surgery. Mean postoperative intraocular pressure was 4.5 mmHg. All four patients had band keratopathy at the 6-month follow-up. Case 3 underwent enucleation surgery in a local hospital for cosmetic reasons 9 months after the vitrectomy. The other three cases had silicon oil-sustained eyes.

Table 1: Clinic characters of 4 patients

	Preoperative					Intraoperative				Postoperative				
Gender	Age	Trauma nature	Extent of the injury	VA	Cornea blood staining	Ciliary detachment	Choroidal schisis and detachment	Vortex vein rupture	Temporary keratoprosthesis	VA	IOP	Choroidal detachment	Band-keratopathy	Consequence	Histopathology examination
Male	28	Blunt trauma	Zone I and II and III	NLP	N	Y	Y	Y	Y	NLP	5	Y	Y	Silicon oil tamponade	HE staining
Male	26	Blunt trauma	Zone I and II and III	NLP	N	Y	Y	Y	N	NLP	4	Y	Y	Enucleation 6 months after the vitrectomy	Trans-electron microscope
Male	15	Blunt trauma	Zone I and II and III	NLP	Y	Y	Y	Y	Y	NLP	4	Y	Y	Silicon oil tamponade	Scanning electron microscope
Female	61	Sharp trauma	Zone I and II and III	NLP	N	Y	Y	Y	Y	NLP	5	Y	Y	Silicon oil tamponade	Trans-electron microscope

IOP=Intraocular pressure, VA=Visual acuity, NLP=No light perception, HE=Hematoxylin and eosin, N=No, Y=Yes

Intraoperative observations

Vortex vein rupture along with opening choroidal injury was observed in all four cases during surgery. Cases 1 and 4 had ora serrata dialysis was observed by peripheral external indentation. After debriding a massive subretinal, intrachoroidal, and suprachoroidal hemorrhage, large-scaled ICS was observed with the midperipheral choroidal region invovled, next to the ruptured vortex vein, and suprachoroidal space was connecting to the vitreous cavity in all these opening choroidal injury eyes. The ICS separated the choroid into two parts, the retinal pigment epithelium (RPE)-Bruch's membrane complex with underlying tissue comprised the inner layer of the ICS while the rest of the detached choroid comprised the outer layer of the ICS [Figure 1a]. Brownish lamina fusca was observed in the choroidal detachment region [Figure 1b]. Large choroidal vessel indentations were observed on the inner part of the ICS layer when the specimen was placed on the cellulose acetate membrane [Figure 2].

Histopathology observation of the inner layer of intrachoroidal splitting

A specimen from Case 1 was examined by HE staining [Figure 3]. The inner part of the ICS included the RPE layer, Bruch's membrane, and medium-sized choroidal vessels surrounded by melanin. Many monocytes accumulated within the lumen of medium-sized choroidal vessels.

Specimens from Cases 2 and 4 were examined by transmission electron microscopy. The RPE cells were either absent [Figure 4b] or filled with multiple vacuoles [Figure 4a]. Bruch's membrane of the older patient [Figure 4b] was thicker than that of, the younger patient [Figure 4a]. Macrophages filled with melanin were observed in both specimens. Medium-sized choroidal vessels with intact vascular walls were observed in both cases [Figure 4a and b] while large-sized choroidal vessels were absent in both specimens.

The specimen from Case 3 was examined by scanning electron microscopy. The two sides of the specimen from the inner part of the ICS were observed. A large RPE denuded area exposing the smooth surface of the Bruch's membrane was observed [Figure 5a]. The other side of the ICS specimen split between the medium sized choroidal vessel layer and the large-sized choroidal vessel layer ,meanwhile multiple large-sized choroidal vessel indentations with one ruptured choroidal vessel aside were observed on the interface[Figure 5b and c].

Discussion

The findings of the present study revealed that traumatic ICS associated with choroidal detachment can occur in

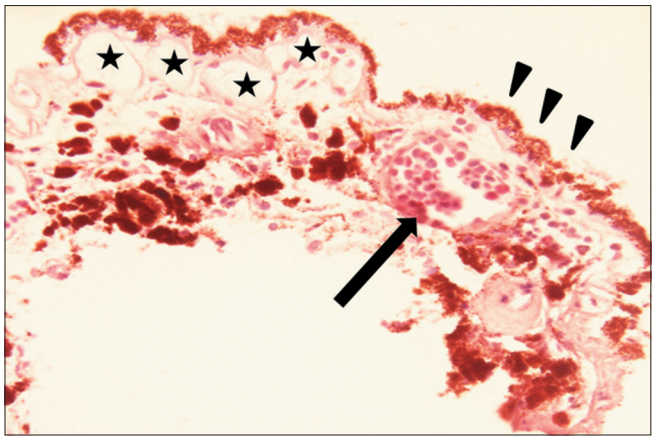

Figure 3: Arrowheads indicate the RPE layer on the top, and the choriocapillaris (★) beneath the RPE layer (HE staining, ×40). A medium-sized choroidal vessel (→) was filled with tens of monocytes. The dark brown cells on the bottom are macrophages with melanin

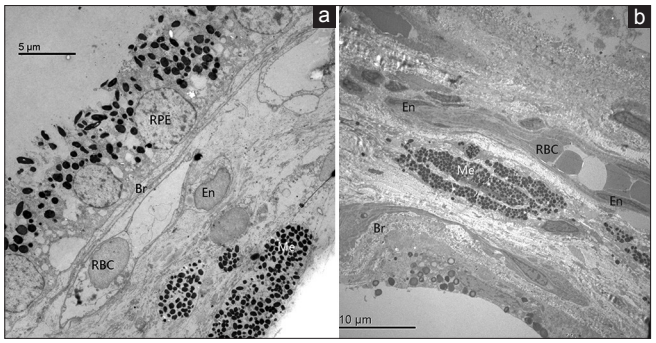

Figure 4: (a) The retinal pigment epithelium cells on the Bruch's membrane have multiple intracellular vacuoles, and a choriocapillaris with red blood cells right beneath the Bruch's membrane. A choroidal melanocyte with abundant pigment particles is observed outside the choriocapillaris (Transmission electron microscopy. Br: Bruch's membrane; En: Endothelium; Me: Melanocyte; RBC: Red blood cell) (b) No retinal pigment epithelium cells were observed on the Bruch's membrane where only some pigment particles remained. Spindle-like endothelium of the choriocapillaris was observed right under Bruch's membrane. An intact medium-size choroidal vessel with vascular endothelium cells attached is filled with red blood cells. Between Bruch's membrane and the choroidal vessels are a choroidal melanocyte that is filled with dark granules (Transmission electron microscopy. Br: Bruch's membrane; En: Endothelium; Me: Melanocyte; RBC: Red blood cell)

severe open-globe injury, and the condition often results in poor visual outcome. Analysis of the histopathology of ICS specimens led us to conclude that the splitting occurred between the medium-sized choroidal vessel layer and the large-sized choroidal vessel layer. To the best of our knowledge, this is the first report describing traumatic ICS in open-globe injury through histopathologic analysis.

Massive choroidal damage often leads to direct retinal injury as well as visual dysfunction.[12-14] In this study, all four cases suffered severe open-globe injuries associated with both retinal injury and choroidal detachment associated with ICS, and the visual function could not be saved. Surgical reattachment of the choroid was very difficult in these cases because: (1) the choroidal tissue and blood vessels contained abundant collagen and elastic fibers, which constricted and shortened the choroidal wound; (2) the ruptured vortex vein enlarged the choroidal detachment; and (3) silicon oil easily enters the suprachoroidal space after surgery, which made the choroid reattachment much more difficult. Therefore, all four cases had a poor outcome, including one enucleation case and three silicon oil-sustained eyes with band keratopathy.

Trauma led directly to the occurrence of ICS along with the rupture of multiple choroidal vessels. The ruptured vortex vein, which is under hypotensive venous pressure compared to arterial pressure, could also cause massive hemorrhage leading to ICS under open-globe injury due to higher venous pressure compared to atmospheric pressure. The plane between the medium-sized choroidal vessels and the large-sized choroidal vessels might be the weakest and thus not stretch under stress or strain during trauma and healing processes. The causes of ICS occurrence during trauma might be relate to factors other than trauma.

The different choroidal vessel layers develop at different points in embryogenesis, which might also contribute to the occurrence of ICS. In the embryo stage, the short posterior ciliary arteries are continuous with the choroidal capillaries by the 3rd month. By the third and 4th months, the choriocapillaris is well developed, and much of its branching network is present. Medium-sized vessels begin forming between the large vessel layer and the choriocapillaris by the 5th month.[15] Therefore, the delayed development of the medium-sized choroidal vessels might produce a potential space with the large-sized choroidal vessel layer. Unlike the split between the choriocapillaris and medium-sized vessel layer achieved by surgical instruments,[16] the ICS described in this study occurred spontaneously during the trauma process without any surgical manipulation.

Choroidal hypoperfusion and ischemia might also cause the occurrence of ICS. Compromise of the short posterior ciliary artery branch is inevitable because the trauma results in choroidal hypoperfusion. Over time, the ischemic choroid tissue may start to curl due to contraction of the collagen fibers, and the unbalanced tractional force might cause the ICS. The smooth layer of the splitting observed by scanning electron microscopy also demonstrated that the ICS process might happen gradually before vitrectomy surgery, which was performed on average about 13 days after the injury.

In highly myopic eyes, the pathologic process of ICS differs from that in open-globe injury although the split layers are the same between the two groups.[17] In highly myopic eyes, choroid tissue around the optic disc becomes thinner as the axial expansion of the eyeball progresses.

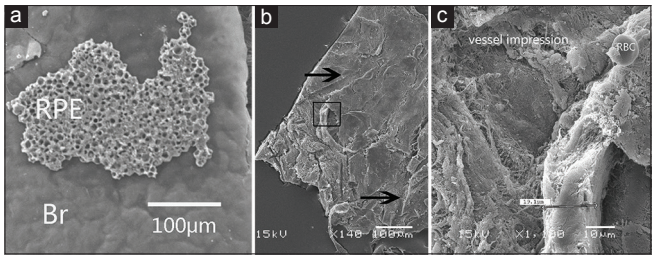

Figure 5: (a) Scanning electron microscopy examination revealed one side of the inner part of the choroidal split. The region covered by the retinal pigment epithelium cells shows multiple interdigitations that were formed by numerous microvilli on top of the retinal pigment epithelium cells while the retinal pigment epithelium-denuded region appears as a smooth interface of Bruch's membrane. Br: Bruch's membrane; retinal pigment epithelium: Retinal pigment epithelium. (b) One side of the intrachoroidal splitting observed by scanning electron microscopy showed the outer intrachoroidal interface. The two arrows point to large-sized choroidal vessel indentations on the surface. One ruptured choroidal vessel tip is observed (▭). (c) Scanning electron microscopy revealed that the diameter of the ruptured choroidal vessel was 19.1 μm, as large as the diameter of the nearby choroidal vessel indentation. A red blood cell is observed near the tip of the ruptured large-sized choroidal vessel

This mechanical traction causes choroidal microtears around the optic nerve allowing vitreous to flow into the intrachoroidal cavitation through the microtear and separate the medium-sized choroidal vessel layer and large-sized choroidal vessel layer. The ICS process in highly myopic eyes is much slower than in traumatic ICS, which occurred within 13 days in the present study. The ICS region around the optic disc in highly myopic eyes is much smaller than the large choroidal detachment associated with ICS in open globe-injury. The incidence of ICS in highly myopic eyes is 1%,[7] while in our study, the incidence of ICS in open-globe injury was 2.42%.

ICS produced by surgical manipulation without complications would potentially be very beneficial for many reasons: (1) a drug delivery tube could be inserted within the choroid space through the ICS rather than into the suprachoroidal space,[18] making treatment more efficient; (2) in autologous RPE transplantation surgery,[16,19] a graft of RPE-Bruch's membrane complex with medium-sized choroidal vessels attached could be performed through the ICS region, and a graft with the underlying vessels attached might increase RPE cell survival.

Conclusion

This study evaluated ICS through histopathologic analysis of four specimens obtained from four patients with open-globe injuries. Future research focused on the pathogenesis of ICS during trauma may contribute to the development of an appropriate treatment strategy.

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Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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