Clinicopathological findings in refractory diabetic macular edema: A case report

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Abstract. The present study describes the case of a patient with refractory diabetic cystoid macular edema who underwent vitrectomy with *en bloc* removal of the cystoid lesion component. The current study also performed histopathological and immunohistochemical analyses of the cystoid lesion component to assess fibrin/fibrinogen and advanced glycation end-products (AGEs) immunoreactivity. A 69-year-old Japanese man presented with visual loss in the left eye due to residual cystoid macular edema (CME) refractory to anti-vascular endothelial growth factor therapy. Best-corrected visual acuity was 1.2 in the right eye (OD) and 0.5 in the left eye (OS). Fundus examination showed dot hemorrhages and hard exudates in the peri-macular region with pan-retinal photocoagulation scars in both eye. Swept-source optical coherence tomography revealed CME with slight hyperreflectivity in the cyst OS. A total of 3 months after the initial visit, pars plana vitrectomy was performed, and the translucent solidified component within the cystoid lesion was isolated. Histopathologically, the excised component was elliptical in shape, measuring 0.7x0.4 mm and exhibited homogeneous eosinophilic material without cellular components. No membranous structure was observed surrounding the component. Immunohistochemistry demonstrated that the tissue was positive for fibrin/fibrinogen and weakly positive for AGEs, but was negative for glial fibrillary acidic protein, type 1 collagen and receptor for AGEs. To the best of our knowledge, the present case report is the first to histopathologically examine the contents of refractory CME, and to immunohistochemically demonstrate that fibrin in diabetic CME may be post-translationally modified by AGEs. These results suggested that fibrin in CME may escape degradation by plasmin due to post-translational modifications.

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Introduction

Diabetic macular edema is a clinical condition that causes severe visual impairment. The vision of patients with diabetic retinopathy (DR) can be maintained through various topical treatments, including intravitreal administration of anti-vascular endothelial growth factor (VEGF) drugs, retinal photocoagulation, sub-Tenon injection of triamcinolone acetonide (STTA) or intravitreal injection of triamcinolone acetonide, and pars plana vitrectomy (1). However, some DR patients may be resistant to standard treatment, leading to refractory cystoid macular edema (CME). Vitrectomy with incision of cystoid lesions has been reported as an alternative treatment for treatment-resistant CME secondary to DR (2-4).

In 2020, Imai *et al* (5) reported on cystoid lesion components in CME caused by diabetic macular edema or branch retinal vein occlusion using transmission electron microscopy (TEM) and mass spectrometry (MS) analysis. TEM revealed that the cystoid lesion components were non-cellular structures composed mainly of microfibrils wrapped in collagen fibers. MS analysis also revealed that the component contains fibrinogen α, β and γ (5). To date, to the best of our knowledge, there have been no histopathological or immunohistochemical studies examining the expression of proteins in cystoid lesion components in patients with diabetes.

Fibrinogen is known to undergo various post-translational modifications (6). Post-translational modifications of fibrinogen affect its function, subsequently contributing to various pathological conditions. For example, it has been reported that glycation and methylglyoxal (MGO)-derived advanced glycation end-product (AGE) modification of fibrinogen occurs in patients with diabetes (6,7).

The present study describes the case of a patient with refractory diabetic CME who underwent vitrectomy with *en bloc* removal of the cystoid lesion component. The present study also performed histopathological and immunohistochemical analysis of the cystoid lesion content to assess its immunoreactivity for fibrin/fibrinogen and AGE.

Case report

Clinical presentation. A 69-year-old Japanese man complained of visual loss and visual field distortion in the left eye. In July 2021, the patient was referred to Hokkaido University Hospital (Sapporo, Japan) due to residual diabetic CME despite receiving intravitreal anti-VEGF injections of aflibercept (IVA) a total of eight times for 3 years prior to referral. The patient had a medical history of diabetes mellitus, dyslipidemia and hypertension, and was diagnosed with diabetes at the age of 56 years. At the time of referral, the patient was being treated for diabetes with subcutaneous injection of dulaglutide, a weekly glucagon-like peptide-1 receptor agonist, 1.5 mg/week and insulin lispro 10 U/morning and 12 U/evening. The serum HbA1c levels were well controlled at 6.0%. The patient underwent cataract surgery on their right eye at the age of 66 years. The best-corrected visual acuity (BCVA) was 1.2 in the right eye (OD) and 0.5 in the left eye (OS). Intraocular pressure was normal in both eyes. Slit-lamp examination demonstrated an intraocular lens (OD) and mild cataracts (OS). Fundus examination showed dot hemorrhages and hard exudates in the peri-macular region with pan-retinal photocoagulation scars in both eyes (Fig. 1A). Swept-source optical coherence tomography demonstrated macular edema with foveal cystoid lesions, where the reflectivity was slightly higher than that of vitreous fluids OS (Fig. 1B). A total of 3 months after the initial visit, the patient underwent pars plana vitrectomy of the left eye with cataract surgery, internal limiting membrane peeling and removal of the cystoid lesion component. The cystoid lesion component was a translucent soft solid (Fig. 1C and D), which was then fixed in 4% formalin at room temperature overnight immediately after removal and embedded in paraffin for hematoxylin-eosin staining at room temperature for a few minutes each, and fluorescence immunohistochemical staining. The BCVA of the left eye 1 month after surgery was 0.3. Postoperatively, the CME subsided but soon recurred. Therefore, the left eye was further treated with STTA, direct photocoagulation for microaneurysms and IVA during the next year. A total of 1 year after surgery, the BCVA of the left eye had decreased to 0.2.

Methods. The formalin-fixed, paraffin-embedded tissue sections (5 μm) underwent pathological diagnosis and immunohistochemical analysis. Immunohistochemical analysis was performed as follows: The sections were dewaxed in xylene, dehydrated in various concentrations of ethanol and rinsed in phosphate-buffered saline after rinsing in Milli-Q water for 5 min. As a pretreatment, microwave-based antigen retrieval was conducted in 10 mM citrate buffer (pH 6.0) for 10 min after boiling. The sections were then incubated with 5.0% normal goat serum (cat. no. 50062Z; Thermo Fisher Scientific, Inc.) for 1 h at room temperature and with the following primary antibodies: Rabbit anti-human fibrin/fibrinogen polyclonal antibody (1:200 dilution; cat. no. A0080; Agilent Technologies, Inc.), rabbit anti-AGEs polyclonal antibody (1:100 dilution; cat. no. ab23722; Abcam), rabbit anti-collagen type 1 polyclonal antibody (1:100 dilution; cat. no. 600-401-103-0.1; Rockland Immunochemicals, Inc.), mouse anti-glial fibrillary acidic protein (GFAP) monoclonal antibody (1:100 dilution; cat. no. 14-9892-82; Thermo Fisher Scientific, Inc.), mouse anti-human receptor for AGE (RAGE) monoclonal antibody (1:100 dilution; cat. no. MAB11451; R&D Systems, Inc.), normal rabbit IgG (1:70 dilution; cat. no. AB-105-C; R&D Systems, Inc.) and normal mouse IgG (1:20 dilution; cat. no. X0931; Agilent Technologies, Inc.) at 4˚C overnight. Data on immunoreactivity for normal mouse IgG are not shown. The sections were then incubated with Alexa Fluor 488-conjugated (1:500 dilution; cat. no. A32723; Thermo Fisher Scientific, Inc.) or Alexa Fluor 546-conjugated (1:500 dilution; cat. no. A11035; Thermo Fisher Scientific, Inc.) secondary antibodies at room temperature for 1 h. Sections were visualized with an inverted fluorescence-phase contrast microscope.

Histopathological findings. Microscopic examination of the excised tissue revealed it to be elliptical in shape, measuring 0.7x0.4 mm. It displayed a homogeneous structure comprising eosinophilic material without cellular components (Fig. 2A). No membranous structure was observed surrounding the component, but a few erythrocyte aggregates were detected at the margin (Fig. 2B, arrows). Immunohistochemical analysis demonstrated that the tissue was positive for fibrin/fibrinogen and weakly positive for AGEs (Fig. 3E and F). By contrast, no immunoreactivity for GFAP, RAGE (data not shown) or type 1 collagen (Fig. 3G) was observed. Immunoreactivity for normal rabbit IgG was shown as a negative control (Fig. 3H). In addition, bright-field microscopic images consistent with Fig. 3E-H are shown in Fig. 3A-D. Histopathological images were obtained using the Biorevo light and fluorescence microscope system (BZ-9000; Keyence Corporation).

Discussion

The present study demonstrated clinicopathological findings of the cystoid lesion component in refractory diabetic CME. The pathological features of the cystoid lesion were homogeneous structures that consisted of acellular eosinophilic material with positive immunoreactivity for fibrin/fibrinogen and weakly positive immunoreactivity for AGEs. Moreover, the excised tissue was a solid material, suggesting that it was insoluble fibrin, not soluble fibrinogen.

In a previous report, TEM for the cystoid lesion component depicted microfibrils wrapped in collagen fibrils (5). In the present case, membranous collagen structures were not microscopically observed. Furthermore, the immunohistochemical analysis revealed no staining for type I collagen or GFAP, a representative retinal intermediate filament, in the component. In contrast to a previous report (5), there is a possible reason why a membranous structure was not observed around the excised components in this case. It is conceivable that the membranous structure was not present from the outset. There are instances where the membranous structure may or may not be present; in this case, it was absent. Furthermore, the erythrocytes interspersed within the tissue were thought to originate from retinal hemorrhages and microaneurysms with high viscosity, suggesting the possibility of fibrinogen leakage from these microvascular lesions.

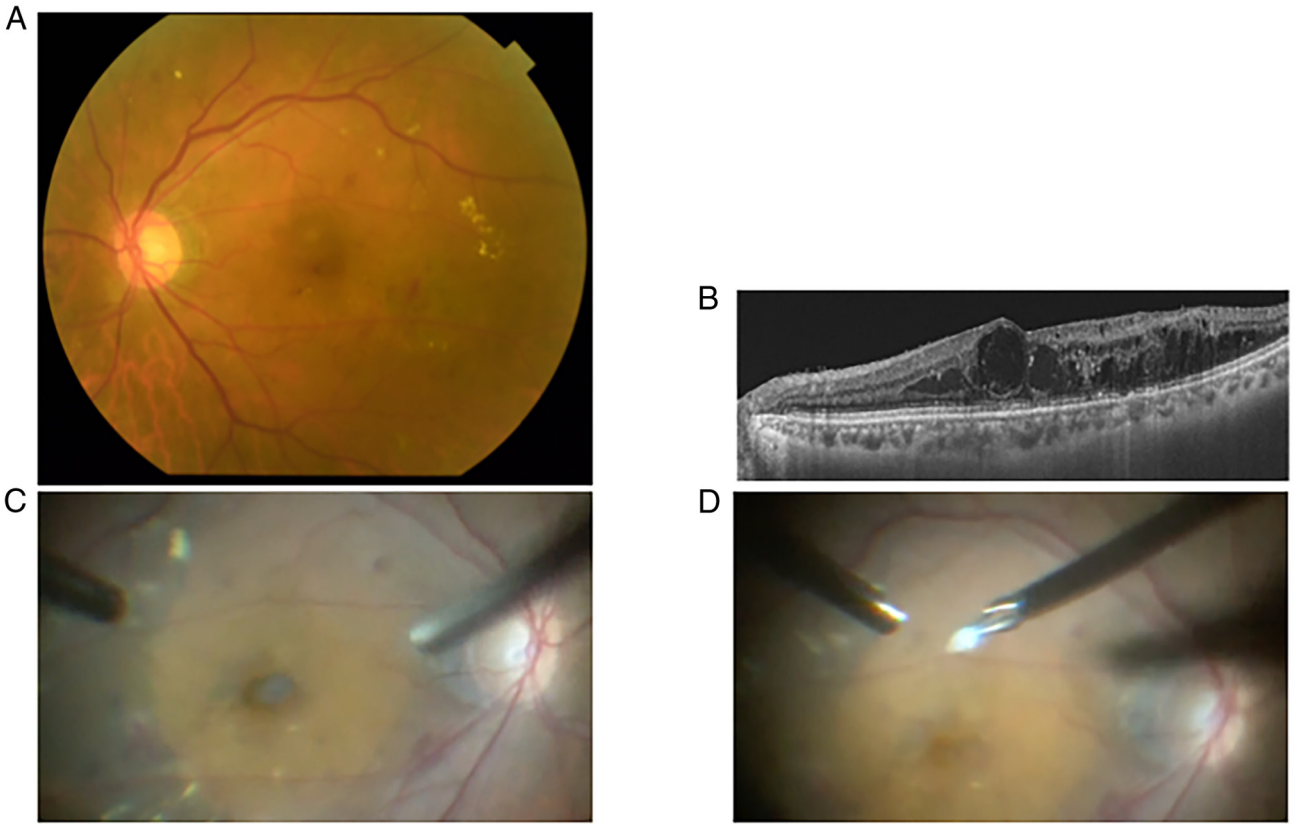

Figure 1. Clinical findings in the present case of diabetic cystoid macular edema. (A) Color fundus photography showed the dot hemorrhage and hard exudates at the peri-macular region in the left eye. (B) Swept-source optical coherence tomography at the horizontal section revealed the presence of spongiform edema with cystoid space in the macula. (C) Cystotomy of the cystoid macular lesion revealed a translucent solid component. (D) The excised cystoid lesion component was soft and solid.

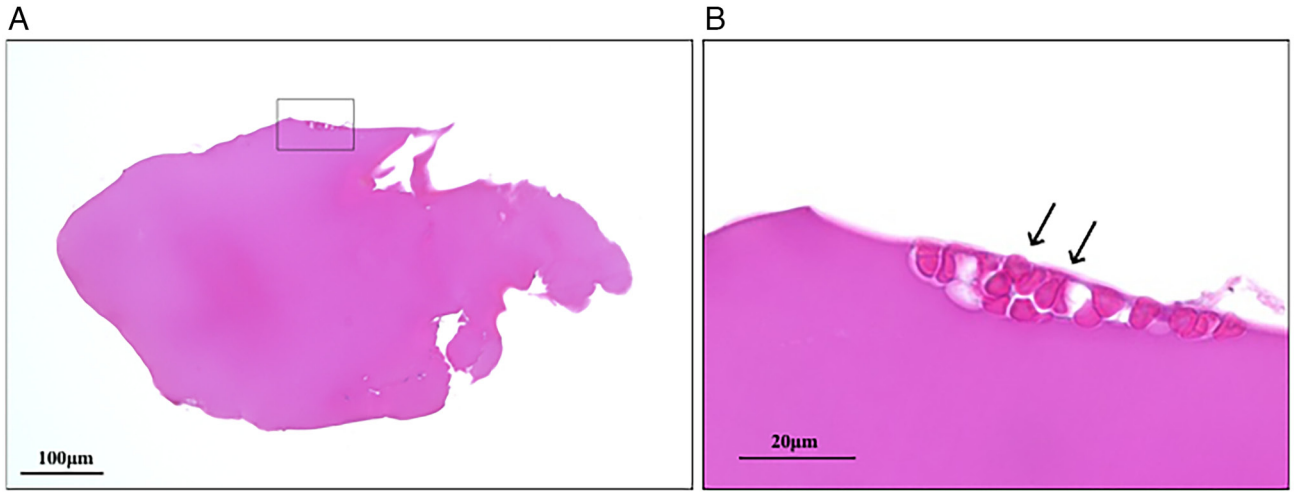

Figure 2. Histopathological findings of the excised tissue (hematoxylin and eosin staining). (A) The excised tissue was elliptical in shape, measuring 0.7x0.4 mm, and displayed a homogeneous structure comprising eosinophilic material without cellular components. Scale bar, 100 μm. (B) A few erythrocyte aggregates were observed at the margin of the component (black arrows). Scale bar, 20 μm.

In the present case, immunohistochemistry of the tissue for AGEs showed weakly positive staining. These results suggested that AGEs may post-transcriptionally modify fibrin clots of the cystoid lesion in diabetic CME. Fibrinogen is a 340-kDa glycoprotein synthesized in hepatocytes. It is secreted from hepatocytes into the blood, with plasma concentrations ranging from 1.5 to 3.0 g/l and a half-life of ~3 days (8,9). The high plasma concentrations and the long half-life allow fibrinogen to undergo various post-translational modifications that affect its function, including susceptibility to fibrinolysis (6). AGEs modification progressively occurs in patients with diabetes and AGEs are considered a significant pathogenic factor for diabetic complications (7,10).

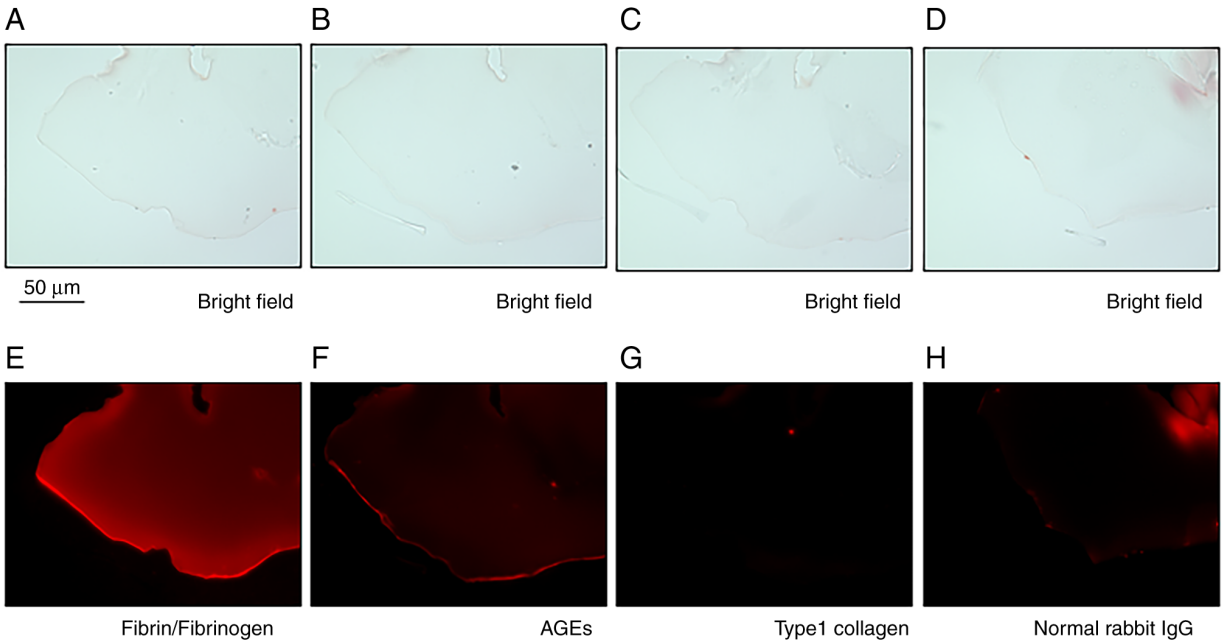

Figure 3. Findings of fluorescence immunohistochemistry and bright-field microscopic imaging of the excised tissue. (A-D) Bright-field microscopic imaging corresponds to the respective images as follows: (A) corresponds to (E), (B) corresponds to (F), (C) corresponds to (G), and (D) corresponds to (H). Bright-field microscopic images of (E-H), respectively. Immunohistochemical staining was (E) strongly positive for fibrin/fibrinogen, (F) weakly positive for AGEs, and (G) negative for type 1 collagen. (H) Immunohistochemistry staining of the negative control rabbit IgG. Scale bar, 50 μm. AGEs, advanced glycation end-products.

Furthermore, studies investigating fibrinogen glycation and MGO-derived AGE modification have shown that the fibrinolysis of the clot is reduced due to modification of the plasmin-cleavage sites (6,7). Therefore, based on the current study, fibrin clots in the cystoid lesion may be fibrinolytic-resistant due to glycation and AGEs modification in patients with diabetes. In addition, AGEs modification may also cause cellular dysfunction (7), possibly leading to retinal damage. Therefore, various post-translational modifications of fibrin/fibrinogen, such as glycation and AGEs modification, may influence the pathogenesis of diabetic CME.

There are several limitations to the present report. First, the glycation of the excised tissue could not be confirmed, although this study discussed post-transcriptional modification by glycation and AGEs. Nevertheless, additional studies could not be performed because the excised tissue was too small to allow further analysis. Second, since this is a single case report, it remains unclear whether this phenomenon generally happens in diabetic CME.

In conclusion, to the best of our knowledge, the current study is the first to provide evidence that the cystoid lesion component in diabetic CME is a fibrin clot post-translationally modified by AGEs. In patients with diabetes, post-translational modifications, such as AGE modification, may lead to resistance to fibrinolysis by plasmin. These findings indicated that it is important to know how the components of the cystoid lesion undergo post-transcriptional modifications, since it may induce alterations in the characteristics of the lesion.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TT, MS, and SK substantially contributed to the conceptualization of the present study and confirmed the authenticity of all the raw data. TT drafted the original manuscript. SK supervised the conduct of the study and contributed to the revision of the manuscript draft. MS performed the surgery in this case and removed the component. IH and MM significantly contributed to the immunohistochemical staining. ET made significant contributions to the pathological diagnosis. SI contributed to interpretation of the results and the revision of the manuscript draft. All authors critically reviewed and revised the manuscript draft, and read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

The patient provided written, retrospective informed consent for publication following detailed explanation of the purpose

of the manuscript and understanding that no identifiable information was going to be released.

Competing interests

The authors declare that they have no competing interests.

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