Optical Coherence Tomographic Reflectivity of Photoreceptors beneath Cystoid Spaces in Diabetic Macular Edema

Tomoaki Murakami, Kazuaki Nishijima, Tadamichi Akagi, Akibito Uji, Takabiro Horii, Naoko Ueda-Arakawa, Yuki Muraoka, and Nagahisa Yoshimura

PURPOSE. To investigate the relationship between the cystoid spaces in the outer plexiform layer (OPL) and the characteristics of the photoreceptors beneath the cystoid spaces in patients with diabetic macular edema (DME).

METHODS. In this observational cross-sectional study, 123 eyes of 96 consecutive patients with clinically significant macular edema were retrospectively reviewed. The characteristics of the photoreceptors on optical coherence tomography (OCT) images represented by the external limiting membrane (ELM) and the junction between inner and outer segments (IS/OS), and their association with the overlying cystoid spaces were investigated.

RESULTS. The areas beneath the cystoid spaces in the OPL had a longer transverse length of disrupted or faint IS/OS and disrupted ELM lines than those without cystoid spaces ($P < 0.001$, $P < 0.001$, and $P = 0.009$). The IS/OS lines beneath the cystoid spaces had higher reflectivity than those in areas without cystoid spaces ($P < 0.001$). Enlarged cystoid spaces extending from the inner nuclear layer to the OPL were associated with disrupted IS/OS or ELM but not faint IS/OS ($P < 0.001$, $P < 0.001$, and $P = 0.467$). The transverse length of disrupted IS/OS at the fovea was correlated with the logarithm of the minimum angle of resolution (logMAR) more than the association between foveal thickness and logMAR ($r = 0.49$, $P < 0.001$ vs. $r = 0.28$, $P = 0.002$). The ELM descended to the RPE more frequently in eyes with single-lobulated fluorescein pooling in the foveal avascular zone than those with multilobulated pooling ($P < 0.001$).

CONCLUSIONS. OCT showed that the cystoid spaces in the OPL were accompanied by photoreceptor damage beneath the cystoid spaces in DME. (Invest Ophthalmol Vis Sci. 2012;53: 1506–1511) DOI:10.1167/iovs.11-9231

Diabetic retinopathy (DR) often leads to severe visual impairments especially in patients of working age. Diabetic macular edema (DME), which is caused by the breakdown of blood-retinal barrier (BRB), deteriorates the neuronal function in the macula and concomitantly the visual acuity (VA). Despite intensive effort, complete resolution of DME has not been achieved, and research into the pathogenesis and treatments must continue.

Although DME was diagnosed originally as macular thickening based on the biomicroscopic examination, optical coherence tomography (OCT) has enabled quantification of the retinal thickness and evaluation of the pathohistologic findings of the neuroglial components. The macular thickness is correlated modestly with poor VA in DME, suggesting that, despite its clinical relevance, mechanisms other than retinal thickening remain to be elucidated. Several pathomorphologic findings have been seen on OCT images (i.e., cystoid macular edema; CME), serous retinal detachments (SRDs), and sponge-like retinal swelling, all which contribute to macular thickening. Cystoid spaces especially are seen often in the inner nuclear layer (INL) and the outer plexiform layer (OPL) on OCT images. These investigators have speculated that the spaces correspond to the accumulated extracellular fluids or intracytoplasmic swelling of the Müller cells, both of which might exacerbate neuroglial dysfunction in signal transduction from the photoreceptors.

Recent advances in OCT technology have provided better delineation of the individual retinal layers and intraretinal lesions with reduced speckle noise, which prompted us to evaluate the junctions between the inner and outer segments (IS/OS) and the external limiting membrane (ELM) as the representative findings of photoreceptor integrity. Qualitative assessment of the foveal photoreceptor status especially might predict the dysfunction of the photoreceptor cells and concomitant visual impairment in retinal diseases such as retinitis pigmentosa, retinal vein occlusion, DME, and age-related macular degeneration (AMD). Despite the clinical relevance of photoreceptor degeneration seen on the latest generation of OCT, it remains to be determined how vascular hyperpermeability in diabetes exacerbates structural and functional changes in the photoreceptor cells.

In the present study, we showed an association between the cystoid spaces and photoreceptor changes in DME, suggesting a pathologic effect of the cystoid spaces on photoreceptor damage.

METHODS

Patients

We retrospectively reviewed 123 eyes of 96 patients (mean age, 65.3 ± 9.9 years; range, 34 to 85 years). Three patients had type 1 diabetes mellitus (DM) and 93 had type 2 DM and visited the Department of Ophthalmology in Kyoto University Hospital from July 2008 to February 2011. Eyes with clinically significant macular edema were included, for which fluorescein angiography (FA) and OCT images of sufficient quality were obtained. Eyes with a SRD were excluded, because it was difficult to determine the IS/OS status in the areas with a SRD. All
research and measurements adhered to the tenets of the Declaration of Helsinki.

**Qualitative and Quantitative Analyses of OCT Images**

After evaluation of the best-corrected VA and fundus biomicroscopy, retinal sectional images (30° cross-hair scans) were obtained using spectral-domain (SD)-OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany). We first determined the presumed foveal center, followed by further analyses using the central 7 millimeters of the vertical images dissecting the fovea. After we excluded the areas in which the inner retinal lesions or structures (vasculature, intraretinal hemorrhages, or hyperreflective foci) decreased the reflectivity levels of the retinal pigment epithelium (RPE), we evaluated the areas with cystoid spaces and the photoreceptor status.

Previous studies often have classified the status of the IS/OS and ELM into three patterns: complete, discontinuous, and absent. Intriguingly, SD-OCT (Spectralis; Heidelberg Engineering) shows the variability of the reflectivity levels in continuous IS/OS lines, which encouraged us to introduce a novel classification and quantify the status of the transverse length of each segment of the IS/OS.

In this classification, an intact line indicates a highly reflective and continuous line, as delineated in the physiologic line; a faint line indicates the presence of a continuous line with lower reflectivity (less than half of the OCT reflectivity in the RPE); and a disrupted line indicates an absent or discontinuous line, as reported previously (Fig. 1). Compared with that in the IS/OS lines, OCT reflectivity in the ELM lines was relatively homogeneous and described as intact, which seems to be a continuous line, or disrupted, when the ELM line was absent, followed by measurement of the transverse length of each ELM section (Fig. 1). We further evaluated the transverse length of the cystoid spaces in the OPL and investigated its relationship with the status of the photoreceptors (Fig. 2).

We also quantified the reflectivity levels of either the ELM or IS/OS lines compared with that of the RPE. After images with inversed signals were imported into ImageJ (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html), one straight line dissecting the retina from the vitreous to the choroid was randomly selected in each image. ‘Prot Profile’ analyses in ImageJ showed the peaks of reflectivity at the ELM, IS/OS, and RPE that were measured auto-

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**FIGURE 1.** Several patterns of photoreceptor status in DME. A representative case of DME (top) and a magnified image (middle left). OCT shows an intact ELM (middle center, arrows) or a disrupted ELM (middle right, between the arrowheads). The junction between the IS/OS is intact (bottom left, arrows), faint (bottom center, between the arrowheads), or absent (bottom right, between the arrowheads). COST, cone outer segment tips.

**FIGURE 2.** Association between the cystoid spaces and photoreceptor status in diabetic macular edema. The cystoid spaces (white arrowheads) are seen mainly in either the outer plexiform layer (top left), the inner nuclear layer (top center), or extending across these layers (top right). The black arrows show the inner portion of the OPL. The black arrowheads indicate the external limiting membrane. Bottom: the status of the junction between the IS/OS is evaluated on the OCT images corresponding to the arrow in infrared image. The red line indicates a disrupted IS/OS. The red dotted line indicates a faint IS/OS. The red dashed line indicates an intact IS/OS. The blue line indicates cystoid spaces in the OPL. The black line indicates the central 7-mm area to be evaluated, without shadowing by the inner lesions.
The relative reflectivity levels were determined according to the formula:

Relative reflectivity (arbitrary unit) = (IS/OS [or ELM] – vitreous) / (RPE – vitreous)

When we could not find any peak of reflectivity level just above the RPE, we designated the relative reflectivity of ELM or IS/OS to be 0.

FA was acquired using confocal scanning laser ophthalmoscope (Heidelberg Retinal Angiography 2; Heidelberg Engineering), followed by determination of the patterns of fluorescein pooling at the foveal avascular zone (FAZ). After we determined the FAZ in the early phases, we classified the patterns of fluorescein pooling in the late phases as single-lobulated or multi-lobulated (Fig. 4).

### Statistical Analysis

The results are expressed as the mean ± standard deviation. The Student’s t-test was used to compare quantitative data populations with normal distributions and equal variance. The data were analyzed using the Mann-Whitney U test for populations with nonnormal distributions or unequal variance. Linear regression analysis was performed to test the statistical correlation. *P* < 0.05 was considered statistically significant.

### RESULTS

#### Optical Coherence Tomographic Reflectivity of the Junction between the Inner and Outer Segments

In addition to several IS/OS patterns on previous-generation OCT images, the latest generation, SD-OCT (Spectralis; Heidelberg Engineering) also showed that different levels of reflectivity in continuous IS/OS lines in DME contain a faint IS/OS line as defined in Methods. Because OCT reflectivity in the photoreceptor OS can represent retinal function,27,28 we evaluated the association between the logarithm of the minimum angle of resolution (logMAR) VA and photoreceptor status within 1 mm of the foveal center (Table 1). Poor VA was correlated with the transverse length of the disrupted IS/OS and was more significant than the association between the

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IS/OS, junction between inner and outer segments; ELM, external limiting membrane.

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**FIGURE 3.** Quantification of the reflectivity of the ELM and the junction between the IS/OS. The right column shows the quantification of the reflectivity levels along the white arrow in the left column in eyes with intact (top), faint (middle), or disrupted (bottom) IS/OS lines.

**FIGURE 4.** Association between fluorescein pooling and the ELM in DME. Top row: the ELM (arrow) has descended to the RPE beneath a cystoid space with single-lobulated fluorescein pooling (arrowheads) in the foveal avascular zone, compared with (bottom row) no change in an eye with multi-lobulated pooling (arrowheads). Left column: early phase in FA; center column: late phase in FA; right column: OCT image.

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**TABLE 1.** Association between Foveal Photoreceptor Status and Visual Acuities in DME

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center point thickness and the logMAR VA ($r = 0.28$; $P = 0.002$). However, the logMAR VA was negatively associated with the length of the faint IS/OS (Table 1). These data suggested the clinical relevance of the disrupted IS/OS or ELM but not the faint IS/OS.

### Disrupted Photoreceptors in Eyes with Extended Cystoid Spaces

Despite the clinical relevance of the photoreceptor status in DME, it remains to be determined how breakdown of the BRB leads to photoreceptor degeneration. As reported previously, cystoid spaces, resulting from vascular hyperpermeability, are mainly in the INL and OPL (Fig. 2).12–16 and large cystoid spaces extending from the INL to OPL sometimes seen on OCT images suggest severe damage of the neuroglial components. Eyes with extended cystoid spaces had a larger transverse length of disrupted IS/OS or ELM than those without cystoid spaces compared with no differences in the length of the faint IS/OS (Table 2), which suggested an association between the extended cystoid spaces and photoreceptor degeneration.

### Photoreceptor Status beneath the Cystoid Spaces in the OPL

Although cystoid spaces in the OPL may have direct contact with the photoreceptor cells, it is unknown how these spaces affect photoreceptor degeneration in macular edema associated with retinal vascular diseases.13 Evaluation of the ELM or IS/OS status beneath the cystoid spaces indicated that the transverse length of the disrupted or the faint IS/OS lines beneath the cystoid spaces in the OPL was significantly larger than that in the area without cystoid spaces compared with that of the intact IS/OS lines (Table 3). Quantification of the reflectivity levels of the IS/OS or ELM lines in each A-scan showed that the relative OCT reflectivity of the IS/OS lines beneath the cystoid spaces was significantly lower than that in the areas without cystoid spaces ($0.46 \pm 0.33$ vs. $0.83 \pm 0.21$; $P < 0.001$); however, there were no differences in the reflectivity levels of the ELM lines ($0.33 \pm 0.20$ vs. $0.38 \pm 0.12$; $P = 0.130$). These data suggested that the cystoid spaces in the OPL were significantly associated with the pathogenesis in the photoreceptor cells.

### Association between Fluorescein Pooling and Descent of the ELM

Recent publications have reported a relationship between the FA and OCT findings in DME.12,16,25,29 This encouraged us to investigate whether the patterns of dye pooling at the FAZ were associated with the morphologic parameters seen in the photoreceptors on the OCT images. After 23 eyes with poorly delineated fluorescein pooling or ELM lines at the fovea were excluded, descending of the ELM to the RPE was seen in 15 of 30 eyes (50.0%) with single-lobulated pooling of fluorescein dye, whereas only 11 of 70 eyes (15.7%) with multi-lobulated pooling showed the ELM descending to the RPE ($P < 0.001$) (Table 4). However, we did not find significant differences in other parameters seen on the OCT images.

### DISCUSSION

Recent qualitative studies have reported the clinical relevance of the photoreceptor status on OCT images,17,19–21 although the mechanisms of photoreceptor degeneration are still poorly defined. The present study showed for the first time several associations between the characteristics of the cystoid spaces and photoreceptor changes beneath them in DME. Our findings suggested that the cystoid spaces might affect the pathogenesis in the photoreceptors, in addition to the signal transduction system, and that the cystoid spaces might be a therapeutic target for improving the photoreceptor function. It had been speculated that the cystoid spaces disturb light transmission at the fovea, and in the present study we showed other mechanisms for the visual dysfunction associated with the cystoid spaces. However, we evaluated the retinal sections using cross-hair mode, which suggests the limited quality of information, compared with the full delineation by three-dimensional scan mode. Because the present study was cross-

### OCT Reflectivity beneath Cystoid Spaces in DME

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sectional and retrospective in nature, another longitudinal study should be planned.

We first investigated the photoreceptor status seen on SD-OCT (Spectralis; Heidelberg Engineering) that is represented by the ELM, IS/OS, and COST lines. Compared with the reproducible and clear delineation of the ELM and IS/OS lines, COST lines were often blurred or diminished, even in vivo. Although further advances in OCT technology would facilitate accurate evaluation of the interface between the RPE and photoreceptors, the present study focused on the ELM and IS/OS lines, both of which might represent individual properties. SD-OCT improves visualization of each retinal layer with reduced speckle noise, and both lines appeared continuously in the physiologic eyes, which enabled us to differentiate the fragmented lines more precisely.

We therefore investigated the transverse length or the OCT reflectivity of the continuous ELM and IS/OS lines. The reflectivity levels of the ELM lines were relatively homogeneous, and their presence could be clearly determined. However, the continuous IS/OS lines had varying reflectivity, and it was difficult to differentiate fragmented IS/OS lines from RPE hyperplasia, hyperreflective foci, or degenerative processes in the neuropilial tissue. All these conditions might be associated with photoreceptor damage, which prompted us to categorize them as disrupted IS/OS, and would correspond to the absent or discontinuous IS/OS lines reported previously. We also evaluated the transverse length of the discontinuous IS/OS, which should depend on the signal intensity, which might have led us to reach erroneous conclusions compared with the clinical relevance in the qualitative analyses. These situations encouraged us to introduce a novel classification for quantitative analyses. However, the reflectivity levels of the continuous IS/OS lines varied, which led us to subdivide them further into faint and intact lines. For the objective evaluation, we performed additional quantification of the IS/OS reflectivity, which also showed differential levels of OCT reflectivity compared with that of ELM lines.

Several mechanisms might lead to photoreceptor changes. Breakdown of the BRB associated with diabetes might increase extravasation of the blood constituents (water, ions, proteins, lipids) and inflammatory cells and concomitantly exacerbate the pathomorphologic changes in the macula. SRDs might decrease the metabolism of the photoreceptor cells, resulting in their degradation. We recently found that hyperreflective foci in the outer retinal layers were associated with photoreceptor damage. The Early Treatment Diabetic Retinopathy Study (ETDRS) reported that foveal hard exudates led to retinal fibrosis and visual impairment, which might depend on photoreceptor degradation, as in AMD. In addition to those possible mechanisms, the present study found that disrupted or faint IS/OS lines were beneath the cystoid spaces more frequently, suggesting that the cystoid spaces might be an additional mechanism contributing to exacerbation of photoreceptor damage.

Enlarged cystoid spaces can extend from the inner retinal layers to the OPL in macular edema associated with retinal vascular diseases. We identified such cystoid spaces extending from the INL to OPL in 18 eyes in the present study; these cystoid spaces had larger transverse length of the disrupted IS/OS or ELM, but not that of the faint IS/OS. Extended cystoid spaces might represent degeneration of the neuropilial components in signal transduction, which could lead to retrograde degeneration of the photoreceptor cells. Otherwise, the degenerative changes in the glia and Müller cells might result in disruption of the ELM, which is the heterophilic adherens junction between the Müller cells and photoreceptors and is a barrier for macromolecules. Concomitantly, blood constituents or inflammatory cells might migrate into the outer retinal layers and exacerbate photoreceptor degeneration, which possibly is supported by the association between the hyperreflective foci in the outer retinal layers and the disrupted photoreceptors. Furthermore, because Müller cells function as living optical fibers, their damage might lead to the reduced transparency of the retina, which explains the reflectivity error in outer retinal layers and visual dysfunction. On the contrary, we had to consider the possibility that photoreceptor degeneration might contribute to the pathogenesis of DME, because retinitis pigmentosa, whose primary lesions are in photoreceptors and RPE, is sometimes accompanied with cystoid spaces. Another explanation might be that a longer duration or more severe DME might result in both extended cystoid spaces and photoreceptor damage.

The present study also showed that decreased OCT reflectivity was specifically associated with IS/OS lines but not ELM or RPE lines beneath the cystoid spaces in the OPL. The classic speculation regarding the disturbed light transmission through the cystoid spaces might be supported by the signal reduction in some areas with cystoid spaces on OCT images. In other words, we could not completely exclude the possibility that faint IS/OS line might correspond to an optical artifact. However, previous studies have reported that OCT reflectivity levels in the outer segments increase after light exposure and are associated with retinal function. Those authors speculated that light-evoked cell swelling and shrinkage and membrane depolarization might result in optical changes in the outer segments (i.e., scattering or transmission). Instead of light exposure, blood constituents or waste in the cystoid spaces might change the cellular metabolism with concomitant cellular swelling or shrinkage, or local imbalance of ions might affect membrane depolarization, although we could not find a definite association between VA and a faint IS/OS line. Further longitudinal analysis might identify factors predictive of photoreceptor degeneration at least in part.

FA has provided important information regarding DME, and several recent publications have reported interesting associations between FA and OCT findings, which encouraged us to investigate whether patterns of fluorescein pooling are related to photoreceptor characteristics seen on OCT images. Although we could not find a relationship between fluorescein pooling and OCT reflectivity of the ELM or IS/OS, the descent of the ELM to the RPE occurred more frequently in eyes with single-lobulated pooling than in those with multilobulated pooling. This suggested that a single-lobulated cystoid space might compress the surrounding retinal parenchyma, resulting in the descent of the ELM, and support the finding that these eyes often have enlarged cystoid spaces. However, it is unclear what determines the patterns of fluorescein pooling. Some eyes with a descended ELM had continuous IS/OS lines beneath the cystoid spaces and others did not, and the transverse length of the disrupted IS/OS beneath the descended ELM was much longer. This suggested that distortion of the photoreceptor layer might contribute partly to visual impairment. Another possible explanation might be that persistent multi-lobulated cystoid spaces might result in a single-lobulated cystoid space, with progression of the atrophic changes in the outer retinal layers. A longitudinal study would elucidate whether these mechanical changes exacerbate photoreceptor degeneration beneath the cystoid spaces.

The present study found a significant association between cystoid spaces and photoreceptor degeneration beneath them in the DME, suggesting that the cystoid spaces in the OPL might be an important mechanism in photoreceptor damage and visual disturbance.
References


