Qualitative and Quantitative Characteristics of Near-Infrared Autofluorescence in Diabetic Macular Edema

Shin Yoshitake, MD, Tomoaki Murakami, MD, Takahiro Horii, MD, Akihito Uji, MD, Ken Ogino, MD, Noriyuki Unoki, MD, Kazuaki Nishijima, MD, Nagahisa Yoshimura, MD

Objective: To study the characteristics of near-infrared autofluorescence (NIR-AF) imaging and its association with spectral-domain optical coherence tomography (SD-OCT) findings and logarithm of the minimal angle of resolution (logMAR) visual acuity (VA) in diabetic macular edema (DME).

Design: Retrospective, observational, cross-sectional study.

Participants: One hundred twenty-one consecutive eyes of 87 patients with center-involved DME for whom NIR-AF and SD-OCT images of sufficient quality were obtained.

Methods: The NIR-AF images were acquired using Heidelberg Retina Angiograph 2 (Heidelberg Engineering, Heidelberg, Germany), and sectional retinal images were obtained using Spectralis OCT (Heidelberg Engineering). The presence of a mosaic pattern and cystoid signs were determined qualitatively. We quantified the average fluorescence intensity in the central 1-mm subfield. The characteristics of the NIR-AF images were compared with the OCT findings and logMAR VA.

Main Outcome Measures: Qualitative and quantitative characteristics of the NIR-AF images and their association with SD-OCT findings and logMAR VA.

Results: Fifty-seven eyes with a mosaic pattern in the NIR-AF macular images had worse logMAR VA (0.355±0.239 vs. 0.212±0.235; $P = 0.001$), a thicker central subfield (CSF) (530±143 μm vs. 438±105 μm; $P < 0.001$), and disrupted external limiting membrane (ELM; $P < 0.001$) compared with 64 eyes without these findings. Forty-one eyes with a cystoid sign in the NIR-AF images had worse logMAR VA (0.393±0.233 vs. 0.221±0.234; $P < 0.001$) and a thicker CSF (557±155 μm vs. 443±100 μm; $P < 0.001$) than those without them; there were no significant differences in the ELM status. The relative fluorescence intensity in the central subfield in the NIR-AF images was correlated negatively with the CSF thickness and logMAR VA ($R = 0.492$, $P < 0.001$ and $R = 0.377$, $P < 0.001$, respectively). Eyes with foveal serous retinal detachment had lower levels of relative fluorescence intensity than those without it (0.751±0.191 vs. 0.877±0.154; $P = 0.007$); there was no association with the presence of foveal cystoid spaces, disrupted ELM, or hyperreflective foci in the outer retinal layers.

Conclusions: Novel qualitative and quantitative NIR-AF characteristics in the macula indicated the clinical relevance and suggested the pathogenesis in DME. *Ophthalmology 2014;121:1036-1044 © 2014 by the American Academy of Ophthalmology.*

Supplemental material is available at www.aaojournal.org.

Diabetic macular edema (DME) is a major cause of severe visual loss in diabetic retinopathy (DR).[1] Diabetes induces breakdown of the blood−retinal barrier (BRB), and accumulation of blood components in the extravascular spaces leads to macular thickening and functional disturbances.[2,3] Because increasing numbers of patients have DR and vision-threatening DR,[4] further research is needed to develop treatments and evaluation systems for DME.

Fluorescein angiography shows various patterns of hyperfluorescence, representing the breakdown of the inner or outer BRB.[5−10] Basic and clinical research has shown that biochemical pathways, growth factors, and cytokines disturb the barrier function in the vascular endothelium, which is reversed by anti−vascular endothelial growth factor treatments.[11] The Early Treatment Diabetic Retinopathy Study (ETDRS) recommended grid-pattern photocoagulation for diffuse fluorescein leakage, which may correspond to some extent to the extravasation of blood components through the retinal pigment epithelium (RPE).[12] Accumulated basic research data have suggested that transepithelial transport or the barrier properties in the RPE in DR are disrupted,[13−19] although in vivo imaging has not confirmed these theories. Another imaging method, spectral-domain optical coherence tomography (SD-OCT), facilitates acquisition of sectional, high-resolution images of retinal pathologic features. Its results have shown a correlation between visual impairment and photoreceptor damage at the fovea in DME.[20−22] Despite the clinical relevance, how the disturbance in the foveal photoreceptor cells, which are nourished by the RPE, occurs remains unknown.

ISSN 0161-6420/14/$ - see front matter
http://dx.doi.org/10.1016/j.ophtha.2013.11.033

The physiologic features of the RPE and photoreceptor interface are important for preserving vision.[23] New techniques for in vivo imaging of fundus autofluorescence (AF), that is, short-wavelength (SW) AF and near-infrared (NIR) AF, have been used to estimate the function and morphologic features of the retina and the RPE in various diseases, such as age-related macular degeneration, retinal dystrophy, and central serous chorioretinopathy.[24–26] It is widely accepted that the signal of SW-AF is derived mainly from lipofuscin in the RPE[27] and the NIR-AF signal from the melanin, a fluorophore in the RPE and the choroid.[8,28] A major component of lipofuscin is N-retinyl-N-retinylidene ethanolamine, which may disturb the function of the RPE cells via various toxic mechanisms, including photo-oxidative damage.[9,29] In contrast, melanin, which accumulates in the apical part of the RPE cells, is thought to protect the RPE cells from various factors, for example, light scattering, radiation, oxidative stress, and light damage, and to counteract phototoxic damage.[10,30] One SW-AF abnormality, increased AF corresponding to cystoid spaces, has been associated with visual acuity (VA), retinal thickening, retinal sensitivity, and photoreceptor integrity in DME.[31–34] However, the distribution or intensity of the fluorescence signals on NIR-AF images and the relationship to the characteristics on SD-OCT images in DME are poorly understood.

Herein we report for the first time the qualitative findings, that is, the mosaic pattern and the cystoid sign, seen in the NIR-AF images, quantify the fluorescence intensity in the macula, and evaluate the association of the findings with macular thickening, photoreceptor damages, and visual dysfunction in DME.

Methods

Patients

One hundred twenty-one eyes of 87 patients (mean age, 63.4±9.9 years; range, 33–83 years; 2 eyes with mild nonproliferative diabetic retinopathy, 72 with moderate nonproliferative diabetic retinopathy, 22 with severe nonproliferative diabetic retinopathy, and 25 with proliferative diabetic retinopathy) who visited the Department of Ophthalmology of Kyoto University Hospital from June 2010 through December 2012 were included in this study. The inclusion criteria were center-involved DME and the availability of SD-OCT and NIR-AF images of sufficient quality that were obtained on the same day. The major exclusion criteria were the presence of any other chorioretinal diseases, including age-related maculopathy and age-related macular degeneration, severe media opacity, a history of treatment of DME, cataract surgery within 3 months, or any major surgery other than cataract extraction within 1 year. All research and measurements adhered to the tenets of the Declaration of Helsinki. The ethics committee of Kyoto University Graduate School of Medicine approved the study protocol.

Near-Infrared Autofluorescence Imaging

After comprehensive ophthalmologic examinations including measurement of the best-corrected VA, slit-lamp biomicroscopy, and color fundus photography, NIR-AF images of the macula were obtained using a scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2; Heidelberg Engineering, Heidelberg, Germany). An area of 30×30° centered on the fovea was scanned using an 800-nm filter and excitation with a 787-nm diode laser. The signal gain was increased sufficiently to delineate the major vessels and the disc, followed by averaging with 30 to 100 scanned images for sufficient quality.

We qualitatively evaluated 2 morphologic characteristics at the fovea, namely, a mosaic pattern and cystoid sign. It is widely accepted that in healthy eyes, the fluorescence signals in the NIR-AF images are almost absent in the optic disc, depending on the distribution of the melanin, and that the signals are blocked by retinal vessels. The signal intensity gradually increased centrifugally with the peak at the fovea (Fig 1, available at www.aaojournal.org). In contrast, we observed the mosaic patterns of granular or patchy hyperfluorescence and hypofluorescence at the fovea in eyes with center-involved DME (Figs 2 and 3), after we carefully excluded the fluorescence blocked by hard exudates and retinal hemorrhages. Another finding was the cystoid sign. In eyes with foveal cystoid spaces, the outlines of the cystoid spaces were sometimes seen at the fovea (Fig 4). Two graders (S.Y. and T.H.) evaluated these qualitative findings in a masked fashion, and when they disagreed, a third higher grader (T.M.) made the decision.

We then quantified the relative fluorescence intensity in the central 1-mm subfield of the ETDRS grid. We excluded 35 eyes with definite intraretinal lesions in the central subfield of the ETDRS grid on color fundus photography, which would have blocked the fluorescence signals from the RPE and the choroid. The mean fluorescence intensity in the central subfield was measured using image processing software (Photoshop; Adobe Systems, San Jose, CA) and was followed by further calculations with the controls. We first measured the mean signal intensity of 1000 pixels within the optic disc, where melanin is absent, as the 0 point in individual images. We then assigned a value of 1 to the signal levels in the areas outside the ETDRS grid (approximately 6 mm) because the pathologic decreases in fluorescence intensity in the NIR-AF images were mainly within the ETDRS grid (approximately 6 mm) and the signal intensity outside the ETDRS grid was not affected markedly in eyes with DME. Intraretinal lesions often block the signals, and the major choroidal vessels reduce the fluorescence from the choroid, which would modify the average fluorescence intensity, but not the maximal signals. We selected and calculated the mean fluorescence intensity in 1000 pixels with the highest signals in the individual quadrants (superior, nasal, inferior, and temporal) using the histogram function in Image J software (National Institutes of Health, Bethesda, MD). We then determined the mean signal intensity of all quadrants (referred to as the fluorescence intensity in the areas outside the ETDRS grid). Finally, we determined the relative fluorescence intensity according to the formula:

$$\text{Relative fluorescence intensity in the central subfield} = \frac{\text{fluo(mean central intensity)} - \text{fluo(optic disc)}}{\text{fluo(areas outside the ETDRS grid)} - \text{fluo(optic disc)}}$$

where *fluo* is fluorescence intensity.

We confirmed the agreement of the values between 2 independent graders (intraclass correlation coefficient, 0.967), and the average was applied to further analysis.

Optical Coherence Tomography

Retinal sectional images of the macula were acquired using SD-OCT (Spectralis OCT; Heidelberg Engineering), and the OCT parameters were evaluated quantitatively and qualitatively. The mean central subfield (CSF) thickness of the ETDRS grid was measured on a 2-dimensional OCT map constructed by raster scans, as described previously.[35] We diagnosed center-involved