Imaging of Melanin Disruption in Age-Related Macular Degeneration Using Multispectral Imaging

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BACKGROUND AND OBJECTIVE: To investigate whether multispectral imaging (MSI) is able to obtain a noninvasive view of melanin disruption associated with age-related macular degeneration (AMD), which could support early diagnosis and potential treatment strategies.

PATIENTS AND METHODS: A single retinal center, retrospective, observational, image analysis study of MSI images of 43 patients was done to determine the extent of melanin pigment exhibited in association with AMD, based on the Age-Related Eye Disease Study classification and grading scale. Corresponding fundus photos were also graded for 12 of the eyes.

RESULTS: Fifty-one of 61 eyes (84%) of 43 patients with AMD were determined to have melanin disruption in their MSI images in at least the central and/or one of four inner ETDRS areas. There was a relationship between severity of disease and the degree of melanin disruption. The sensitivity of fundus photography for melanin pigment as compared to MSI was only 62.5%, with three false-negatives.

CONCLUSION: A direct, noninvasive, unobstructed view of melanin disruption associated with AMD can be observed using MSI.


INTRODUCTION

Research into novel treatments that target the retinal pigment epithelium (RPE) for the management of dry age-related macular degeneration (AMD) is moving away from solely vitamin-based therapy to interventional pharmaceutical therapy.1 Pathological changes to the RPE have traditionally been difficult to image in vivo, particularly those related to abnormal melanin distribution in the form of pigment clumping, stacking, or migration.2 Visualization of changes to the RPE are vital because these, along with the formation of drusen, are the two major factors used to determine the risk of advancing AMD.3 The issue of imaging melanin pigment changes may be resolved with multispectral imaging (MSI), a novel imaging modality that allows for early detection and diagnosis of retinal pathologies. MSI potentially provides direct observation of melanin disruption in the RPE using long wavelength light and could also potentially aid the clinician in predicting the direction of advancing retinal pathology, particularly geographic atrophy (GA), as seen in Figure 1. Histology has shown thickening of Bruch’s membrane and RPE pigmentation abnormalities, including melanin stacking, whereas MSI may be able to show melanin disruption clinically.4

MSI is hypothesized to noninvasively provide an unobstructed view of the RPE, particularly melanin disruption in AMD, allowing for early diagnosis and monitoring the effects of targeted therapeutic treatment strategies.
PATIENTS AND METHODS

A single retinal center, retrospective, observational image analysis of MSI images taken during a 6-month period between May 19, 2014, and Nov. 5, 2014, was done. Images were obtained during the normal course of practice with informed consent. Of the 85 patients who were imaged with MSI during that period, 43 were diagnosed with AMD in one or both eyes by a retinal specialist (PUD) using a combination of fundus examination, optical coherence tomography (OCT), fundus autofluorescence (FAF), and intravenous fluorescein angiography (IVFA). All patients with pathologies other than AMD were excluded from this study.

Classification and Grading

The MSI images of the 61 eyes with AMD were subdivided into three groups — dry AMD, GA, and neovascular AMD — using the Age-Related Eye Disease Study (AREDS) Classification/Category system, based on defining features. Additionally the melanin disruption seen on the MSI images was graded using the AREDS Grading Scale specifically for level of increased pigmentation, a six-point scale where grade 0 describes no pigmentation and grade 6 shows the highest amount of pigmentation (Table). An Early Treatment Diabetic Retinopathy Study (ETDRS) grid overlay was used for this purpose (Figure 2). Only the central (1), inner nasal (2), inner superior (3), inner temporal (4), and inner inferior (5) areas were graded. Corresponding fundus photos were available for 12 of the 61 eyes. These were also graded for increased level of pigmentation using the same AREDS Grading Scale and then compared to the MSI images for sensitivity and specificity. Grading of MSI images and fundus photos was done by a masked optometrist (CNZ).

Multispectral Imaging

The MSI images were taken with the RHA (Annidis Corporation, Canada), a U.S. Food and Drug Administration-approved, commercially available MSI system that employs 12 specific individual, nonoverlapping, narrow-band light sources using light emitting diodes (LEDs) in a range of wavelengths from 520 nm through 940 nm. MSI creates discrete en face images through the posterior pole of the eye, from the internal limiting membrane (ILM) through to the choroid. The carefully selected spectral bands used by MSI target the clinically relevant structures and metabolic characteristics of the retina and choroid, particularly the ocular chromophores melanin and hemoglobin, as well as the fluorophore lipofuscin. Narrow spectral bands and a combination of illumination processes, including direct feature backscatter, retroillumination with feature silhouetting, and transmission imaging (in which the incident light travels through the sclera, rather than through the pupil), are used to produce a series of images.

Figure 1. Melanin pigment disruption along the nasal border of an area of geographic atrophy using MSI-690.

Figure 2. Grid with central and inner quadrants labelled and standard circles used in assessing size, area, and location of abnormalities. The radii of the grid circles are one-third, 1, and 2 disc diameters, respectively, and their areas are four-ninths, 4, and 16 disc areas (DAs). When the diameter of the optic disc is assumed to be 1,500 µm, the radius of the central circle of the grid is 500 µm, that of the middle (inner) circle is 1,500 µm, and that of the outer circle is 3,000 µm. The standard circles have the following diameters and areas: C-0, 63 µm and 0.0017 DA; C-1, 125 µm and 0.0069 DA; C-2, 250 µm and 0.028 DA; I-2, 354 µm and 0.056 DA; O-2, 650 µm and 0.19 DA; and 0.5 DA, 1,061 µm and 0.50 DA. (Adapted with modifications.)
The ability to optimize the visibility of these retinal pigments with MSI allows for highly contrasted views. Hemorrhages, RPE disruption, and GA boundaries, for example, generally have maximum differential visibility within a relatively narrow spectral range for a given individual but have a wider spectral range when considered across the population. The degree of absorption depends on the impinging wavelength, as well as on the thickness and composition of the structure.

Eight red and infrared spectral bands ranging in wavelength from 620 nm to 850 nm are used to ensure RPE visibility across a large range of patient demographics. The patented viewing software allows the full image set to be rapidly reviewed, removing the requirement for individualized wavelength optimization by patient. A comparison between a traditional fundus photograph and MSI-690 is shown in Figure 3, where mild RPE disruptions, including melanin pigment changes, are seen on the long wavelength MSI. Subtle RPE atrophy is visible on the fundus photo, but the changes in melanin are not readily observed.

**TABLE**

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Adapted without modification from: Davis MD, Gangnon RE, Lee L, et al. The age-related eye disease study severity scale for age-related macular degeneration. The RPE structure, in particular, is highlighted through optimization of the incident wavelengths. The MSI images of 61 eyes of 43 patients who were diagnosed with AMD were subdivided into three groups using the AREDS classification/category system, based on defining features. The results were as follows: dry AMD ($n = 25; 41\%$), GA ($n = 9; 15\%$), and neovascular AMD ($n = 27; 44\%$). Of the 43 patients, 21 were male. The mean age (± standard deviation) was 78 years ± 11.4 years. Fifteen of the 43 patients were phakic, and the remainder were pseudophakic.

Melanin disruption was defined as level of increased pigmentation, particularly melanin clumping, stacking, or migration, but not specifically atrophy. Fifty-one of 61 MSI images (84%) showed some
form of melanin disruption in at least the central and/or four inner ETDRS quadrants. The other 10 MSI images were identified as having AMD by the presence of drusen, GA, or choroidal neovascularization in the absence of melanin disruption. Examples of the increased pigmentation grading system are shown in Figure 4. Anomalous pigment disruption and accumulation was differentiated from the normal background pigment variation based on contrast, density, and contextual differences. The average grading for each group is summarized in Figure 5. There was generally more melanin disruption centrally than in the inner quadrants, with an average central grade of 1.6, 3.4, and 2.7 out of 6 for the MSI images for dry AMD, GA, and neovascular AMD, respectively. There was a relationship between severity of disease based on category and the degree of melanin disruption. In other words, with advancing disease, there is an increase in abnormal melanin pigment distribution in all quadrants.

The corresponding fundus photos for 12 of the 61 eyes were also graded for level of increased pigmentation using the same AREDS pigmentation grading scale. The results are shown in Figure 6. The MSI images were more likely to show the presence and severity of melanin disruption than the fundus photos. The sensitivity of fundus photography for pigmentation, as compared to MSI, was only 62.5% with three false-negatives. The specificity for both was 100%, as there were no false-positives. Figure 7 shows a comparison of the graded MSI-690 and fundus photos for an 86-year-old female, as well as a transparent registered overlay supporting the results.

The mean MSI wavelength where melanin disruption could best be viewed was 682.6 nm ± 52.4 nm. This corresponds most closely with the MSI-690 image, but varied due to patient pigmentation variability.

**DISCUSSION**

**Pathophysiology of the RPE in Dry AMD**

There is a synergistic relationship between the functionally dependent photoreceptors, RPE, Bruch’s membrane, and choriocapillaris (CC) complex. As such, if any one of these components is damaged, the remaining structures fail to function adequately. The RPE is a monolayer of highly pigmented hexagonal cells lining the inner layer of Bruch’s membrane. One of the RPE’s many roles is to phagocytize the byproducts produced by the outer segment of the photoreceptors, particularly retinylidene-N-retinyl-ethanolamine (A2E), which is a created through the conversion of 11-cis-retinal to all-trans-retinal during the visual cycle. With age, these outer segment byproducts are not properly phagocytized. The RPE also transports nutrients from the vascular CC, across Bruch’s membrane to the photoreceptors. Bruch’s
membrane thickening due to lipid and protein accumulation results in the formation of sub-RPE drusen.\textsuperscript{1} The further the photoreceptors are from their source of nutrients in the CC, the greater the chance of defective nutrient transport, increased oxidative stress, and cell death.\textsuperscript{13}

Melanin, an insoluble, high-molecular-weight, brownish-black heterogeneous polymer derived from the enzymatic oxidation of tyrosine and dihydroxyphenylalanine scavenges free radicals and reactive oxygen species, protecting the cells from oxidative stress, light toxicity, and the cytotoxic effects of ocular inflammation.\textsuperscript{10,11,14,15} Lipofuscin, a nondegradable intralysosomal substance that forms as a result of oxidation and polymerization of proteins and lipids, may interfere with the phagocytic properties of the RPE and sensitize the eye to blue light.\textsuperscript{16}

Experimental studies have indicated that RPE atrophy causes secondary photoreceptor degeneration and loss of the CC, leading to advanced AMD.\textsuperscript{10,12,13,17} The loss of the RPE and deposition of soft drusen may be predictive of progression to GA and choroidal neovascularization.\textsuperscript{18}

Histologically, the RPE at the borders of GA may show dysmorphic, hypertrophic, or hyperplastic cells.\textsuperscript{19} These RPE cells have been shown to be vertically superimposed with a resultant discontinuous layer of large, rounded, sloughed RPE cells next to clumped, small RPE cells.\textsuperscript{19,20} Stacking of melanin has also been shown.\textsuperscript{21} In May 2013, Adaptive Optics near-infrared, high-resolution imaging showed that hyporeflective clumps precede and occur in conjunction with progression to GA in AMD.\textsuperscript{22}

These changes in RPE cell morphology result in an increased autofluorescence at the transition zone between normal RPE and advancing GA, with a sensitivity and specificity toward cell death of approximately 75\% each.\textsuperscript{19} It has been theorized that autofluorescence in the transition zones may serve as a disease marker to predict GA progression.\textsuperscript{23} Melanin disruption in the same vicinity may also be an indication of advancing GA. This is illustrated in Figure 8, which shows successive MSI-690 images of a 76-year-old female during a 19-month period. Abnormal melanin pigment distribution can be observed at the inferior border of the atrophic lesion in the first image. The second and third images show progressing atrophy in the direction of the increased pigmentation.

**RPE and Choroidal Melanin**

Prota published the absorption spectra for various retinal components.\textsuperscript{24} Maximizing the differential visibility requires utilizing small spectral slices. Melanin absorption falls within the wavelength spectrum used by traditional fundus cameras (450 nm to 700 nm). In the wavelength range from 450 nm to 600 nm, however, melanin is obscured by other dominant components of the eye including the lens, hemoglobin, zeaxanthin, lutein, and rhodopsin.\textsuperscript{24} Beyond 600 nm, melanin is the prevailing retinal pigment. The
long wavelength light received by a retinal camera using a white light flash system is typically dominated by the fundus reflex, which results from melanin-filtered scleral reflection, the magnitude of which is dependent upon the choroidal melanin contribution. The larger the choroidal contribution of melanin, the poorer the visibility of subtle retinal melanin features.

**Current Clinical Imaging Modalities and Challenges With Respect to RPE Imaging**

Traditional color fundus photography provides a visual representation of a clinical observation using the visible spectrum, with a peak centered at 555 nm to match the peak sensitivity of the human eye. Early and subtle pathologies may be difficult to isolate, particularly in the case of comorbidity. In addition, when white light digital photography is used, normal retinal pigment, particularly in highly pigmented individuals, can restrict the view of deeper retinal structures. Taking a traditional color fundus photo and subsequently using software to split the image into red, green, and blue channels or utilizing filters to provide monochromatic renderings does not provide the same beneficial effects of MSI. First, there is a loss of spatial resolution. There are also the short wavelength issues of scatter and the inability to view overlapping retinal structures and pathologies. In addition, the wide bandwidth of the red channel does not provide sufficient discrimination to allow subtle differences in melanin distribution to be clearly imaged.

SD-OCT has limitations with respect to illustrating subtle alterations in RPE morphology because of the similar reflectivity of normal RPE and adjacent RPE with atrophy or changes in melanin distribution. Histologically, GA appears as focal retinal thinning over an atrophic zone of RPE. Small areas of atrophy of the RPE may not be visible with OCT due to difficulties differentiating the Bruch’s membrane-choriocapillaris complex from the RPE. Polarization-sensitive SD-OCT can detect the depolarizing properties of the RPE and may be able to determine areas of atrophy but is limited by summation of OCT images.

**MSI Attributes Pertaining to RPE Disruption**

Most forms of retinal imaging illuminate the retinal layers through the pupil and then collect the counter propagating light exiting the pupil to form the image. Each interface and structure in the retina produces scattering, which is the essence of the OCT signal. This scattering occurs in all directions exhibiting both specular and nonspecular components. In particular, Bruch’s membrane and the sclera act as broad band reflectors that can provide background illumination to the inner layers in the eye.

The key element behind MSI performance and differential visibility, especially with respect to the outer retinal layers, is a fine balance between reflection, transmission, and absorption of ocular features against the background. In general, the shorter wavelengths are back-scattered and absorbed by the

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*Figure 6. Retinal pigment epithelium melanin disruption grade for multispectral imaging versus fundus photos centrally (1).*
inner layers to generate a conventional scattered-light image with little retro-illumination. As the MSI wavelengths increase in length, the absorption of the surface layers is reduced and more light is able to be back scattered by deeper layers and structures, gradually increasing the degree of retro-illumination. The back scatter and absorption depends on reflection from Bruch’s membrane, with varying degrees of RPE absorption adding wavelength dependence to the reflection strength.

Embryologically, the RPE is derived from the neuroepithelium and, therefore, there is no racial difference in RPE pigmentation. On the other hand, the neural crest is the origin for melanocytes in the choroid, as such, choroidal ethnic differences may be noted. Above a certain wavelength — which tends to be patient-specific due to the aforementioned variations in choroidal melanin concentration, but averages 680.8 nm, as revealed by this study — the back reflection from the sclera becomes more significant and the ability to observe melanin disruption is realized. The red and infrared wavelengths from 620 nm to 850 nm bypass shallower structures and are absorbed by the melanin in the RPE in the outer retina, revealing metabolic and structural defects that may not be seen otherwise.

**Current and Future Management of Dry AMD**

There is a multifactorial mechanism to RPE melanin disruption that includes oxidative stress, light exposure, genetics and environmental factors. Reducing oxidative stress may be a way to prevent the disease. The current management for dry AMD consists of nutritional therapy as supported by AREDS.
I and II.28 Fortunately, supplementation with lutein and zeaxanthin, absorbed by macular pigment, helps to reduce oxidative stress by blocking blue light.1

The focus of future management of dry AMD may veer away from preventative vitamin therapy toward targeted pharmaceutical therapy. These treatments will likely work one of two ways: either by modifying the pathophysiological damage to the photoreceptor/RPE/BrM/choriocapillaris complex or by suppressing the inflammatory component of dry AMD.29 Visual cycle modulation involves slowing the accumulation of A2E in the RPE or amyloid beta in drusen.2 Corticosteroids and complement pathway inhibition may address the inflammatory nature of dry AMD.1,29 In either case, adequate clinical visualization of the RPE, which can best be obtained using MSI, is required to monitor progress.

CONCLUSION

This study illustrates that MSI shows the presence of RPE melanin disruption, particularly pigment accumulation, in dry AMD, GA, and neovascular AMD more readily than traditional fundus photography. The pathophysiology of AMD is complicated and current therapeutic management strategies remain limited. MSI could ultimately replace traditional fundus photography due to its versatility and ability to image specific retinal structures. It is a useful screening and therapeutic management tool able to provide a direct, noninvasive, unobstructed view of the RPE, particularly melanin disruption. This is particularly useful for early diagnosis and monitoring the effects of forthcoming treatment strategies.

REFERENCES