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Correlation between Spectral Optical Coherence Tomography and Fundus Autofluorescence at the margins of Geographic Atrophy

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Abstract

Purpose—We studied the appearance of margins of Geographic atrophy in high- resolution optical coherence tomography (OCT) images and correlate those changes with fundus autofluorescence imaging.

Design—Retrospective observational case study.

Methods—Patients with geographic atrophy secondary to dry age related macular degeneration (ARMD) were assessed by means of Spectral Domain OCT (Spectralis HRA/OCT; Heidelberg Engineering, Heidelberg, Germany or OTI, Inc, Toronto, Canada) as well as Autofluorescence Imaging (HRA or Spectralis Heidelberg Engineering, Heidelberg, Germany): The outer retinal layer alterations were analyzed in the junctional zone between normal retina and atrophic retina, and correlated with corresponding fundus autofluorescence.

Results—23 eyes of 16 patients aged between 62 years to 96 years were examined. There was a significant association between OCT findings and the fundus autofluorescence findings($r=0.67$, $p<0.0001$). Severe alterations of the outer retinal layers at margins on Spectral OCT correspond significantly to increased autofluorescence; Smooth margins on OCT correspond significantly to normal fundus autofluorescence. ($Kappa=0.7348$, $p<0.0001$).

Conclusion—Spectral OCT provides in vivo insight into the pathogenesis of geographic atrophy and its progression. Visualization of reactive changes in the retinal pigment epithelial cells at the junctional zone and correlation with increased fundus autofluorescence; secondary to increased lipofuscin may together serve as determinants of progression of geographic atrophy.

Introduction

Geographic atrophy is responsible for approximately 20% of legal blindness attributable to age related macular degeneration (ARMD) and will become even more prevalent with the growth of the elderly population^{1 2}. The pathophysiologic mechanism of retinal pigment epithelium (RPE) cell atrophy with corresponding atrophy of the choriocapillaries and outer neurosensory

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retinal layers in this disorder still remain unclear. Several lines of experimental and clinical evidence indicate that lipofuscin accumulation in the lysosomal compartment of RPE cells plays a critical role in the disease process^{3,4,5,6}. This is supported by in vitro studies.⁷

Various studies done in the past using fundus auto fluorescence have shown that lipofuscin laden RPE cells in the junctional zone of geographic atrophy correspond to the band of increased autofluorescence^{8,9,10}. Optical coherence tomography (OCT) is another way to study tissue morphology in situ and in real time without the need to excise and process specimens. With the advent of spectral domain OCT (SD-OCT) it is now possible to differentiate 11 structural characteristics within the retina including visualization of photoreceptor and pigment epithelial morphology as well as subtle changes associated with disease process¹¹⁻¹². The purpose of our study was to characterize the OCT changes occurring in the junctional zones of geographic atrophy using spectral OCT images and correlate it with the fundus autofluorescence findings.

Methods

We retrospectively examined Spectral OCT scans, fundus autofluorescence images, color fundus photographs and fluorescein angiography images of 16 consecutive patients with geographic atrophy. All studies were performed at the UCSD Jacobs Retina Center and all imaging and clinical examinations were performed within 4 weeks of each other. All patients included in our study underwent a thorough clinical examination with best corrected visual acuity using ETDRS charts, indirect ophthalmoscopy, color fundus photography and fluorescein angiography to confirm the diagnosis of geographic atrophy.

Inclusion Criteria

Eyes with geographic atrophy secondary to dry ARMD were included. Geographic atrophy was defined as one or more discrete areas of loss of RPE measuring 0.5mm or more in greatest linear diameter, with a color change relative to the surrounding RPE and more prominent visualization of the choroidal vessels¹³. Only eyes with a good quality fundus autofluorescence images were studied.

Exclusion Criteria

Eyes with atrophy of RPE secondary to wet AMD, hereditary retinal diseases and laser photocoagulation were excluded from the study. In addition eyes with pathological myopia, inflammatory choroidopathies, retinal detachment, and ocular trauma or vitreoretinal surgeries were not included in the study.

Instruments

Spectral OCT images reviewed in the study were obtained using the Spectralis HRA/OCT (Heidelberg Retinal Angiograph, Heidelberg Engineering, Heidelberg, Germany) or Spectral OCT (OTI, Inc, Toronto, Canada.). Spectralis combines high-resolution spectral domain OCT with a scanning laser ophthalmoscope (SLO). The system allows for simultaneous OCT scans with fundus autofluorescence imaging, Infrared imaging, Fluorescein angiography or indocyanine green (ICG) angiography. The instrument uses a broadband 870nm super luminescent diode laser (SLD) for the OCT channel. The retina is scanned at 40,000 A-scans per second, creating highly detailed images of the structure of the retina with an optical depth resolution of 7µm. Twenty five OCT scans were averaged to reduce speckle noise. The OTI spectral domain OCT captures pseudo-simultaneous OCT and SLO image pairs and also allowed us to co-localize the spectral domain OCT with retinal lesions and autofluorescence images¹⁴ {Mojana F, et al. IOVS 2008; ARVO E-abstract # 260, Brar M, et al. IOVS 2008; ARVO E-abstract # 926}. It uses a modified superluminescent diode with a 40nm

bandwidth centered at 830nm wavelength, thus achieving an axial resolution up to 5-7 microns and a transverse one of 16 microns. It has a scanning speed of up to 28,000 A-scans per second. Each B-scan is composed of 512 A-scans and requires 0.182 seconds to acquire. A set of 128 B-scans is acquired covering an area of 8mm × 8mm through the macula. The operator, who manually sweeps the line cursor overlapping the fundus images, chooses the scan orientation in the x-y plane. The fundus images are obtained with a confocal scanning laser ophthalmoscope combined with the OCT system. Fundus autofluorescence images were recorded using Spectralis or a confocal Scanning Laser Ophthalmoscope (HRA2, Germany), the optical and technical principles of which have been described previously^{15 16 17}. The field size was selected as 30° × 30° at 1,536 × 1,536 pixels. A time sequence of 9-25 frames was captured. The exact number of frames varied between patients and was dependent on media clarity.

Image Selection and Grading

Two reviewers independently graded the fundus autofluorescence and OCT images of each patient and were not aware of the results of one modality when scoring for the other. They reviewed OCT images at the junctional zone between normal retina and atrophic area. Outer retinal layer alterations were studied in the junctional zone and margins of GA lesion were divided into two groups. Horizontal and vertical scans approximately passing through the center of the lesion were chosen and all the four (superior, inferior, nasal, temporal) margins were reviewed and each margin was classified into type 1 or type 2.

Patients in whom margins of GA were found to be smooth with no alterations of the outer retina were classified as type 1 (figure 3) and the other group with severe alterations in the outer retinal layers; irregular margins with or without increased optical reflectivity of the RPE were classified into type 2 (figure 4). Corresponding auto fluorescence at all the four margins was reviewed separately by two independent reviewers and classified again into two types. Type 1 comprise of no abnormal fundus autofluorescence (figure 3) and type 2 (figure 4), for increased autofluorescence at the margins of GA. The Spectralis HRA/OCT allows simultaneous OCT scans and fundus autofluorescence. But for those set of patients who had spectral OCT scans done using OTI, (OCT/SLO, OTI Inc, Toronto, Canada) fundus autofluorescence images were overlaid on the corresponding SLO image considering the vessel as a reference for a correct alignment.

Statistical analysis

Two observers graded the same patient on the four margins of the same lesion. In order to examine if there is a general association between OCT findings and fundus autofluorescence findings, Cochran-Mantel-Haenszel test was performed using observer and patient as the strata.

The agreement of ratings by each observer based on two instruments was measured by calculating the Kappa within each observer. The agreement was also measured and tested between the two observers. In addition, correlation among the four margins of each eye on both OCT and fundus autofluorescence was analyzed using Spearman correlation test. All tests were two tailed and p<0.05 was considered statistically significant (SAS software v 9.2, Cary, North Carolina).

Results

23 eyes of 16 patients (9 women, 7 men) aged between 62 to 96 years with unifocal or multifocal geographic atrophy due to dry AMD were included in the study. We examined 4 margins per eye, if both eyes were included in the study i.e. 8 margins per patient. There was a good

agreement between two observers in terms of grading the lesions on OCT ($\kappa = 0.8644$, $p < 0.0001$) and fundus autofluorescence image ($\kappa = 0.9762$, $p < 0.0001$).

After adjustment (using Cochran-Mantel-Haenszel test) of inter-observer and intra patient there was a significant association between OCT findings and the fundus autofluorescence findings ($r = 0.67$, $p < 0.0001$). OCT seen changes in the junctional zone including outer retinal layer alterations were found to correlate well with increased fundus autofluorescence and smooth margins with no structural changes were appreciated at points correlating to areas with normal fundus autofluorescence ($\kappa = 0.7348$, $p = 0.0001$) (Table 1). In most cases with increased fundus autofluorescence at the margin there was a corresponding structural alteration at the outer retina (93.64%) (Figure 3,5).

Highly reflective material at the level of RPE and irregularities found in the junctional zone, are possibly caused by RPE hypertrophy and /or phagocytosed melanin by RPE / macrophages^{18, 19}. In the lesions showing no abnormalities in autofluorescence, the smooth OCT margins suggest no abnormal accumulation of lipofuscin and thus no changes in the RPE cells (78.38%) (Figure 2). We also statistically analyzed the correlation between fundus autofluorescence and the type of OCT margin by randomly picking up one margin (temporal) per eye per patient. Kappa value was 0.7089, $p < 0.0002$. In addition to this, there was found to be a correlation although not strong among four margins of each eye on OCT and fundus autofluorescence (Table 2).

Discussion

Geographic atrophy is defined as any sharply delineated round or oval area of hypopigmentation or depigmentation or apparent absence of the RPE, in which choroidal vessels are more visible than in the surrounding areas and which must be at least 175 μ m in diameter²⁰. However, since such a small area could result from the regression of a single drusen, other proposed dimensions have been wider, varying from 200 μ m²¹, 500 μ m¹³, 700 μ m²² to 1mm²³. We studied eyes with geographic atrophy at least 500 μ m in its greatest linear diameter as was done in the recent past by other study groups²⁴.

With the advent of novel combined high resolution OCT-SLO system it has become possible to get simultaneous fundus autofluorescence / OCT images making it easier to find small changes in fundus autofluorescence and analyze them on OCT²⁵. In our study, we have shown that there is a statistically significant correlation between pattern of fundus autofluorescence and type of OCT margin in patients of geographic atrophy secondary to AMD. In our cross-sectional analysis we identified two distinct patterns of structural changes at the margins of geographic atrophy on spectral OCT corresponding to specific fundus autofluorescence patterns. Those areas showing increased fundus autofluorescence have a corresponding irregular margin on OCT as compared to the second group with no abnormal fundus autofluorescence at the margins and corresponding smooth margin on OCT.

Geographic atrophy in association with AMD has been shown to gradually enlarge over time^{26, 13, 21}. Recent study done by Sunness et al using stereo color fundus photographs has reported the median overall enlargement rate of geographic atrophy to be 2.1mm²/year²⁴. Further studies using fundus autofluorescence have shown that increased fundus autofluorescence patterns at the junctional zone precede the development and enlargement of preexisting geographic atrophy⁹. It has also been indicated that progression is smaller in eyes with minimal or no fundus autofluorescence alterations at the junctional zone as opposed to eyes with widespread diffuse changes²⁷.

Our study using spectral OCT provides in vivo insight into the morphological alterations at the margins of geographic atrophy. Findings in our study correlate well with the

histopathological studies performed in the past ⁷. In those studies, lipofuscin and melanolipofuscin-filled RPE cells were observed in the junctional zone between the atrophic and the normal retina. In the zone of severe degeneration towards the atrophic region, RPE cells were found to become increasingly abnormal in shape and cell drop out was evident. Close to the edge of the atrophic area large hyper-pigmented RPE cells were observed to be shed into the subretinal space. Many of those cells contained large membrane bound bodies filled with fused lipofuscin and the subsequent increased autofluorescence granules in these cells is not only the result of autophagy and outer segment phagocytosis but also of engulfment of cellular debris, including spent RPE cells. In our study outer retinal changes seen on Spectral OCT might suggest abnormal hypertrophic and damaged RPE cells with increased lipofuscin correlating to increased auto fluorescence on fundus autofluorescence image as well.

In summary, combined OCT-SLO system used in our study allows detection of small structural changes at the margins of geographic atrophy. Currently there is no gold standard predictor of vision loss in geographic atrophy, but large studies using fundus autofluorescence has shown that progression rates in eyes with increased fundus autofluorescence were significantly higher compared to eyes without fundus autofluorescence abnormalities ^{9,27}. Fundus autofluorescence is a useful predictor for assessing the progression of geographic atrophy but spectral OCT gives us a low power in vivo insight into the structural changes at the junctional zone. Since spectral domain imaging allows visualization of the pathological structural changes it is intriguing to speculate that OCT changes might be a better predictor than autofluorescence of geographic atrophy prediction. Our study only shows that the two changes are correlated but does not allow determination of which was the better predictor of clinical progression. The ability to anatomically classify the margins of geographic atrophy on Spectral OCT might serve as a prognostic determinant of geographic atrophy progression together with fundus autofluorescence. Future longitudinal studies may further contribute to understand the progression of geographic atrophy.

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Biography

Manpreet Brar, MD, graduated from Govt. Medical College, Chandigarh, India. She completed her residency at the Dept. of Ophthalmology, Christian Medical College, Ludhiana, India in December 2004 and is presently undergoing a retina fellowship at Jacobs Retina Center, Shiley eye Center, University of California San Diego, USA.

Figure 1.

Normal retina as imaged by HRA + OCT (SPECTRALIS). Arrows: Different outer retinal layers as imaged by spectralis. External limiting membrane (ELM), IS/OS (inner segment outer segment junction) and RPE (retinal pigment epithelium layer). Inset; Magnified image of the outer retina.

Figure 2.

Fundus autofluorescence and Spectral OCT images from a 74 year old man with geographic atrophy. Fundus autofluorescence image showed no abnormal increased auto fluorescence at the margins. Horizontal scan on OCT showed atrophic outer retina with loss of normal reflectivity of the ELM, IS/OS, ONL and thin RPE with increased choroidal transmission. Outside the geographic atrophy area, retinal layer structures appear normal and margins are smooth and sharp with no abnormal activity at the level of RPE. (Arrows), corresponding to none fundus autofluorescence at the margins. (Arrowheads)

Figure 3.

Fundus autofluorescence and spectral OCT images from an 87-year woman with geographic atrophy. Fundus autofluorescence image shows a continuous band of abnormal increased autofluorescence at the margins of geographic atrophy. Corresponding Spectral OCT image shows severe alterations in the outer retinal layers at the margins of geographic atrophy. Arrows indicate increased hyper reflectivity, irregularities and thickening at the level of RPE with loss of ELM. Arrowheads: Corresponding areas on fundus autofluorescence image.

Figure 4.

Fundus autofluorescence and Spectral OCT images with horizontal scans at two different levels in a patient with geographic atrophy. TOP: Spectral OCT shows irregular margins with structural alterations at the outer retina (Black arrow) corresponding to increased Fundus autofluorescence (black arrowhead) at the nasal margin. BOTTOM: Horizontal scans through the area of no abnormal fundus autofluorescence (white arrowhead) shows smooth margins on the Spectral OCT. (White arrow)

Figure 5. Simultaneous fundus autofluorescence and Spectral OCT image from Spectralis (HRA + OCT) in a patient with geographic atrophy: Fundus autofluorescence image shows diffusely increased FAF (arrowhead) and corresponding horizontal scan on Spectral OCT shows atrophic outer retina with an irregular border. Arrows: structural alterations in the outer retina at the margins of geographic atrophy.

Table 1

Correlation between Spectral OCT and Fundus autofluorescence at the margins of Geographic atrophy in 23 eyes (92 margins) *.

OCT MARGIN	FUNDUS AUTOFLUORESCENCE		TOTAL
	NORMAL	INCREASED	
SMOOTH	29 (78.0%)	8 (22%)	37 (100%)
ACTIVE	3 (6.0%)	52 (94.0%)	55 (100%)
TOTAL	32	60	92 100.00%

KAPPA = 0.7348, $P < 0.0001$.

Values in the table represent average score of two observers. Brackets: percent values.

* Analysis of one margin per patient selected randomly (n= 23) showed, Kappa = 0.7089, $p < 0.0002$.

Table 2

Correlation of four margins of each eye with geographic atrophy on Spectral OCT and fundus autofluorescence.

2A			
OCT MARGIN		R	P VALUE
Nasal	Superior	0.36364	0.013
Nasal	Inferior	0.52089	0.0002
Nasal	Temporal	0.54449	<0.0001
Superior	Inferior	0.32786	0.0261
Superior	Temporal	0.37776	0.0096
Inferior	Temporal	0.5708	<0.0001
R- Spearman correlation coefficient.			
2B			
FAF PATTERN		R	P VALUE
Nasal	Superior	0.69957	0.0038
Nasal	Inferior	0.67737	<0.0001
Nasal	Temporal	0.73446	<0.0001
Superior	Inferior	0.64284	<0.0001
Superior	Temporal	0.58252	<0.0001
Inferior	Temporal	0.53808	<0.0001

FAF-fundus autofluorescence, R- Spearman correlation coefficient