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Correlations between Optical Coherence Tomography Angiography Findings and Multifocal Electroretinogram Parameters in Retinitis Pigmentosa Patients

한정우, 이상언, 최은우, 윤영훈

Jung Woo Han, Sang Earn Woo, Eun Woo Choi, Young-Hoon Ohn

순천향대학교 부천병원 안과

Department of Ophthalmology, Soonchunhyang University Bucheon Hospital, Bucheon, Korea

Purpose: We investigated the correlations between macular function recorded with a multifocal electroretinogram (mfERG) and macular structure evaluated by optical coherence tomography angiography (OCT-A) in patients with retinitis pigmentosa (RP).

Methods: In total, 30 eyes of 15 RP patients and 40 eyes of 20 healthy subjects were enrolled in this study. OCT-A and mfERG were performed for all patients. The area and circularity of the foveal avascular zone (FAZ) were measured, and the vessel density (VD) and perfusion density (PD) of the superficial capillary plexus and deep capillary plexus (SCP and DCP, respectively) were estimated. The average amplitude and implicit time of N1, P1, and N2 in rings 1 and 2 were obtained from the mfERG findings. Correlation between the OCT-A results and mfERG parameters were analyzed in RP patients and normal subjects.

Results: The mean FAZ area in RP patients increased significantly compared with that in normal subjects ($p < 0.001$). Both the PD and VD in the SCP and DCP were significantly reduced in RP patients compared with normal subjects ($p < 0.001$ and $p < 0.001$, respectively). The mean amplitude of N1, P1, and N2 from rings 1 and 2 were significantly reduced in RP patients compared with normal subjects. The superficial FAZ and deep FAZ area were significantly correlated with P1 amplitude ($r = -0.682$, $p = 0.000$ and $r = -0.612$, $p = 0.000$, respectively). Both the VD and PD of the superficial plexus were correlated with P1 amplitude on mfERG ($r = 0.188$, $p = 0.021$; $r = 0.215$, $p = 0.013$; $r = 0.323$, $p = 0.002$; and $r = 0.362$, $p = 0.001$, respectively).

Conclusions: The P1 amplitude from mfERG was significantly correlated with FAZ area and the PD and VD of the SCP and DCP in RP patients. Together, these results suggest that OCT-A together with mfERG is a useful modality for evaluating macular structure and function in RP patients.

Keywords: Multifocal electroretinogram; Optical coherence tomography angiography; Retinitis pigmentosa

Introduction

Retinitis pigmentosa (RP) refers to a heterogeneous group

of inherited dystrophies that is characterized by progressive primary degeneration of rods and secondary but critical degeneration of cones. The visual impairment typically

Address reprint requests to Young-Hoon Ohn, MD, PhD

Department of Ophthalmology, Soonchunhyang University Hospital Bucheon, #170 Jomaru-ro, Wonmi-gu, Bucheon 14584, Korea
Tel: 82-32-621-5053, Fax: 82-32-621-5435
E-mail: yjohn@schmc.ac.kr

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involves night vision and midperipheral vision, with gradual deterioration of central visual acuity (VA) [1-3]. The pathogenesis of RP is complex, with loss of rods and cones accompanied by changes in the retinal pigment epithelium and retinal glia. Ultimately, the inner retinal neurons, blood vessels, and optic nerve head are affected by the disease.

The severity of RP can be evaluated using different examination and imaging modalities, including VA testing, visual field (VF) testing, electroretinography, optical coherence tomography (OCT), and multifocal electroretinograms (mfERGs) [4]. The mfERG is a valuable test for detecting outer retinal disorders [5], and it has been used to quantify the remaining cone-mediated function in RP patients [6-8]. It is especially useful in advanced stages of the disease [9].

Study of retinal and choroidal vasculatures has become more feasible with the advent of OCT angiography (OCT-A). OCT-A is a noninvasive and ideal alternative to fluorescein angiography because it produces results more quickly and avoids the potential side effects of fluorescein angiography, including vomiting and hypersensitivity reactions. OCT-A detects streaming blood flow and constructs an image of the retinal vasculature, facilitating visualization of the superficial capillary plexus and deep capillary plexus [10]. The choriocapillaris also can be visualized, but the small size and intersinusoidal spacing of its blood vessels cause it to appear homogenous with bright areas that represent blood flow [11]. The technology of OCT-A has been applied broadly to study vasculature changes in inherited retinal dystrophies, including RP, Stargardt disease, and choroideremia [12-16].

However, it is unclear how these microcirculatory changes correlate with morphological and functional changes. Our study investigated the correlations between macular function recorded with mfERG and macular structure that was determined using OCT-A in RP patients.

Materials and Methods

Ethics statement

This study was approved by the Institutional Review Board of our institute (2020-01-010). Informed consent was waived due to the retrospective nature of the study. The research and data collection were conducted in accordance with the tenets of the Declaration of Helsinki from the World Medical Association.

Participants

The present study included 15 consecutive RP patients who visited the Department of Ophthalmology at our institution from September 1, 2017, to December 31, 2019. Clinical diagnosis of RP was based on ocular history, family history, funduscopy findings, VF testing, and International Society for Clinical Electrophysiology of Vision standard full-field electroretinograms (ffERGs). The exclusion criteria were as follows: significant media opacity, presence of maculopathy involving cystoid macular edema, glaucoma, nystagmus, myopia greater than -6.0 diopters, or any systemic or neurological disease that could affect the tests. In total, 40 eyes of 40 age-matched subjects without any history of chronic or systemic disease or pathological features identified after a complete ophthalmic examination were enrolled as controls. All patients and controls underwent a complete ophthalmic examination, including best-corrected visual acuity (BCVA) using the logarithm of the minimum angle of resolution (logMAR), intraocular pressure, refraction, slit lamp biomicroscopy, dilated stereoscopic fundus examination, OCT-A, and mfERG. FAG was not performed on all patients.

OCT-A image analysis

OCT-A was performed using a Zeiss Cirrus 5000 with Angioplex (Carl Zeiss Meditec, Dublin, CA, USA). The Angioplex used optical microangiography, a recently developed imaging technique that produces 3D images of the dynamic blood flow within the microcirculatory tissue beds. A scanned area of 3×3 mm centered on the fovea was used. All acquisitions were performed using FastTrac™ (Carl Zeiss Meditec) retinal tracking technology to reduce motion artifacts. Segmentation of the retinal layers was automatically performed by the embedded software. In addition to the non-segmented en-face images (whole retina), en-face images of the superficial retinal capillary layer plexus and the deep retinal capillary layer plexus were analyzed. The superficial foveal avascular zone (FAZ) area, superficial vessel density (VD), superficial perfusion density (PD), deep FAZ area, deep VD, and deep PD were also evaluated. We did not analyze the choriocapillaris vessels in this study.

mfERGs

mfERGs (VERIS 6.4.4; EDI, Redwood City, CA, USA) were performed with Burian-Allen bipolar electrodes using standard protocols. Pupils were fully dilated with topical

application of 1% tropicamide and 2.5% phenylephrine hydrochloride. The stimulation matrix consisted of a 103-cell hexagonal element pattern array displayed on a cathode ray tube monitor at a frame rate of 75 Hz at 32 cm from the participants' eyes. Each hexagon was modulated between black ($<10 \text{ cd/m}^2$) and white (200 cd/m^2) according to a binary m-sequence. The duration of data acquisition was 4 minutes and was divided into eight sessions of 30 seconds. The waveforms were recorded, amplified ($\times 200,000$), and band pass-filtered (5-100 Hz). The responses were analyzed according to ring averages. The average amplitude and implicit time of N1 and P1 in rings 1 and 2 were measured (Fig. 1).

Early experience with the method has shown no detectable multifocal responses in a small proportion of patients with retinitis pigmentosa. Therefore, we maximized the fixation target and performed the tests by guiding the patients to gaze

Figure 1. Spatial correlation between optical coherence tomography angiography and hexagonal elements of multifocal electroretinogram (mfERG).

Table 1. Study group characteristics

Characteristic	RP patients (n = 15)	Control group (n = 20)	p-value*
Age (years)	42.34 ± 11.19	43.11 ± 13.83	0.314
Sex (male:female)	5:10	10:10	-
Spherical equivalent (D)	-1.54 ± 2.45	-0.95 ± 2.56	0.175
BCVA (logMAR)	0.56 ± 0.16	0.00 ± 0.18	0.001 [†]

Values are mean ± standard deviation unless otherwise indicated. RP = retinitis pigmentosa; D = diopters; BCVA = best corrected visual acuity; logMAR = logarithm of minimal angle of resolution.

*Mann-Whitney *U* tests with Bonferroni correction; [†]The significance level cutoff of $p \leq 0.05$.

as far forward as possible, for a more accurate examination.

Statistical analyses

Data are presented as mean ± standard deviation. The BCVA values were converted to logMAR for statistical analysis. The Mann-Whitney *U* test and Bonferroni correction were used to compare data sets, as appropriate. The degree of correlation between two variables was expressed as linear regression analysis. A value of $p < 0.05$ was considered statistically significant.

Table 2. Quantitative analysis of macular vascular density and FAZ between retinitis pigmentosa patients and controls

	RP patients	Control group	p-value*
SFAZ area (mm^2)	0.39 ± 0.12	0.27 ± 0.12	<0.001 [†]
DFAZ area (mm^2)	0.41 ± 0.11	0.29 ± 0.13	<0.001 [†]
FAZ circularity	0.66 ± 0.11	0.66 ± 0.15	0.280
SPD	0.38 ± 0.05	0.44 ± 0.04	<0.001 [†]
DPD	0.35 ± 0.08	0.42 ± 0.03	<0.001 [†]
SVD (mm^{-1})	16.88 ± 1.93	19.46 ± 0.70	<0.001 [†]
DVD (mm^{-1})	13.51 ± 2.91	17.23 ± 1.41	<0.001 [†]

Values are mean ± standard deviation.

FAZ = foveal avascular zone; SFAZ = superficial FAZ; DFAZ = deep FAZ; SPD = superficial perfusion density; DPD = deep perfusion density; SVD = superficial vessel density; DVD = deep vessel density.

*Mann-Whitney *U* tests with Bonferroni correction; [†]The significance level cutoff of $p \leq 0.05$.

Table 3. mfERG parameters in rings 1 and 2 in retinitis pigmentosa patients and controls

	RP patients	Control group	p-value*
Response density (nV/deg ²)			
N1	17.44 ± 7.94	28.38 ± 6.30	<0.001 [†]
P1	26.50 ± 13.30	44.75 ± 15.7	<0.001 [†]
N2	27.01 ± 4.80	78.59 ± 16.3	<0.001 [†]
Implicit time (ms)			
N1	15.87 ± 2.11	14.55 ± 1.10	0.087
P1	32.64 ± 9.30	28.82 ± 1.01	0.059
N2	47.21 ± 9.51	43.48 ± 2.21	0.080

Values are mean ± standard deviation.

mfERG = multifocal electroretinogram; RP = retinitis pigmentosa.

*Mann-Whitney *U* tests with Bonferroni correction; [†]The significance level cutoff of $p \leq 0.05$.

Results

In total, 25 eyes of 15 patients (5 males and 10 females) who met the inclusion criteria and 40 eyes of 20 normal subjects (10 males and 10 females) were enrolled. The mean age was 42.34 ± 11.19 years in the affected patients and 43.11 ± 13.83 years in normal subjects ($p = 0.314$). The average logMAR BCVA was 0.56 ± 0.16 for the affected patients and 0.00 ± 0.18 for the normal subjects (Table 1).

The macular VD and FAZ in the healthy subjects and RP patients are shown in Table 2. There were significant dif-

ferences in FAZ between normal subjects and RP patients. However, there was no significant difference in FAZ circularity between normal subjects and RP patients. There were significant differences in PD and VD of the superficial and deep layers between normal subjects and RP patients.

The mean amplitudes of N1, P1, and N2 in rings 1 and 2 were reduced significantly in RP patients compared with normal subjects ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively). The mean implicit times of N1, P1, and N2 were also reduced in RP patients compared with normal subjects, but the difference was not significant ($p = 0.087$, $p = 0.059$, and

Figure 2. Relationship between foveal avascular zone (FAZ) and average P1 amplitude of multifocal electroretinogram (mfERG) (Ring 1-Ring 2). FAZ in the superficial and deep capillary plexuses correlated significantly with average P1 amplitude of mfERG.

Table 4. Correlations between multifocal electroretinogram and OCT angiography

	SFAZ area	DFAZ area	FAZ circularity	SPD	DPD	SVD	DVD
Response densities (nV/deg2)							
N1	0.045*	0.079	0.770	0.277	0.432	0.600	0.141
P1	0.000*	0.000*	0.606	0.002*	0.001*	0.021*	0.013*
N2	0.174	0.470	0.851	0.414	0.505	0.995	0.453
Implicit times (ms)							
N1	0.072	0.090	0.403	0.091	0.136	0.058	0.034*
P1	0.789	0.624	0.633	0.156	0.378	0.937	0.316
N2	0.650	0.652	0.851	0.141	0.414	0.995	0.453

OCT = optical coherence tomography; SFAZ = superficial FAZ; DFAZ = deep FAZ; FAZ = foveal avascular zone; SPD = superficial perfusion density; DPD = deep perfusion density; SVD = superficial vessel density; DVD = deep vessel density.

*Linear regression analysis.

Figure 3. Relationship between vessel density (VD), perfusion density (PD) and Average P1 amplitude of multifocal electroretinogram (mfERG) (Ring 1-Ring 2). VD and PD in the superficial and deep capillary plexuses correlated significantly with Average P1 amplitude of mfERG.

$p = 0.080$, respectively) (Table 3).

Both VD and PD of the superficial plexus and deep plexus were correlated with P1 amplitude on mfERG ($r = 0.188$, $p = 0.021$; $r = 0.215$, $p = 0.013$; $r = 0.323$, $p = 0.02$; and $r = 0.362$, $p = 0.001$, respectively). The FAZ area showed significant correlation with P1 amplitude in the superficial and deep plexuses ($r = -0.682$, $p = 0.000$ and $r = 0.612$, $p = 0.000$, respectively) (Table 4, Fig. 2, 3). The strongest correlation with P1 amplitude on mfERG was found in the FAZ area. Representative cases of patients with retinitis pigmentosa and normal subjects are shown in Fig. 4.

Discussion

OCT-A is a novel imaging technique that enables visualization of blood flow in retinal and choroidal vessels and vessels of the optic nerve head without recourse to an intravenously injected dye. An interesting feature of OCT-A is its ability to quantitate blood flow. Its application has been described in normal subjects and in patients with ocular and systemic diseases [17-21]. Several studies have recently reported abnormalities in OCT-A images of eyes of RP patients, indicating a reduction in retinal blood flow and an increase in FAZ size

Figure 4. Vascular network differences between retinitis pigmentosa (RP) patient and healthy control at the superficial and deep capillary level. (A, B) 3×3 optical coherence tomography (OCT) of the superficial and deep capillary plexus of a healthy subject. (C) Trace arrays of multifocal electroretinogram (mfERG) of a healthy subject. (D, E) 3×3 OCT angiography of the superficial and deep capillary plexus of a patient with RP. (F) Trace arrays of mfERG of a patient with RP.

[12,14,22,23]. Our results using OCT-A showed an enlarged FAZ and reduced macular perfusion in RP patients compared with normal subjects. These findings are consistent with previously published results that used different methods and imaging technologies. OCT-A has the advantage of being a noninvasive imaging technique and is available in an increasing number of hospitals and medical centers. It is suitable for routine clinical examinations and can be performed quickly and easily.

Standardized fERG is a mass response that reflects total retinal area. It is possible to estimate small residual responses in advanced RP patients in combination with computer averaging and use of analog or digital filters [24], but without this technique, a recordable response cannot be obtained from advanced RP patients. In contrast to fERG, multifocal electroretinography tests local retinal function and is sensitive enough to quantitate cone-mediated local retinal function, even in advanced RP patients who have severely

constricted VF with non-recordable fERG [6,8,9]. Therefore, mfERG might be a more useful modality than fERG to evaluate retinal function in RP patients.

The present study examined retinal structure using OCT-A and retinal function using mfERGs in the foveal region (Rings 1 and 2) of RP patients, followed by determination of correlations between these measurements. mfERGs and OCT-A have previously been used to evaluate individual retinas in RP patients. However, to the best of our knowledge, no study has identified correlations between OCT-A parameters and mfERGs. In the present study, we identified correlations between mfERG and macular structure as revealed by OCT-A in RP patients. The results show a significant correlation between FAZ, PD, VD, and mfERG. The strongest correlation was found between FAZ in the superficial and deep plexuses and P1 amplitude on mfERG. The PD and VD were also correlated with P1 amplitude on mfERG. Overall, the results demonstrated a possibility for evaluating the mac-

ular function of RP patients using OCT-A.

Attenuation of retinal blood vessels is a funduscopic hallmark of RP. Using different types of OCT-A instruments, it has been reported that there is a reduction in retinal blood flow in the eyes of RP patients [12]. Our results also show that the PD and VD of the superficial and deep layers in the macular region were reduced in RP patients compared with normal controls.

The mechanism underlying the vascular changes in RP patients is unclear, and studies have discussed possible mechanisms of pathogenesis [12,25]. Studies have indicated that blood flow is altered in patients with RP, and this process was not localized to the eye but generalized across the body [26,27]. In RP patients, even in the early stages of disease, plasma level of endothelin-1, a strong endogenous vasoconstrictor, is increased and has been correlated with a decrease of blood flow in the ophthalmic and posterior ciliary arteries [26]. The decrease in retinal blood flow in RP may be aggravated by metabolic alteration of retinal endothelial cells and by an increase in oxygen level that also determine an increase in retinal production of endothelin-1. The observed differences in our study could be explained by vascular changes in RP patients and by reduced blood flow in the damaged retina.

In this context, it has also been noted that FAZ area in RP patients is significantly greater than that in normal controls. This has to be considered when analyzing our results because flow density measurements of the fovea could be affected by an enlarged FAZ in RP patients. In the present study, the superficial and deep layers in the FAZ were increased in RP patients compared with normal controls.

Our study is limited by its small sample size and lack of genetic characterization. Additional studies addressing these limitations with a longer follow-up period are needed to better evaluate the usefulness of OCT-A in diagnosis and follow-up of patients with RP.

In conclusion, RP patients displayed decreased macular perfusion compared with normal subjects. The flow density measured using OCT-A correlated with functional parameters recorded on mfERGs. These results suggest that OCT-A performed with multifocal electroretinography is a useful modality for evaluating both macular structure and function in RP patients.

Conflicts of Interest

The authors declare no conflicts of interest relevant to this article.

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