Original Article

Lipofuscin- and Melanin-related Fundus Autofluorescence in Patients with Submacular Idiopathic Choroidal Neovascularization

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Abstract

Purpose: To compare melanin-related near-infrared fundus autofluorescence (NIA; excitation 787 nm, emission> 800 nm) with lipofuscin-related fundus autofluorescence (FAF; excitation 488 nm, emission > 500 nm) in patients with idiopathic choroidal neovascularization (ICNV).

Methods: FAF, NIA, fundus fluorescein angiography (FFA) and indocyanine green angiography (ICGA) were obtained using a confocal scanning laser Ophthalmoscope HRA2 (Heidelberg Retina Angiograph 2) in 18 eyes of 18 patients with ICNV.

Results: Eighteen eyes had classic CNV, and autofluorescence imaging showed hypoautofluorescence at the site of CNV. A well-defined hyperautofluorescent ring was detected surrounding the CNV in all 18 eyes with NIA imaging. In our sample, the FAF patterns around the CNV were classified as normal (n=1, 5.56%), well-defined hyperautofluorescent ring (n=7, 38.89%), or ill-defined hyperautofluorescent ring (n=10, 55.56%).

Conclusion: The patterns of FAF and NIA indicated different involvement of lipofuscin and melanin in the pathophysiological process of ICNV. Compared to FAF imaging, NIA imaging appears to be a superior noninvasive method for in vivo visualization of retinal pigment epithelium (RPE) abnormalities in ICNV patients. (***Eye Science 2012*; *27*:*138-142***)

Keywords: idiopathic choroidal neovascularization, ICNV; lipofuscin-related fundus autofluorescence, FAF; melanin-related near-infrared fundus autofluorescence, NIA; retinal pigment epithelium, RPE

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Choroidal neovascularization (CNV) is a common pathologic change that occurs in many chorioretinal diseases that cause severe vision loss[1,2]. In the elderly, CNV is most commonly caused by exudative age-related macular degeneration (AMD)[3]. Idiopathic CNV (ICNV) is an uncommon condition that affects patients under the age of 50 years, with out retinal pigment epithelium (RPE) or Bruch's membrane alterations predisposing to CNV[1-5].

Fundus fluorescein angiography (FFA) and indocyanine green angiography (ICGA) have been used to determine the type and location of the CNV[4,6]. Autofluorescence (AF) imaging has been developed as a tool to evaluate RPE during aging and in ocular disease.

Using a confocal scanning laser ophthalmoscope (cSLO), two forms of autofluorescence can be obtained: melanin-related near-infrared fundus autofluorescence (NIA) and lipofuscin-related fundus autofluorescence (FAF)[7-11]. The FAF imaging results of CNV secondary to exudative AMD were reported in detail[8,12], but there is no data comparing the characteristics of FAF and NIA images in ICNV patients. We examined autofluorescence from ICNV patients using both FAF and NIA imaging to demonstrate the distinct patterns of autofluorescence in the same clinical cases.

Methods

Patients

Eighteen eyes from 18 patients with ICNV were included in the study from January 2010 to February 2011. There were 8 male and 10 female patients. The mean age of the patients was 31.7 years (range, 19-

45 years). The procedures conformed to the tenets of the Declaration of Helsinki and informed consent was obtained from each patient before enrollment in this study.

The diagnosis of ICNV was made when there was an active CNV in patients less than 50 years without signs of age-related macular degeneration, intraocular inflammation, angioid streaks, choroidal rupture, chorioretinal scars, or chorioretinal dystrophy[1].

FAF, NIA, FFA and ICGA examination

NIA, FAF, FFA, and ICGA images were obtained in both eyes using a cSLO (Heidelberg Retina Angiograph 2, HRA2; Heidelberg Engineering, Heidelberg, Germany).

Before examination, the pupil was dilated to a diameter of at least 6.5 mm with tropicamide. Autofluorescence images were produced using a 30° field-of-view mode. Autofluorescence imaging was performed before FFA and ICGA imaging. FAF imaging was performed as previously described[7–12]. Briefly, optically pumped solid-state laser light (488 nm) was used to excite lipofuscin autofluorescence. A band-pass filter with a cut-off at 500 nm, included in the system, was inserted in front of the detector. NIA imaging was performed as described by Keilhauer and Delori[9]. Diode laser light (787 nm) was used to excite melanin related autofluorescence. A band-pass filter with a cut-off at 800 nm, included in the system, was inserted in front of the detector. In order to amplify the autofluorescence signal, the best 9 single images of FAF and 18 single NIA images were averaged, and the mean images were generated by using "compute mean" processing controlled by the Heidelberg Eye Explore software (version: 1.5.8.0).

After FAF and NIA imaging was completed, fluorescein (3 ml, 20%) and indocyanine green (25 mg) were injected into the antecubital vein, and simultaneous images of ICGA and FFA were recorded using standard techniques.

Findings on FFA, ICGA, FAF, and NIA images were compared. The distribution of autofluorescence was evaluated at the site of the CNV, around the CNV, and outside the area affected by the CNV[7,12]. Abnormal autofluorescence was classified as hyperautofluorescence or hypoautofluorescence compared to autofluorescence outside the lesion, the latter being labeled as normal autofluorescence[7].

Results

All patients had a normal fundus in the fellow eye. In the normal eye, the FAF and NIA distributions were different. The FAF intensity was highest in the perifoveal region and decreased toward the periphery. The fovea showed relative hypoautofluorescence in FAF (Figure 1A). In contrast, the fovea exhibited hyperautofluorescence in NIA images. There was a marked decline in NIA intensity towards the perifoveal region (Figure 1B). The optic disc and both large and moderate-sized retinal vessels showed hypoautofluorescence in both FAF and NIA images (Figure 1 A,B).

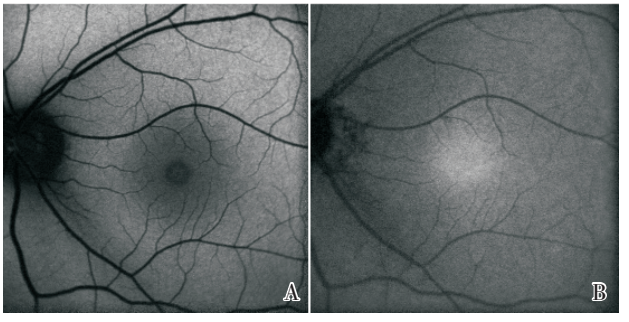

Figure 1 The fellow eye of a 26-year-old female with ICNV shows normal FAF (A) and NIA (B).

Fundus examination in the affected eyes exhibited a submacular yellowish lesion with subretinal hemorrhage or fluid.

In FFA, 18 eyes showed a well-demarcated area of vascular hyperfluorescence in the early frames (Figures 2A, 3A and 4A) and progressive leakage of the fluorescein in the late angiographic images. ICGA images confirmed a distinct choroidal neovascular membrane (CNVM) in all 18 eyes (Figures 2B, 3B and 4B). A dark rim was detected surrounding the CNVM in 17 eyes in the early phase of ICGA (Figures 2B,3B and 4B)

In FAF and NIA imaging, all 18 eyes (100%) showed hypoautofluorescence at the site of the CNV (Figures 2C, 2D, 3C, 3D, 4C, 4D). Around the CNV, 18 eyes (100%) displayed a well-defined hyperautofluorescent ring in NIA images (Figures 2D, 3D, 4D), while 17 eyes (94.44%) showed a hyperautofluorescent ring (Figures 2D, 4D) and one eye (5.56%)

showed normal autofluorescence (Figure 3D) in FAF images. This hyperautofluorescent ring in FAF was scored as well-defined (n=7, 38.89%) (Figure 2C) or ill-defined (n=10, 55.55%) (Figure 4D).

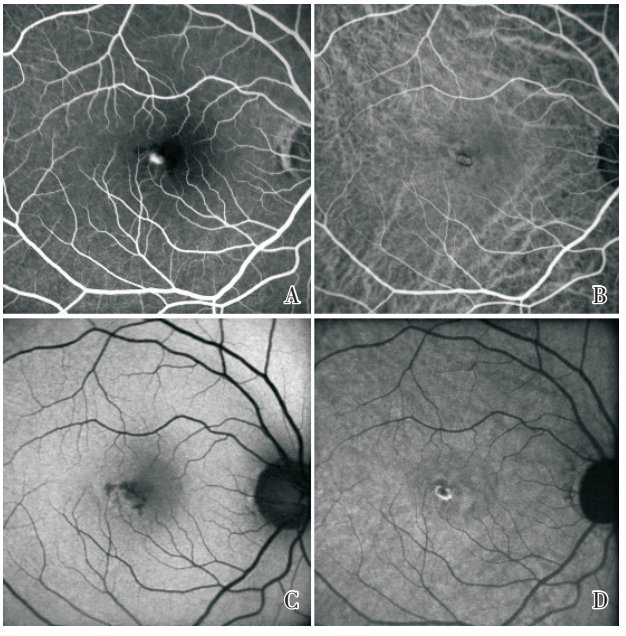

Figure 2 A 39-year-old male with ICNV. A: Early phase FFA reveals a juxtafoveal classic CNV; B: Early phase ICGA confirms a well-defined CNVM with a narrow dark rim; C: FAF shows hypoautofluorescence at the site of CNV. Around the CNV, the distribution of autofluorescence is normal. Note the hypoautofluorescent areas adjacent to CNV caused by subretinal hemorrhage; D: NIA show hypoautofluorescence at the site of CNV. A well-defined hyperautofluorescent ring around the CNV is present.

Discussion

NIA and FAF imaging are noninvasive techniques that facilitate in vivo evaluation of lipofuscin and melanin, respectively[7-11]. In normal autofluorescence images, optic disc and vessels appear dark due to the absence of autofluorescent fluorophores. FAF reflects the lipofuscin distribution and is higher in the parafoveal area and decreases toward the periphery[10,13], while NIA reflects the RPE melanin distribution and is higher in the macula and decreases from the periphery to the posterior pole[9,10,13].

Lipofuscin accumulation in RPE reflects the level of metabolic activity, which is largely determined by the quantity of photoreceptor outer segment renewal[14,15]. Hyperautofluorescence in FAF images in-

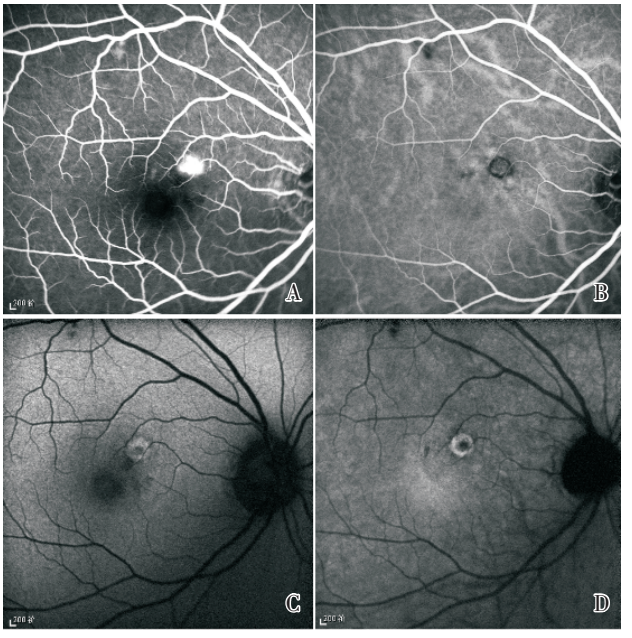

Figure 3 A 26-year-old female with ICNV. A: Early phase FFA reveals a juxtafoveal classic CNV. B: Early phase ICGA confirms a well-defined CNVM with a dark rim; C, D: FAF and NIA show hypoautofluorescence at the site of CNV. A well-defined hyperautofluorescent ring is seen surrounding the CNV in both FAF and NIA images.

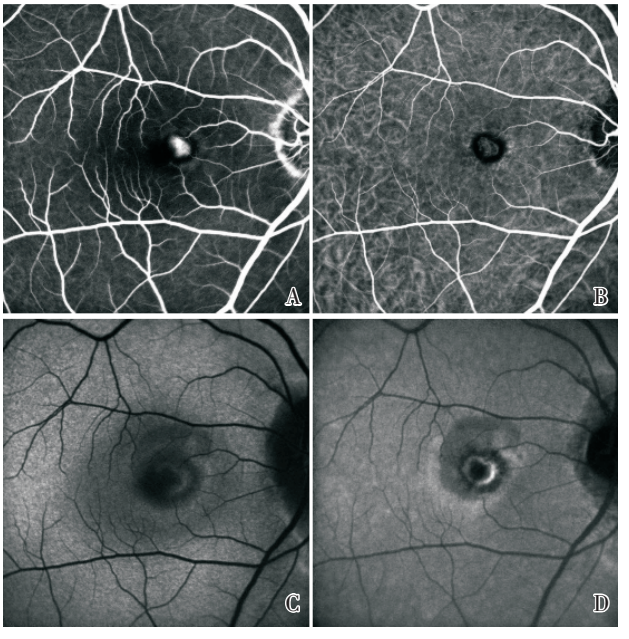

Figure 4 A 34-year-old male with ICNV. A: Early phase FFA reveals a juxtafoveal classic CNV; B: Early phase ICGA confirms a well-defined CNVM. A distinct dark rim around the CNV is present; C, D: FAF and NIA images exhibit hypoautofluorescence at the site of CNV. An ill-defined hyperautofluorescent ring is observed in FAF image(C), while a well-defined hyperautofluorescent ring is observed surrounding the CNV in NIA image (D).

dicates a disease process in the photoreceptor/RPE-complex with increased phagocytotic activity and subsequent lipofuscin accumulation in the retinal pigment epithelial cells. Conversely, hypoautofluorescence in FAF images indicates reduced phagocytotic activity or the absence of RPE cells[8,10,11,14].

Melanin is the major photoprotective agent in RPE cells[8]. Hyperautofluorescence in NIA images corresponds to areas with higher melanin concentration or areas with an ongoing degenerative process. Hypoautofluorescence in NIA may correspond to a reduction in melanin concentration or activity, whereas the absence of a NIA signal indicates the absence of RPE cells[8,10,11].

Idiopathic CNV is characterized by small neovascular lesions under the sensory retina in the macular area[16]. The neovascularization is always classic[17] and type 2[18] that is located between the sensory retina and the RPE[4].

In this study, all affected eyes had purely classic CNV based on FFA findings.

In FAF and NIA imaging, 18 eyes (100%) showed hypoautofluorescence at the site of CNV. This finding was also seen in patients with classic CNV secondary to AMD[8,12]. McBain et al[12]. reported that 90% of eyes with exudative AMD showed hypoautofluorescence at the site of the classic CNV. They suggested that the hypoautofluorescence at the site of the classic CNV was probably related to blockage of autofluorescence caused by the CNV growing in the subretinal space rather than severe damage to the RPE by the neovascularization process[12]. Our results are quite similar to their findings. We speculate that the hypoautofluorescence at the site of CNV in these ICNV patients is probably as the same as the CNV secondary to AMD.

A dark rim surrounding the CNVM and independent of subretinal blood was observed in ICGA imaging. Histopathologic experiment indicated that proliferating RPE cells around the CNV blocked the fluorescence of the choroid and caused the dark rim[5].

In NIA images, a well-defined hyperautofluorescent ring was detected in 100% eyes surrounding the CNV, while a well-defined or ill-defined hyperautofluorescent ring was observed in 94.44% eyes by FAF imaging. In both images, this hyperautofluorescent ring exactly corresponded to the site of the dark rim observed in ICGA images.

McBain et al[12]. also observed a hyperautofluorescent ring in FAF images surrounding the classic CNV secondary to exudative AMD. They speculated that the hyperautofluorescent ring probably corresponds to proliferation of RPE cells around the CNV. In the same way, we propose that proliferated RPE cells around the CNV increased the local density of lipofuscin and melanin, leading to the hyperautofluorescent ring in NIA and FAF imaging.

The emission and fluorescence spectra of NIA are in the near infrared region, the long wavelength light can better penetrate blood, exudate, serous fluid, xanthophylls, and the RPE than short wavelength light used for FAF[19]. So the hyperautofluorescent rings in NIA were well-defined and easy to identify, while most rings in FAF were ill-defined and sometimes difficult to identify.

Contrary to AMD, ICNV is self-limited, with spontaneous regression in the majority of cases[4]. Iida et al[4]. noted that during the follow-up period in ICNV, CNVM regressed in 9 eyes with a dark rim, while CNVM remained active or enlarged in 3 eyes without dark a rim. They concluded that the dark rim around the CNVM in ICGA images appeared to reflect a regression process of ICNV. Gharbiya et al.[6] also considered that the dark rim was a healing response and ICGA was useful to define and follow up the true extent of this healing response around the CNVM of ICNV. So the hyperautofluorescent ring can also reflect a regression of ICNV.

In conclusion, FAF and NIA imaging can be used to evaluate RPE alterations in patients with ICNV, but NIA imaging provides a noninvasive method in vivo visualization of RPE abnormalities that may be superior to FAF imaging in some clinical cases.

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