·Clinical Research·

Evaluation of fundus autofluorescence patterns in age-related macular degeneration

Pradeep Venkatesh, Pradeep Sagar, Rohan Chawla, Varun Gogia, Rajpal Vohra, Yog Raj Sharma

Department of Ophthalmology, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India

Correspondence to: Pradeep Venkatesh. Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, Room 477, Ansari Nagar, New Delhi 110029, India. venkyprao@yahoo.com

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Abstract

• AIM: To study the various morphological patterns of fundus autofluorescence (FAF) images in patients with age-related macular degeneration (AMD) in Indian population.

• METHODS: Totally 179 eyes of 104 patients with clinical diagnosis of AMD were recruited into the study. Autofluorescence images were captured using confocal scanning laser ophthalmoscope and the patterns of FAF were classified.

• RESULTS: Of 179 eyes, 27 (15.08%) were early AMD, 58 (32.41%) were intermediate AMD, 94 eyes (52.51%) were late AMD. Of 94 eyes with late AMD, 79 (84.04%) were neovascular AMD and 15 (15.96%) were central geographic atrophy. In eyes with early and intermediate AMD, 9 patterns of FAF were noted. Six patterns (normal, minimal change, focal increased, patchy increased, linear, reticular) were similar to that in the published classification. Two patterns (lacelike and speckled) described in the published classification were not found. Three new patterns (focal hypo-fluorescence, patchy hypo-fluorescence, mixed focal hypo-fluorescence and hyper-fluorescence) were detected. In eyes with neovascular AMD, 6 morphological patterns of FAF were noted. Two patterns (mixed hypo-fluorescence and hyper-fluorescence, central hypo-fluorescence with hyper-fluorescent rim) were similar to that in published classification. Two patterns (normal, near normal or normal background fluorescence in the centre of hypo-fluorescent area) described in the published classification were not found. Four new patterns (minimal change, hypo-fluorescent patch, central hypo-fluorescence with surrounding reticular, bull´s eye) were recognized. In eye with central geographic atrophy 5 morphological patterns were noted and these were similar to that in published classification.

• CONCLUSION: Phenotypic differences in the pattern of FAF exist in the study population compared to existing classification systems.

• KEYWORDS: age-related macular degeneration; fundus autofluorescence; lipofuscin; choroidal neovascularization

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INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in western population. The disease adversely affects activities of daily living, causing the affected ones to lose their independence in their old age. The prevalence of early AMD and late AMD in India [1] is noted to be similar to that in western population. Fundus autofluorescence (FAF) is due to the presence of fluorophores particularly lipofuscin [2] which is a mixture of auto-fluorescent pigments that accumulate in postmitotic cells throughout life. In the retinal pigment epithelium (RPE), lipofuscin granules accumulate in the lysosomal compartment mainly as a by-product of constant phagocytosis and incomplete digestion of discs and their retinoid content shed from photoreceptor outer segments [3-4]. Formation of these fluorophores is dependent upon a normal visual cycle [5] and lipofuscin is therefore an indirect marker of metabolic activity between photoreceptor outer segment turn over and phagocytic ability of RPE. FAF imaging with a confocal scanning laser ophthalmoscope is an established modality used for evaluation of patients with AMD as it provides much more information than the conventional methods like color fundus photography and fundus fluorescein angiography [6-7]. FAF is a measure of retinal pigment epithelial loss and a significant predictor of visual acuity [8]. Autofluorescence can itself be used to measure the area of geographic atrophy as the area of geographic atrophy measured by autofluorescence and that measured by spectral-domain optical coherence tomography (SD-OCT) is noted to be similar [9]. Various morphological patterns of FAF have been described in cases with early AMD [10]. In cases with geographic atrophy presence of diffuse irregular increased autofluorescence around unifocal or multifocal patches of atrophy is associated

with development of new areas of atrophy over time [11-12]. Similarly FAF image in eyes with geographic atrophy have been classified into different morphological patterns and presence of specific patterns are associated with higher rate of progression of atrophy compared to other types [13]. FAF image in eyes with classic choroidal neovascularization (CNV) exhibits either a slightly decreased fluorescence with near normal or background signal at the centre of the lesion or decreased fluorescence at the centre of lesion and an increased FAF signal towards the lesion edge and in occult CNV either heterogeneous fluorescence at the lesion site or normal FAF pattern[14].

Various patterns of FAF in early and intermediate AMD, central geographic atrophy, neovascular AMD have been described. In central geographic atrophy, specific pattern of FAF like banded and trickling are associated with higher rate of progression[13,15]. In early AMD specific patterns like linear, patchy, reticular are associated with higher risk of development of CNV [16]. So identification of patterns has a prognostic significance and there is no published work studying the various patterns of FAF images seen in patients of AMD in Indian population.

SUBJECTS AND METHODS

Ethical clearance was obtained from Institute Ethics Committee of All India Institute of Medical Science (AIIMS). We included patients presenting to Outpatient Department and vitreo-retinal services of Dr. Rajendra Prasad Centre for Ophthalmic Sciences, AIIMS, New Delhi, over a period of 9mo from February 2014 to October 2014, who were diagnosed clinically as a case of AMD.

Best corrected visual acuity (BCVA) was recorded using Snellen's visual acuity chart at a distance of 6 m. Pupils were dilated with 1% tropicamide or a combination of 1% tropicamide and 2.5% phenylephrine to achieve adequate dilatation. Type of AMD based on age-related eye disease study (AREDS) classification system, presence of other ocular pathology and associated retinal pathology were recorded. Fundus colour photographs of central 50° field were captured using Zeiss fundus camera FF 450^{plus}IR, Carl Zeiss Meditec, Jena, Germany. FAF images of central 30°×30° square field were captured using confocal scanning laser ophthalmoscope, SPECTRALIS® HRA+OCT, Heidelberg Engineering. Macular linear scans of SD-OCT with enhanced depth imaging were captured using SPECTRALIS® HRA+ OCT, Heidelberg Engineering. In cases with neovascular AMD fundus fluorescein angiograms were captured using Zeiss fundus camera FF 450^{plus}IR or SPECTRALIS® HRA+ OCT, Heidelberg Engineering whenever required.

The images were evaluated and classified into 3 groups: early and intermediate AMD (group 2 and group 3 of AREDS classification system), neovascular AMD, central geographic atrophy based on fundus color photograph and SD-OCT images. Morphological patterns of FAF were classified based on the classification systems available in the literature. Classification system proposed by Bindewald *et al* [10] was used to classify FAF patterns in eyes with early and intermediate AMD. Morphological patterns of FAF in eyes with neovascular AMD were classified based on the work of Peng *et al* [14]. Classification system by Holz *et al* [13] was used to classify FAF patterns surrounding the area of atrophy in eyes with central geographic atrophy.

RESULTS

We recruited 104 patients with clinical diagnosis of AMD in at least one eye into the study. Totally 179 eyes (104 patients) were included in the study. Twenty-nine eyes were excluded. The causes of exclusion were poor FAF image quality (8 eyes, 27.58%), corneal opacity (5 eyes, 17.24%), phthisis bulbi (4 eyes, 13.79%), no AMD (group 1 of AREDS classification system) (5 eyes, 17.24%), advanced cataract (2 eyes, 6.9%), ischemic central retinal vein occlusion (1 eye, 3.45%), operated vitreo retinal surgery for retinal detachment (1 eye, 3.45%), retinal detachment (1 eye, 3.45%), epiretinal membrane (1 eye, 3.45%), other cause (1 eye, 3.45%). Poor image quality was due to poor fixation in 4 eyes, significant cataract in 3 eyes and posterior capsular opacification in 1 eye.

Out of 104 patients 72 (69.2%) were male and 32 (30.8%) were female. The patients were in the age group of 50 to 85y (range 50 to 85y) with a mean age of 68.35 ±6.89y and median age of 68y.

Out of 104 patients 47 (45.20%) had symmetrical disease in both eyes, 36 (34.62%) had asymmetrical disease in both eyes, 5 (4.8%) were unilateral and in 16 (15.38%) the presence or type of AMD could not be determined due to other ocular pathology. Out of the 47 patients with symmetrical disease 5 (10.64%) had early AMD (group 2) in both eyes, 17 (36.17%) had intermediate AMD (group 3) in both eyes, 22 (46.81%) had neovascular AMD in both eyes and 3 (6.38%) had bilateral central geographic atrophy. Out of 36 patients with asymmetrical disease 14 (38.89%) had early AMD in one eye with neovascular AMD in other, 15 (41.67%) had intermediate AMD in one eye with neovascular AMD in other, 6 (16.67%) had early or intermediate AMD in one eye with central geographic atrophy in other, and 1 (2.77%) had neovascular AMD in one eye with central geographic atrophy in other. Out of 179 eyes 27 (15.08%) were early AMD (group 2 of AREDS), 58 (32.4%) were intermediate AMD (group 3 of AREDS), 94 eyes (52.52%) were late AMD (group 4 of AREDS). Out of 94 eyes with late AMD, 79 (84.04%) were neovascular AMD and 15 (15.96%) were central geographic atrophy.

FAF images in eyes with early and intermediate AMD were classified based on the work of Bindewald *et al* [10] into 9 patterns normal FAF, minimal change pattern, focal

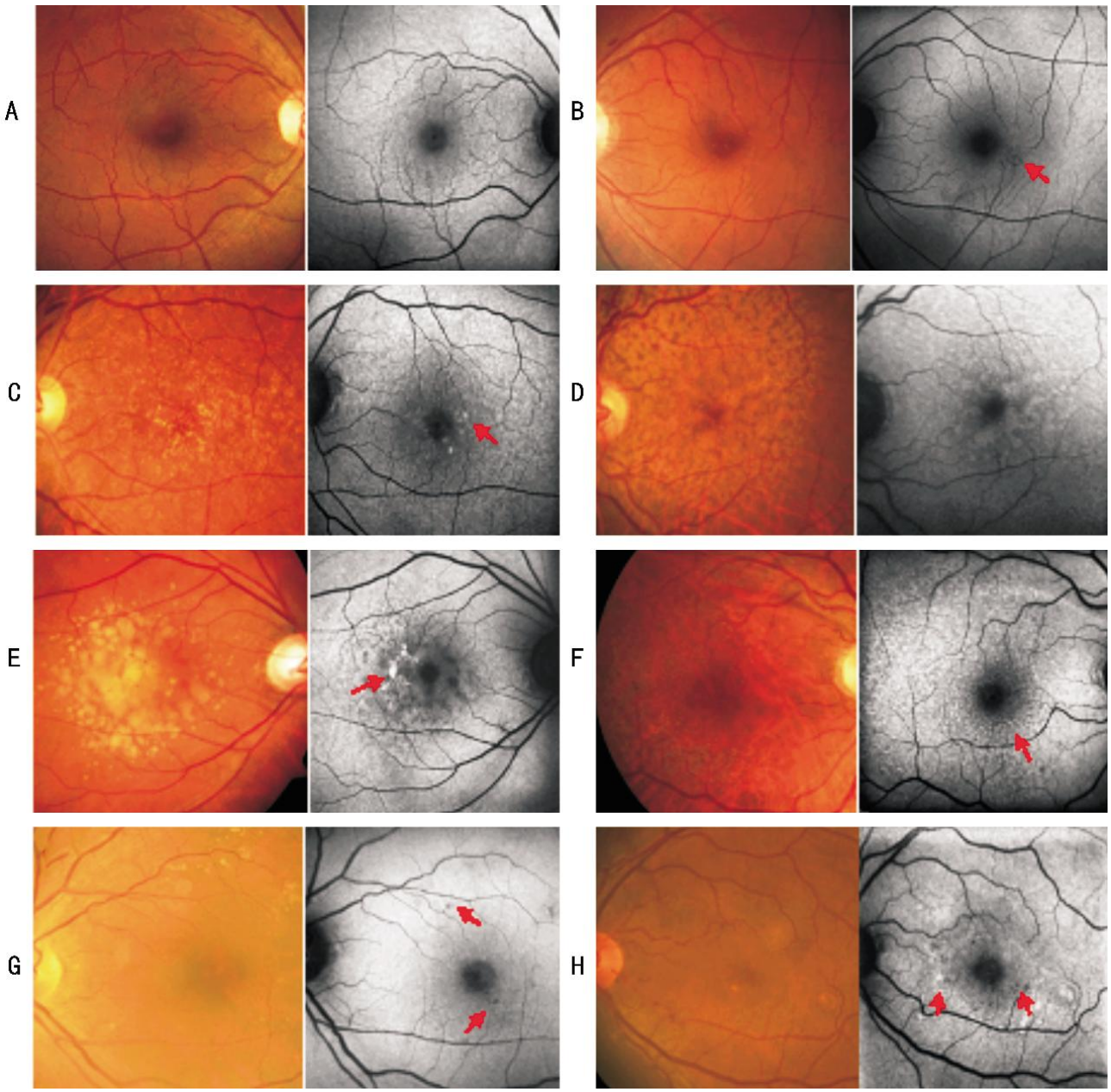

Figure 1 Patterns of FAF in early and intermediate AMD A: Normal FAF; B: Minimal change; C: Focal hyper-fluorescence; D: Patchy hyper-fluorescence; E: Linear; F: Reticular; G: Focal hypo-fluorescence; H: Mixed focal hyper-fluorescence and hypo-fluorescence.

hyper-fluorescence, patchy hyper-fluorescence, linear pattern, reticular pattern, focal hypo-fluorescence, patchy hypo-fluorescence and mixed focal hypo-fluorescence and hyper-fluorescence (Figure 1).

Normal FAF: it is characterized by the presence of homogenous background fluorescence and a gradual decrease of fluorescence in the inner macula towards the foveola.

Minimal change pattern: it is characterized by the presence of minimal variations from the normal background FAF.

Focal hyper-fluorescence: it is characterized by the presence of at least one well defined spot of markedly increased fluorescence from background fluorescence measuring less than 200 μm in diameter. Usually these spots have a well defined border. These areas of hyper-fluorescence may or may not correspond to visible abnormalities on colour fundus photograph such as drusen. Four eyes had a rim of decreased fluorescence around an area of increased fluorescence. Such lesions corresponded to drusen on colour fundus photograph.

Patchy hyper-fluorescence: it is characterized by the presence of at least one area with markedly increased fluorescence from background measuring more than 200 μm in diameter. Usually the borders are less well defined compared to focal hyper-fluorescence.

Linear pattern: it is characterized by the presence of at least one linear area of markedly increased fluorescence with well defined borders and usually corresponds to hyper-pigmented lesions on colour fundus photograph.

Reticular pattern: it is characterized by the presence of multiple small areas of decreased fluorescence less than 200 μm in diameter with brighter lines in between.

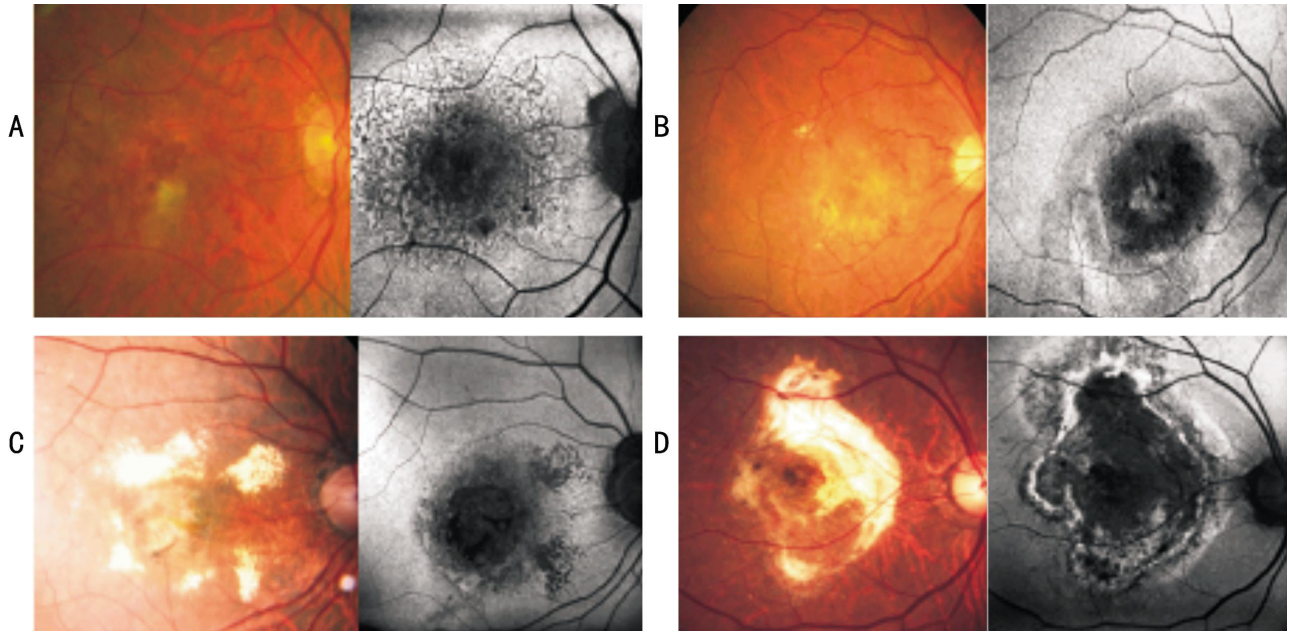

Figure 2 Patterns of FAF in neovascular AMD A: Central hypo-fluorescence with surrounding reticular; B: Bull's eye; C: Hypo-fluorescent patch; D: Central hypo-fluorescence with hyper-fluorescent rim.

Focal hypo-fluorescence: it is characterized by the presence of at least one well defined area of markedly decreased fluorescence measuring less than 200 μm in diameter. These areas have well defined borders. These areas may not correspond to visible lesions on fundus colour photographs.

Patchy hypo-fluorescence: it is characterized by the presence of at least one area of markedly decreased fluorescence measuring more than 200 μm in diameter.

Mixed focal hypo-fluorescence and hyper-fluorescence: it is characterized by the presence of multiple areas of both focal hyper-fluorescence and focal hypo-fluorescence.

Out of 85 eyes with early and intermediate AMD 1 (1.18%) had a normal FAF pattern, 12 (14.12%) had minimal change pattern, 18 (21.18%) were focal hyper-fluorescence, 6 (7.06%) were patchy hyperfluorescence, 2 (2.35%) were linear, 13 (15.29%) were reticular, 8 (9.41%) were focal hypo-fluorescence, 1 (1.18%) had patchy hypo-fluorescence, 14 (16.47%) were mixed focal hypo-fluorescence and hyper-fluorescence. Nine (10.59%) had combination of 2 patterns and in 1 (1.18%) the FAF pattern could not be classified.

The FAF images in eyes with neovascular AMD were classified based on the work of Peng *et al* [14] into 6 patterns: minimal change pattern, mixed focal hypo-fluorescence and hyper-fluorescence pattern (heterogeneous pattern), hypo-fluorescent patch, central hypo-fluorescence with hyper-fluorescent rim, central hypo-fluorescence with surrounding reticular and bull's eye (Figure 2).

Minimal change pattern: it is characterized by the presence of minimal variations from the normal background FAF.

Mixed focal hypo-fluorescence and hyper-fluorescence (heterogeneous fluorescence): it is characterized by the presence of multiple areas of both focal hyper-fluorescence and focal hypo-fluorescence.

Hypo-fluorescent patch: It is characterized by the presence of a large area of decreased fluorescence with normal background FAF surrounding the patch.

Central hypo-fluorescence with hyper-fluorescent rim: it is characterized by the presence of a central hypo-fluorescent patch with a rim of increased fluorescence at the margin of patch. The rim can be complete or incomplete.

Central hypo-fluorescence with surrounding reticular: it is characterized by the presence of a large central patch of decreased fluorescence surrounded by multiple small areas of decreased fluorescence with brighter lines in between.

Bull's eye: it is characterized by the presence of a central area with normal background fluorescence surrounded by concentric alternating zones of decreased fluorescence and increased fluorescence.

Out of 79 eyes with neovascular AMD, 1 (1.27%) had minimal change pattern, 5 (6.33%) had mixed focal hypo-fluorescence and hyper-fluorescence, 5 (6.33%) had hypo-fluorescent patch, 17 (21.52%) had central hypo-fluorescence with hyper-fluorescent rim, 8 (10.13%) had central hypo-fluorescence with surrounding reticular, 6 (7.59%) had bull's eye. Twenty-four (30.38%) eyes had combination of two patterns and in 13 (16.46%) the FAF pattern could not be classified into any specific pattern.

FAF in cases with central geographic atrophy were classified based on the work of Holz *et al* [13] into those with increased fluorescence only at the margin of atrophy and those with increased fluorescence both at the margin of atrophy and elsewhere (diffuse). The eyes with increased FAF only at margin were sub classified into banded and patchy pattern. The eyes with increased FAF both at the margin of atrophy and elsewhere were sub classified into trickling, reticular and

peripheral punctate. Out of the 15 eyes with central geographic atrophy 3 (20.00%) were banded, 7 (46.67%) were trickling, 1 (6.67%) was patchy, 1 (6.67%) was reticular, 1 (6.67%) was diffuse peripheral punctuate and in 2 eyes (13.33%) the FAF pattern could not be classified into specific pattern.

DISCUSSION

AMD is one cause of irreversible blindness with a prevalence of late AMD noted to be 1.2% in the age group of 60-79y and 2.5% in population aged 80y and above in Indian population[1]. No gender difference in the prevalence of AMD was noted in a few studies[1,17] and few other studies[18-19] have showed slight female preponderance. In our study there was a male preponderance but as our study was a hospital-based study the proportion may not be representative of general population. Other than clinical examination with indirect ophthalmoscope and imaging modalities like SD-OCT and fundus fluorescien angiography, FAF with a confocal scanning laser ophthalmoscope is an established modality used for evaluation of patients with AMD[6,20].

In a study by Bindewald *et al*[10], patterns of FAF in 100 eyes with early AMD (according to International Epidemiological Age-related Maculopathy Study Group) were classified into 8 patterns normal, minimal change, focal increased, patchy increased, linear, reticular, lacelike and speckled. Speckled pattern was the most common pattern noted and it was seen in 26% of cases. In our study population 27 cases with group 2 and 58 cases with group 3 AMD were included and FAF images were captured using confocal scanning laser ophthalmoscope. The patterns of FAF were classified into 9 patterns. Five patterns: normal, minimal change, focal increased, patchy increased, linear, reticular were similar to the available classification. Three new patterns were detected: focal decreased, patchy decreased, mixed focal hypo-fluorescence and hyper-fluorescence. Two patterns: lacelike, speckled were not seen. These findings suggest that morphological differences in the pattern of FAF exist compared to the existing classification. The areas of hyper-fluorescence may or not correspond to visible abnormalities on fundus colour photograph. This finding is similar to the available studies.

In a study by Peng *et al* [14], 72 eyes were classified into classic CNV and occult CNV based on fundus fluorescein angiography. In cases with classic CNV, 2 patterns were noted: a rim of hyper-fluorescence at the margin of hypo-fluorescence and near normal or background fluorescence in the centre of hypo-fluorescent area. In cases with occult CNV 2 patterns were noted: normal FAF and heterogeneous fluorescence. Central hypo-fluorescence with hyper-fluorescent rim was noted in 50% of cases. In our study FAF patterns in 79 cases with neovascular AMD were classified into 6 patterns. Two patterns: central hypo-fluorescence with hyper-fluorescent rim and mixed focal hypo-fluorescence and hyper-fluorescence (heterogeneous) were similar to that in the literature. Four new patterns were detected: minimal change, hypo-fluorescent patch, central hypo-fluorescence with peripheral reticular and bull's eye. Two patterns noted in the literature were not found: normal and near normal or background fluorescence in the centre of hypo-fluorescent area were not seen. Combination of 2 patterns was seen in 30.38% of cases.

In a study by Holz *et al*[13], 195 eyes with geographic atrophy were classified into two groups based on the configuration of increased FAF surrounding the areas of atrophy: eyes with increased fluorescence only at the margin of atrophy and diffuse which was defined as eyes with increased fluorescence adjacent to margin of the atrophic patch and elsewhere. Eyes with increased FAF only at the margin of atrophy were sub classified into 3 types-focal, banded and patchy. The eyes with diffuse type were classified into four subtypes- reticular, branching, fine granular and fine granular with peripheral punctuate spots and trickling. In our study group only 15 eyes had central geographic atrophy and 5 patterns were noted: banded, patchy, trickling, peripheral punctuate and reticular. In view of less sample size this classification will not be representative of the larger population.

In conclusion, phenotypic differences in the pattern of FAF exist in the study population compared to existing classification systems. FAF patterns may have ethnic variations and reasons for such phenotypic differences may be based on genetic/environmental factors and this need to be explored in future studies.

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