Abnormal Fundus Autofluorescence Results of Patients in Long-term Treatment with Deferoxamine

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Purpose: To describe and classify patterns of abnormal fundus autofluorescence (FAF) of patients with β-thalassemia receiving long-term treatment with deferoxamine (DFO).

Design: Prospective, cross-sectional, case-control study.

Participants: A total of 197 consecutive patients with β-thalassemia major or intermedia with at least 10 years of treatment with DFO were recruited in a tertiary referral center in Milan, Italy, and were investigated. Seventy-nine thalassemic patients without a history of chelation therapy were included as a control group.

Methods: All of the patients were investigated using best-corrected visual acuity (BCVA), fundus photography, and FAF imaging by confocal scanning laser ophthalmoscopy (cSLO) and were compared with the control group.

Main Outcome Measures: Identification of abnormal FAF patterns in thalassemic patients treated with long-term DFO and their progression and relationship with visual function.

Results: Abnormal FAF not related to other diseases was observed in 18 of the 197 patients (9%) and was classified into 4 phenotypic patterns: minimal change, focal, patchy, and speckled. The abnormal increased or decreased FAF was bilateral in all the cases, and only in some cases did it correspond to funduscopically visible alterations. There were no FAF abnormalities in the control group. During the follow-up, progressive FAF changes related to retinal pigment epithelium (RPE) damage occurred in the patchy pattern, associated with decreasing BCVA. Patients with speckled and focal patterns showed limited or no changes in FAF during the follow-up. No changes in FAF were found in patients with a minimal change pattern. No treated patient with a normal baseline examination demonstrated FAF changes. Patients with patterns other than the minimal change showed significant BCVA deterioration (P<0.001).

Conclusions: Various phenotypic patterns of abnormal FAF can be identified with cSLO imaging. Fundus autofluorescence is a helpful, fast, and noninvasive tool for monitoring the status of the macula in patients at risk of DFO toxicity. It may be useful in the decision to discontinue or switch the therapy in cases of particular high risk for disease progression. The progressive alteration of the RPE suggests an important role of pathologic RPE changes in the evolution of visual loss during long-term treatment with DFO.

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With the advent of confocal scanning laser ophthalmoscopy (cSLO), it is possible to visualize fundus autofluorescence (FAF) and its spatial distribution in vivo. As an index of lipofuscin accumulation, the FAF signal generally provides indirect information on the level of metabolic activity of the RPE. This suggests that in vivo, FAF imaging may represent a suitable noninvasive diagnostic tool to detect early RPE abnormalities in various retinal disorders, including drug-related retinal toxicity. Therefore, the present study was undertaken to evaluate the possible use of FAF in thalassemic patients using long-term DFO treatment.

This article describes the spectrum of fundus alterations using FAF in thalassemic patients treated with long-term DFO. Moreover, it analyzes the clinical course of these alterations and their relationship with visual function.

Patients and Methods

β-Thalassemic patients treated with regular and long-term DFO intake for a minimum of 10 years, with or without visual symptoms suggestive of retinal dysfunction, were recruited in a tertiary referral center in Milan (Department of Internal Medicine, Centro Anemie Congenite, Fondazione IRCCS Ca’ Granda—Ospedale Maggiore Policlinico, Italy) and were evaluated consecutively at the Department of Ophthalmology of the same hospital between March 2007 and December 2010. Patients with signs of other retinal diseases (e.g., inherited retinopathy, age-related macular degeneration (AMD), or diabetic retinopathy) were excluded, as were patients with media opacities not allowing good-quality FAF imaging. Clinical examinations were conducted after explanation of the procedures and receipt of written informed consent. Patients with FAF abnormalities were examined approximately every 6 months, whereas other patients were observed every year during the routine hematologic 1-day hospital examination. The research adhered to the tenets of the Declaration of Helsinki, and approval by the investigational review board of the Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, was obtained.

The collected data included detailed information regarding demographics, medical history, nonocular side effects of DFO treatment, age at start of treatment, dosage (mean daily dose of the last 5 years) and mode of infusion of DFO, and previous ophthalmic examinations. In contrast to patients with thalassemia major (TM), only some patients with thalassemia intermedia (TI) need repeated transfusions. Thus, a control group of patients with TI but without history of chelation therapy also was analyzed. All of the patients underwent a complete eye examination, including best-corrected visual acuity (BCVA), slit-lamp ophthalmoscopy, FAF imaging, and fundus photography.

The in vivo FAF imaging was performed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2 or Spectralis HRA; Heidelberg Engineering, Heidelberg, Germany) in a routine clinical setting. Different camera objectives for either a 30° or a wide-angle field-of-view mode were used, and the image resolution was 768×768 pixels. The maximum illumination of a 10×10° field of view was approximately 2 mW/cm². Focusing was achieved using the near-infrared reflectance mode at 815 nm. An argon laser light (488 nm) was used to excite lipofuscin autofluorescence. A wide-band pass filter with a cutoff at 500 nm was inserted in front of the detector. Using automated eye tracking and image alignment based on cSLO images, the software used the Automatic Real Time module (Heidelberg Engineering) to average a variable number of single images in real time.

Abnormal FAF results were defined as either an increased or decreased FAF signal compared with the normal background FAF outside such areas as described previously in detail. The macula was defined as the part of the retina centered on the foveola with a diameter of approximately 5500 μm. Statistical analysis was performed with SAS statistical software version 9.2 (SAS Inc, Cary, NC).

Results

A total of 197 β-thalassemic patients with regular and long-term DFO treatment for transfusional hemochromatosis were investigated consecutively. Of the patients, 146 (74%) had a TM and 51 (26%) had TI. The average age ± standard deviation of patients at the time of analysis was 41.0 ± 9.5 years (range, 20–66 years). The average duration of DFO treatment was 36 ± 8.5 years (range, 10–55 years). The daily dosage of DFO ranged from 1 to 4.5 g.

Of 197 patients, 18 (9%; 35 eyes) demonstrated FAF alterations (Table 1, available at http://aaojournal.org). Fourteen were women and 4 were men, ranging in age from 27 to 64 years (average, 45.1 ± 9.7 years). Among this group there were 11 of 146 patients (7.5%) with TM and 7 of 51 patients (13.7%) with TI. There was no significant difference in the rate of abnormal FAF results between the TM and TI groups (P = 0.79). At the time of the analysis, they had received subcutaneous DFO therapy for an average of 32.7 ± 8.7 years (range, 20–55 years). The daily dosage of DFO ranged from 1.5 to 4.5 g. The ocular history was insignificant for all of the patients with FAF abnormalities. Two patients (patients 1 and 2; Table 1, available at http://aaojournal.org) with sporadic high levels of ferritin occasionally received short-term intravenous therapy during the study period. When their iron overload allowed, DFO therapy was reinstituted by subcutaneous injection.

At the baseline visit, the BCVA ranged between 20/63 and 20/20, except for 1 eye with severe low vision resulting from advanced cataract (patient 8, left eye). This eye consequently was excluded from the analysis. Another 3 eyes were diagnosed with mild cataract, but nonetheless were included in the study because it did not affect the FAF imaging. At the baseline visit, 5 of the 18
patients with FAF alterations reported visual symptoms, including decreased visual acuity, night vision blindness, and blurry vision.

The control group included 79 patients with TI without a history of chelation therapy and without other retinal pathologic features. The average age of the control group was 40.35 ± 13.8 years (range, 18–67 years), including 39 men and 40 women. None of these patients had FAF abnormalities.

**Fundus Autofluorescence**

The normal, nonthalassemic FAF pattern was characterized by a homogeneous background autofluorescence with a gradual decrease in macular FAF intensity toward the foveola resulting from the masking effect of yellow macular pigment. The topographic alterations were classified in FAF into 4 different patterns using a slightly modified classification for AMD recently published by the FAM Study group.17 Briefly, the characteristics of each pattern were as follows.

**Minimal Change Pattern.** This pattern included eyes with only minimal variations from the normal pattern appearance, showing irregularly increased or decreased background FAF (Fig 1A, B). Increased FAF signal, presumably resulting from the mottling of the RPE, was characterized by relatively small spots of less than 100 μm in diameter in the macular area. The spots had well-defined borders and in some cases corresponded to visible alterations on color fundus photographs, such as focal hyperpigmentation.

Decreases in the FAF signal were characterized by multiple small reticular areas at the posterior pole that extended beyond the arcades with a small decrease of FAF intensity compared with background. The borders of these areas and the physiologic reasons for their presence were difficult to determine. Other decreases were associated with single or multiple small dark spots without obvious topographic pattern, presumably corresponding to atrophy of RPE cells. In funduscopic appearance, these areas did not correspond to visible alterations.

**Focal Pattern.** This pattern was defined by the presence of at least 1 medium-sized area, more than 100 μm but less than 200 μm in diameter, of markedly increased FAF that was much brighter than the surrounding background fluorescence (Fig 1C, D). The borders were well defined, with no gradual decrease of FAF observed between the background and the area with focally increased FAF. On color fundus photographs, these areas sometimes corresponded to visible alterations, such as focal hyperpigmentation. Again, multiple small reticular areas of decreased FAF intensity compared with background levels were present at the posterior pole.

**Patchy Pattern.** This pattern was characterized by the presence of at least 1 large area, more than 200 μm in diameter, of markedly increased FAF (Fig 1E, F). These areas were brighter than the surrounding background fluorescence. The borders typically are well defined, but a coalescence of these areas usually occurred, giving the appearance of a pattern-like dystrophy. The corresponding abnormalities were visible on color fundus photographs and included hyperpigmentation and hypopigmentation. Notably, the affected area was usually larger in FAF imaging than that expected from the color fundus photographs. It sometimes included different intensities of hyperautofluorescence.

**Speckled Pattern.** The speckled pattern was defined by the simultaneous presence of a variety of FAF abnormalities that extended beyond the macular area (Fig 1G, H). Typically, these abnormalities included multiple small areas of irregularly increased and decreased FAF. On color fundus photographs, these abnormalities sometimes corresponded to visible alterations such as focal hyperpigmentation and hypopigmentation. As with the patchy pattern, the pathologic areas seem to be fewer in the corresponding color fundus photographs.

The detected alterations in FAF always were bilateral in each patient, with slight variations of the total affected area. The most frequent pattern was the minimal change pattern (56%), followed by the focal pattern (17%) and the patchy pattern (16%). The speckled pattern was found in only 2 patients (11%).

At the baseline examination, the patients with the patchy or the speckled pattern demonstrated the most severe visual dysfunction. An exception was patient 8, who demonstrated the focal pattern and in whom low visual function mainly was the result of the concomitant cataract. After cataract surgery, his BCVA increased from 20/63 to 20/25 in his right eye, which was included in the study, and from 20/400 to 20/25 in his left eye, which was excluded from the study.

**Clinical Course**

Follow-up examinations were available in 15 of the 18 affected patients, with a mean ± standard deviation duration of 18.46 ± 6.86 months (range, 10–31 months). For patients 1 through 3 of the affected group, each had the patchy pattern at the baseline examination, and the FAF abnormalities progressed through the entire follow-up (Table 1, available at http://aaojournal.org). Neither patient 1 nor patient 2 could discontinue or switch the DFO treatment because of their systemic medical conditions, including renal failure.

For patient 1, the FAF area enlarged from the previously affected areas around the fovea and increased in intensity. There was also a progressive enlargement of the affected foveal area in which the FAF signal decreased. This corresponded to an initial RPE atrophy in the center after 1 year (Fig 2A–C). The BCVA of her right eye decreased from 20/63 to 20/100 and was slightly affected in the left eye (from 20/50 to 20/63).

For patient 2, after the 6-month follow-up visit there was a rapid decrease of the FAF signal in the foveal area. This decrease corresponded to development of initial RPE atrophy that enlarged progressively during the ensuing visits (Fig 3A–C). It was associated with vision loss after 1 year (from 20/40 to 20/63 in the right eye and from 20/25 to 20/40 in the left eye). Moreover, multiple small areas of irregularly increased or decreased FAF, similar to the FAF abnormalities described previously in the speckled pattern, occurred around the fovea at the posterior pole after 20 months (Fig 3D–F). The final BCVA after 31 months was 20/100 in her right eye and 20/63 in her left eye.

Patient 3 was the only one of the 3 patients with the patchy pattern of FAF who was able to switch therapy to oral deferasirox. During the 23 months of follow-up, there was a slight progressive enlargement of the foveal area with hyperautofluorescence and an increase of FAF intensity. Nevertheless, his vision remained stable at 20/32 in both eyes.

Limited FAF changes occurred in 3 other cases (patients 5–7) after 19 to 31 months of follow-up. Of these, only patient 7 was able to switch therapy to oral deferasirox. She experienced only mild BCVA impairment (from 20/20 to 20/25 in the right eye and from 20/20 to 20/32 in the left eye).

In the remaining 9 patients, FAF abnormalities remained unchanged during the follow-up (range, 10–23 months). The BCVA was stable except for the right eye of patient 4, the result of progression of the cataract, and for patient 8, for whom the BCVA improved after cataract surgery.

In the group of the 179 patients without macular FAF abnormalities at baseline, follow-up was available in 144 (80.4%), with a mean ± standard deviation duration of 15.66 ± 4.78 months (range, 12–29 months). During the follow-up, no cases of new onset of FAF abnormalities occurred in this group.
Statistical Analysis
Combining the 3 categories of patchy, speckled, and focal pattern into a single group to allow a dependable statistical analysis, the BCVA deterioration of this group at baseline was higher when compared with that of the minimal change pattern (odds ratio, 30 to 1; \( P < 0.001 \)). No significant differences were found in the duration (\( P = 0.18 \)) or in the dose (\( P = 0.19 \)) of the iron-chelating therapy between the 2 groups.

The BCVA impairment at the end of the follow-up was associated with neither age (\( P = 0.48 \)) nor duration of the therapy (\( P = 0.53 \)). However, higher daily doses were related to significantly higher reduction of the BCVA (\( P = 0.02 \)).
Discussion

The survival of patients with β-thalassemia and other hematologic conditions requires long-term transfusions with consequent iron overload of vital organs, for which chelation therapy is mandatory. Despite new promising chelating agents in the market, DFO remains the standard of care for most of these patients. Thus, the risk of drug-related toxic effects in the retina remains a clinical reality. The mechanism of DFO toxicity is unknown, but it is commonly thought that it manifests mainly in the RPE, afterward involving Bruch’s membrane and photoreceptors. Therefore, the early diagnosis of DFO retinopathy is extremely important because, as Haimovici et al have noted, patients who do not discontinue DFO after the development of retinopathy risk further RPE injury and visual deterioration. Recently, FAF has emerged as a suitable noninvasive diagnostic tool to evaluate the physiologic state of the RPE and possibly to detect the incipient stages of adverse changes in response to chelation therapy.

The present study identified 18 consecutive patients and defined 4 patterns of abnormal FAF in eyes with long-term DFO treatment. The type of thalassemia does not influence the rate of pathologic alteration. Alterations in FAF were not necessarily associated with corresponding funduscopically visible lesions such as irregular pigmentation. This indicates that FAF imaging gives information over and above the clinical examination and may allow early detection of DFO toxicity in the retina. Furthermore, the extent of the RPE alterations was much more widespread on FAF than the fundus examination suggested.

Interestingly, at presentation, only 5 of the patients with FAF alterations reported visual symptoms, including decreased BCVA, night vision blindness, and blurry vision. Therefore, changes in FAF may precede the occurrence of progressive visible lesions and, in some cases, also may precede the onset of visual symptoms.

This study shows that both FAF changes in the macular area and DFO daily dose are indicators for BCVA changes. The small sample size of eyes with the patchy, speckled, and focal FAF patterns does not allow dependable statistical comparisons between these groups. Patients with a minimal change pattern have significantly lower BCVA deterioration at baseline than patients with other patterns. Moreover, a higher DFO daily dose was associated with higher visual loss during the follow-up. However, this study considered the mean daily dose of the last 5 years for each patient. The cumulative dose would be a more accurate parameter for this correlation, but data about changes in the DFO doses during most of the treatment period of the patients (range, 20–55 years) are lacking. Thus, it is not possible to determine how previous dramatic changes in the dose regimen could be associated with visual deterioration or with progression of the macular disease.

In these patients, FAF imaging was helpful in monitoring the rate of progression of the disease. It remains an open question whether the 4 patterns are different stages of DFO maculopathy. Few patients with the focal and speckled pattern developed minimal progression of the FAF abnormalities without shifting into different patterns. During the follow-up, only in 1 patient with the patchy pattern (patient 2) did concomitant FAF changes typical of the speckled pattern occur without stopping or changing therapy; the other 2 patients showed a progression of the pattern without shifting into a different one. Patients with the minimal pattern of FAF abnormalities at presentation did not experience any progression in that pattern during the follow-up period, the longest of which was 23 months. Multifactorial variables may be related to the onset of a particular pattern, including genetic mutation, race, cumulative dose of the iron-chelating therapy, ferritin levels, or other variables. Furthermore, longitudinal data may clarify whether the presence of early abnormalities in FAF represents a transitory disease stage and can be a prognostic sign for progressive stages of DFO retinopathy.

In other diseases involving the RPE, such as AMD, areas of hyperautofluorescence can switch to areas of hypoautofluorescence as atrophy supervenes. In 2 patients with the patchy pattern who did not switch or discontinue DFO (patients 1 and 2; Table 1, available at http://aaojournal.org), areas with increased FAF in the macula clearly preceded and were replaced by hypoautofluorescent areas during the follow-up, corresponding to progressive loss of RPE and associated with reduction of visual function. However, it is not possible to determine if other factors, for example, predisposing genetic factors, concomitant disease, age, smoking, or storage of DFO in the RPE, may be associated with this progression.

Deferoxamine is a life-saving treatment. Thus, the authors agreed with the hematology team of their hospital that for patients with asymptomatic FAF exhibiting the minimal change pattern, it was not necessary to discontinue or switch the drug. Clearly, the stability of the functional and morphologic conditions should be monitored during closer follow-up.

Understanding the nature of hyperautofluorescence in the fundus is important in determining its prognostic value. Fundus autofluorescence signals derive mainly from lipofuscin granules in the RPE, and increased FAF can arise from increased accumulation of this pigment. Such increases can have several origins, including abnormal RPE metabolism with increased phagocytosis of photoreceptor outer segments or an inherited or acquired defect of the phagocytic processes. However, other factors can modulate FAF intensity. Among them are fluid under RPE detachments, as well as longstanding subretinal hemorrhages containing autofluorescent fluorophores. However, neither of these factors was present in the current patients. Fluorophores other than RPE lipofuscin are present in various anatomic layers, such as Bruch’s membrane, choroid, and sclera, but they have different excitation and emission spectra from the fluorophores detected in the present study.

Histopathologic evaluations of eyes with DFO toxicity have shown that retinal changes in the RPE include degeneration and patchy depigmentation in the equatorial as well as the posterior fundus. Retinal pigment epithelial cells appear enlarged and projected into the subretinal space, which sometimes has detached and rounded RPE cells containing typical melanin accumulation.
Supported by postmortem findings and based on instrument characteristics, the dominant fluorophore responsible for the elevated autofluorescence signals may originate either from an accumulation of lipofuscin or melanolipofuscin, or both in the normal RPE cell layer, as well as from RPE cells that have migrated into the neurosensory retina. Adding high-resolution optical coherence tomography to the cSLO FAF imaging will allow investigation of corresponding changes at a semihistologic level and may reveal added detail in the underlying cause of an abnormal FAF signal. Further technological improvements of imaging tools, including adaptive optics systems coupled to SLO, also may contribute to the understanding of these findings. An enhanced understanding of the tissues and fluorophores associated with DFO-related FAF could be derived from histopathologic correlation based on donor eyes that were characterized clinically before enucleation.

Although different hypotheses about the toxic mechanism leading to retinal changes have been reported, the specific cause likely is multifactorial. Because iron is necessary for normal cellular functions in the retina, it seems highly probable that any dysregulation of iron metabolism could contribute to retinal toxicity through the generation of oxygen free radicals. The role of iron and the pathophysiologic connection between ultrastructural changes in Bruch’s membrane and RPE dysfunction is of general interest beyond its importance in DFO retinopathy. Such a connection also has been hypothesized in AMD. This is supported by postmortem findings of iron in drusen, RPE, and Bruch’s membrane of patients affected by AMD. Of note, recent evidence suggests that iron overload may promote retinal and RPE degeneration with features similar to DFO retinopathy as well as dry AMD.

However, in young patients treated with DFO, it is accepted that these changes in RPE cells may be characteristic of DFO toxicity and not of iron overload. This is because of the absence of histochemical evidence of iron deposition in the degenerate RPE cells. Moreover, in the current patients, FAF detected no retinal alterations in the control group. Recent in vitro studies seem to confirm a DFO-related direct toxic effect on RPE cells that is mediated by phosphorylation of p38. However, retinal toxicity also may result from retinal iron deficiency if DFO doses are too high, which could modify retinal iron homeostasis and may lead to changes in the RPE.

Thus far, to the authors’ knowledge, this is the largest prospective study of patients with long-term DFO treatment screened for toxic retinopathy. Excluding patients with the minimally changed FAF pattern who presumably had early-stage DFO retinopathy, 8 patients (2.4%) were diagnosed with marked retinal and RPE alterations. Consistent data about the incidence of DFO-related retinopathy are lacking in the literature. This is particularly so among adult patients receiving standard subcutaneous therapy and patients with complex systemic medical conditions. In the 1990s, 2 studies reported contradictory rates of DFO-related ocular toxicity: 15% and 87%, respectively. However, in those studies, the standard dose of DFO, the method of administration, and the conditions for which the therapy was instituted were different compared with those of the current study. In fact, these dialysis patients with chronic renal failure were treated for aluminium overload. Notably, previous reports indicated that ocular DFO toxicity in dialysis patients can occur with as little as a single intravenous dose. This suggests that the underlying condition also may play a role in the pathophysiology of DFO retinopathy.

In the control group of patients without DFO treatment, no retinal abnormalities were detected by ophthalmoscopy or FAF. However, all of these patients had TI, and in contrast to patients with TM, the former did not require continuous treatment with DFO or other chelating agents.

The literature contains conflicting reports regarding the severity and reversibility of DFO-related ocular toxicity, and the optimal dosage and method of infusion need to be evaluated further. Considering reports that analyzed patients with transfusion-dependent anemias, the largest series to date was reported by Haimovici et al., in which 14 of their 16 cases had DFO retinopathy. However, their retrospective observational study did not report on the incidence because it did not consist of a consecutive series of patients. In another study by Olivieri et al., symptomatic changes in RPE were reported in 5 of 89 patients receiving nightly subcutaneous DFO for transfusion-dependent anemia. A subsequent study by Cohen et al. showed a much lower incidence of toxicity, suggesting that these risks can be minimized by not exceeding doses of 50 mg per kilogram of body weight in patients with iron overload.

In pediatric groups examined recently, DFO-treated patients demonstrated rare and mild findings of ocular toxicity. Chen et al. reported that none of 30 transfusion-dependent patients receiving subcutaneous DFO (40–50 mg/kg per dose) showed abnormalities in visual acuity or funduscopy results during the 2 years of the follow-up. Recently, Baath et al. found only 1 case of DFO-related retinopathy among 84 pediatric patients reviewed retrospectively. A short-term, reversible retinal toxicity developed in the patient after high doses of intravenous therapy.

In conclusion, FAF imaging is a more sensitive method than ophthalmoscopy for detecting RPE abnormalities. It is a helpful, fast, and noninvasive tool for monitoring the status of the macula in patients at risk of DFO toxicity. In general, a better-defined phenotype identified with novel, noninvasive diagnostic tools is important for understanding the pathophysiologic mechanisms of DFO-dependent retinal changes. It is also a prerequisite for identifying specific high-risk characteristics that may be helpful in the decision to discontinue or switch therapy for patients to prevent disease progression. Progressive pathologic alteration of the RPE suggests an important role of this retinal layer in the evolution of visual loss during long-term treatment with DFO. Further studies are needed to answer various open questions, including the incidence of DFO-dependent retinopathy, the optimal dosage of the drug, as well as the best tool for screening and monitoring changes over time.

References


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