

Nutraceuticals for dry age-related macular degeneration: a case report based on novel pathogenic and morphological insights

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ABSTRACT

Age-related macular degeneration represents the main retinal disorder leading to irreversible blindness in people over the age of 50 in the Western World. Here we describe a case report, which suggest that specific nutraceutical compounds may exert beneficial effects on the progression of dry age-related macular degeneration (AMD), an eye disease with no approved treatment or cure. Specific antioxidants, such as lutein, resveratrol and Vaccinium Myrtillus, which are known to reduce the risk of developing AMD, when co-administered alone, were supplemented to diet of an informed patient suffering from dry AMD. The case report indicates an improvement of visual acuity and a long lasting decrease in druse volume and number. The concomitant intake of lutein, resveratrol and Vaccinium Myrtillus when administered for six months produced a marked decrease in the drusen observed at OCT at the 6-month follow-up. At this time interval, the patient experienced a noticeable improvement in visual acuity, a decrease in eye strain, more color contrast, higher visual definition. The case report indicates the potential benefit for a non-invasive treatment with improved quality of vision in dry AMD. A larger population followed over a long-term period is warranted. The support of nutraceuticals could therefore offer a new non-invasive, adverse-effect-free which may restore the pathology affecting the cross talk between choroid and retinal cells. The results of this case report are discussed within the frame of molecular mechanisms synergizing site-specifically at the anatomical border between the outer retina and inner choroid.

Key words

Age-related macular degeneration • Neurodegeneration • Autophagy • Lutein • Vaccinium Myrtillus • Resveratrol • Anthocyanins • Antioxidants • Carotenoids

Abbreviation list

AMD (age-related macular degeneration); OCT (optical coherence tomography); RE (right eye); LE (left eye); UCVA (uncorrected visual acuity); BCVA (best-corrected visual acuity).

Introduction

Age-related macular degeneration (AMD), is a disorder affecting the retina, which progressively leads to loss of vision and represents the main retinal

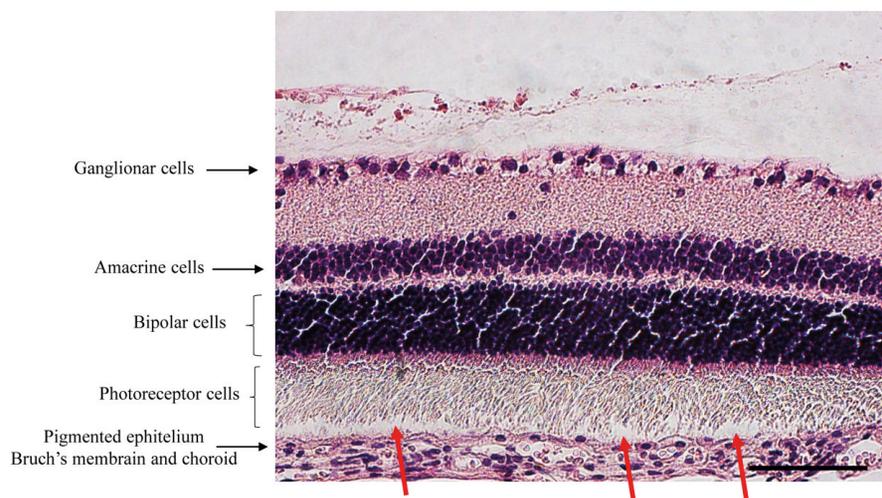
disorder leading to irreversible blindness in people over the age of 50 in the Western World (Pascolini et al., 2004; Congdon et al., 2004; Jager et al., 2008). In fact, the prevalence of the disease in the first decade of the new millennium was estimated over 8 millions in

the United States, affecting 4% of the population over 60 years (de Jong, 2006). However, it is predicted that AMD will increase over time among Western Countries, with an overall estimated prevalence up to 240 millions of people worldwide by 2040 (Datta et al., 2017). Thus, the incidence of AMD is increasing dramatically, mostly concerning the atrophic variant. In fact, the definition of age-related macular degeneration includes roughly two main disease phenotypes, which differ concerning pathology, time course, and severity. These two classic forms, are shortly defined as “dry” (or atrophic) AMD and “wet” (exudative or neovascular) AMD. Indeed, a variety of overlapping phenotypes are described between these two forms (Ambati and Fowler, 2012), which lead to be cautiously when applying such a clear-cut distinction in defining pathology and patients prognosis. In keeping with the classic dual phenotype, the dry form is prevalent, since it affects about 85% of individuals with AMD (Seddon, 2001); it tends to progress slower, thus producing a slower loss of visual acuity than the wet type (Jager et al., 2008). Nonetheless, the eventual progression of dry AMD impairs visual acuity and produce blindness over time. Both the wet and dry phenotypes of AMD share a pathological hallmark known as “drusen”. These consist of polymorphous debris enlarging the space between the retinal pigment epithelium and Bruch’s membrane. It is established that drusen *per se* worsen to a certain extent visual acuity. In fact, drusen consist of polymorphic extracellular aggregates, which directly relate to the disease state since the overall amount, site specificity (behind the macular region

of the retina), and their size all determine the severity of AMD. In the wet AMD drusen are associated with neo-angiogenesis trespassing the choroid-retina border. These newly formed overwhelming vessels often lead to bleeding and fluid accumulation in the extracellular space, which is absent in the dry variant. The anatomical site, which needs to be considered when studying AMD is placed around the retinal pigment epithelium, the Bruch’s membrane and the capillary vessels of the inner choroid as visible at light microscopy (Figure 1). In the wet AMD phenotype, the specific alteration subsides within the semipermeable Bruch’s membrane, which occludes the spreading of blood vessels from capillary of the inner choroid towards the pigmented epithelium. In physiological conditions the Bruch’s membrane modulates the exchanges between the blood and the retina but it also acts as a barrier, which separates the retinal pigment epithelium from the choroid. In contrast, in wet AMD an overproduction of blood vessels supplying the outer border of the retina occurs. This surpasses the border of choroid through ruptures of the Bruch’s membrane, thus destroying the anatomical border between the choroid and the retina.

This represents the key distinguishing point between wet and dry AMD. In fact, in dry AMD, choroid vessels are not primarily recruited and pathology is solely grounded on drusen deposits externally to the pigment epithelium, which disrupt the Bruch’s membrane without angiogenesis, bleeding or extracellular fluid accumulation. In the wet variant drusen associate with bleeding of small vessels in

Fig. 1. - Light microscopy of a mammalian retina, which includes also Bruch’s membrane and the capillary vessels of the inner choroid. Arrows indicate empty spaces, which are the key sites where drusen occur most frequently. **Scale bar** = 50 mm.



the inner choroid layer, which in turn stimulate angiogenesis, which occurs following an abnormal pattern often leading to a detachment of the pigment epithelium. In dry AMD, drusen appear as small white or yellowish deposits, beneath the macula, the central area of the retina.

Despite being present also in extra-macular regions the macular placement and the size of drusen characterizes advanced stages of AMD (Jager et al., 2008). This generates a progressive loss of central vision over time with deterioration in near and far visual acuity, loss of contrast and distortion of images.

In both phenotypes, the disease course is irreversible and, despite transient changes in the slope of the progression curve, no spontaneous recovery takes place. This led to recent efforts to rescue cell transplantation by using stem cell-related approaches for restoring or relenteing macular degeneration in AMD (Zarbin et al., 2019). At present, there is no established or approved treatment for dry AMD. This is mainly based on a lack of a deep insights into the pathogenic mechanisms, which drive the disease, both concerning the wet and dry variants. The detailed advancement in the fine anatomy of the outer part of the retina joined with a deeper knowledge of those metabolic pathways acting as a kernel between the pigmented epithelium and the inner layers of the choroid allow to challenge novel therapeutic approaches.

While the overactive angiogenesis occurring in wet AMD can be mitigated by administering anti-angiogenic drugs, this is not the case for dry AMD. Thus, alternative strategies are needed including the use of flavonoids and related compounds. In fact, evidence-based studies show that macular progressive degeneration can be reduced through the consumption of antioxidants such as flavonoids and anthocyanins in the diet (Forte et al., 2011; Richer et al., 2014; Abu-Amero et al., 2016; Tao et al., 2016; Buscemi et al., 2018; Bungao et al., 2019; Khoo et al., 2019).

These compounds mitigate oxidative stress and inflammation, which play a critical role in the initiation and progression of dry AMD (Donoso et al., 2006; Jarrett and Boulton, 2012). Therefore, we explored the beneficial effects of a combined administration of carotenoids (lutein) and anthocyanins (resveratrol and Vaccinium Myrtillus), with proven antioxidant

and anti-inflammatory activities (Natoli et al., 2010; Forte et al., 2011; Richer et al., 2014; Abu-Amero et al., 2016; Tao et al., 2016; Riva et al., 2017; Buscemi et al., 2018; Bungao et al., 2019; Khoo et al., 2019; Pawlowska et al., 2019). This specific pathogenesis-based dietary treatment was prescribed to an informed patient suffering from advanced dry AMD where big drusen were observed beneath the macular region.

This study was planned to challenge the potential beneficial effects of antioxidants in the course of dry AMD as documented by OCT of the macular region and the improvement of eyesight in compliance with patients health and upon informed consent.

Methods

Optical coherence tomography (OCT)

It consists in a non-invasive imaging procedure based on visible light waves which are reflected at different layers of the retina and adjacent choroid structures. The technique allows to obtain cross-section images. The OCT provides morphological data useful to interpret retinal pathology since it demonstrate the loss of integrity within specific retinal layers as well as the presence of abnormal structures (such as drusen) within the outer retina or the adjacent choroid. Is a gold standard exam in the diagnosis of age-related macular degeneration providing the direct visualization and measurement of drusen and the altered flatness of the retinal surface and derangements of specific layers. It is specifically useful for macular scanning. Imagine produced allows for real time, non-invasive evidence of basic retinal structure and internal layers of the choroid.

Amsler Grid test

The Amsler Grid test was used to detect visual alterations resulting from damage to the macula or the optic nerve. The apparatus consists in a white square-shaped grid divided by horizontal and vertical black lines in approximately 20 small squares in each side of the grid. A central black dot is present for fixation. The illumination of the chart was kept steady and optimal to allow the best resolution. The grid is kept at least 33 cm far from the eye. The patient is asked to close one eye and each eye is tested separately.

The patient is asked to look at the central dot of the grid. In patients with altered vision the lines of the square appear distorted, otherwise they look parallel (Su et al., 2016).

Pelli-Robson Contrast sensitivity test

– **Contrast sensitivity** is the ability to perceive slight change in luminance between regions, which are not separated by sharp borders. It is just as important as the visual acuity (i.e. the ability to discern and perceive sharp outlines of small objects).

– **Pelli-Robson test** measures contrast sensitivity using a single large letter size (20/60 optotype), with contrast varying across groups of letters. Specifically, the chart uses letters (6 per line), arranged in groups which vary in contrast from high to low. Patients read the letters, starting with the highest contrast, until unable to read two or three letters in a single group. Each group has three letters of the same contrast level, so there

are three trials per contrast level. The subject is assigned a score, which is based on the contrast of the last group in which two or three letters were read correctly. The score, a single number, is a measure of the subject’s log contrast sensitivity. Thus, a score 2 means that the subject was able to read at least two out of the three letters with a contrast of 1 percent (contrast sensitivity = 100% or log 2). A Pelli-Robson score 2.0 indicates normal contrast sensitivity of (100%). Scores less than 2.0 signify poorer contrast sensitivity. Pelli-Robson contrast sensitivity score of less than 1.5 is consistent with visual impairment and a score of less than 1.0 defines a visual disability.

Snellen Chart test

A retro-illuminated wall-mounted Snellen chart was used with the patient standing at 6 m from the chart. The tool consists on chart including capital letter degrading in size from the top to the bottom.

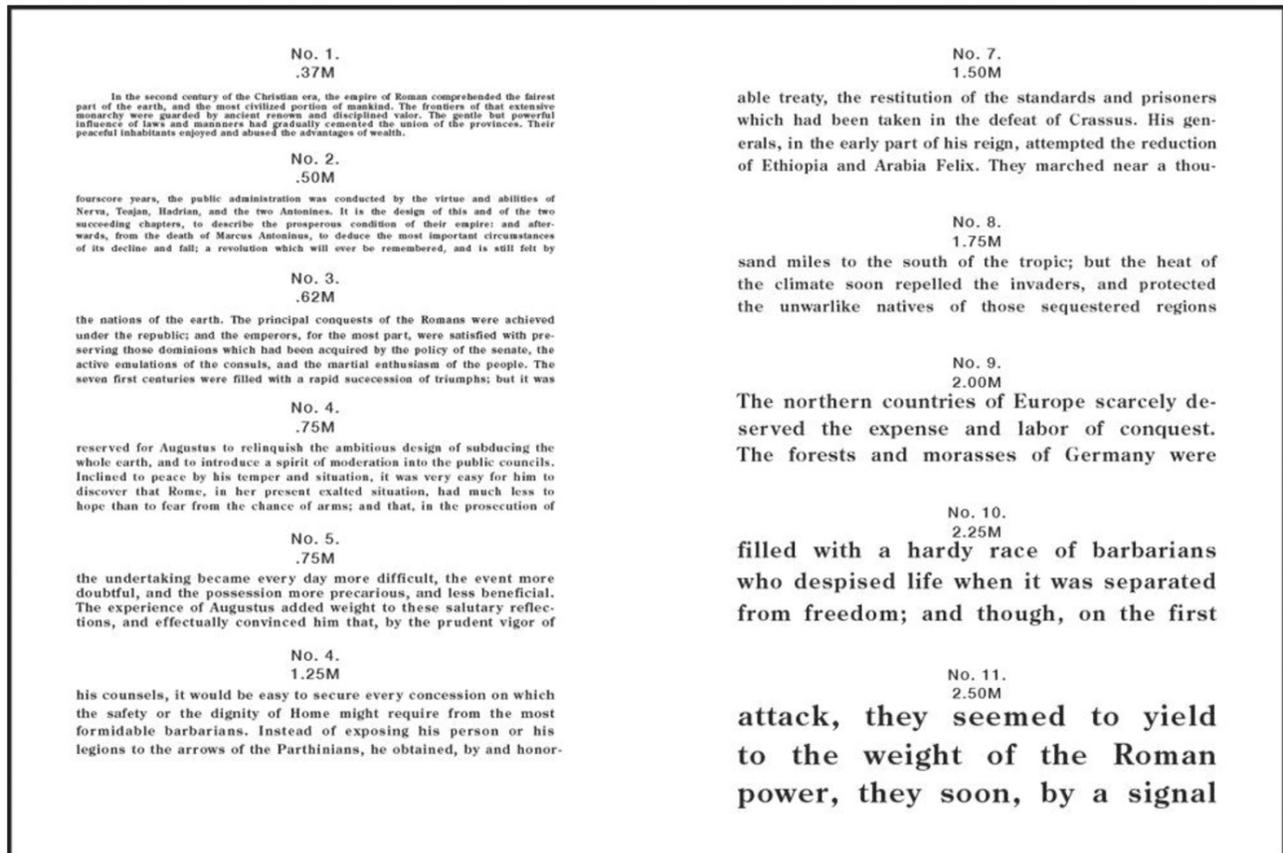


Fig. 2. - The Jaeger chart (see Methods for details).

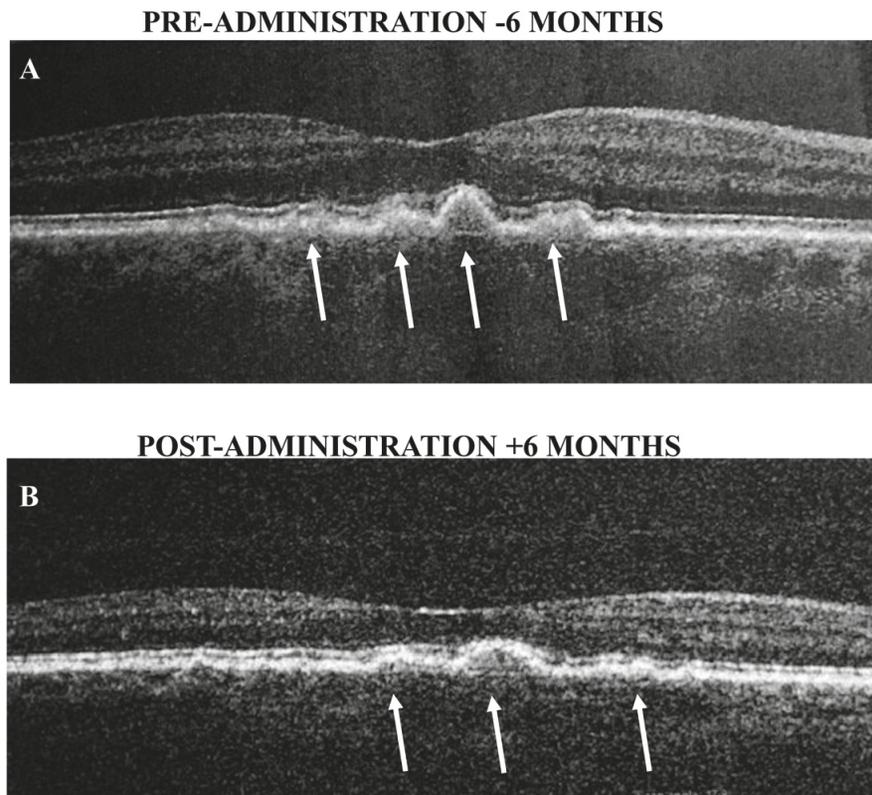


Fig. 3. - A) Left eye macular profile before treatment. B) Left eye macular profile 6 months after treatment.

All measurements were performed in non-dilated eyes. Usually, the right eye was tested first and, consecutively, the left one. The patient is instructed to read from the top of the chart to as far down as they can see. Vision test is stopped when the patient is no longer able to read any letters. (Johnson et al. 1998; Chen et al. 2014). 20/20 Vision is considered “normal” vision meaning that it is possible to see at 20 feet a letter that most human beings should be able to read at 20 feet. Eye charts can be configured in various ways, but generally, if during an eye test the patient can read the big E at the top but none of the letters lower than that, the vision is considered 20/200. That means that the patient can read at 20 feet a letter that people with “normal” vision can read at 200 feet.

The Jaeger eye chart test

The Jaeger chart consists of short blocks of text in various type sizes (Figure 2).

The type scale on a modern Jaeger eye chart usually ranges from J10 (approximately 14-point type for

Times New Roman font) to J1 (approximately 3-point type, Times New Roman). Some Jaeger charts have an additional paragraph labeled “J1+” that may be even smaller than the J1 block of text.

The J1 paragraph on a Jaeger card typically is considered the near vision equivalent of 20/20 visual acuity on a distance eye chart. On some Jaeger cards, the J1+ paragraph is the 20/20 equivalent. Common newsprint generally ranges in size between J7 (10-pt type) and J10 (14-pt type), which are the equivalent of 20/70 and 20/100 on a distance eye chart. The chart is held at a specified reading distance (such as 35 cm) and you are asked to read the passage with the smallest type you can see.

Patient history, dietary treatment and case presentation

A 72-year-old female was diagnosed with dry AMD in her RE about 3 years previously. She used to wear only reading glasses. The main complaint of the patient was that she had a lower quality of vision,

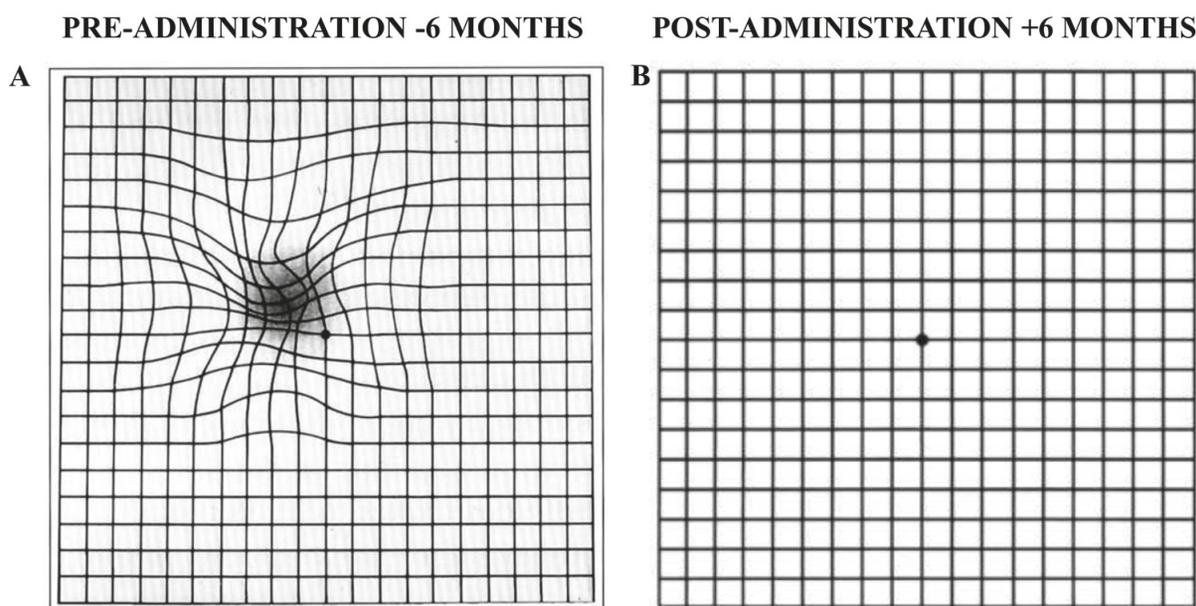


Fig. 4. - Amsler Grid test at the beginning of the treatment (A) and 6 months after nutraceuticals (B).

a loss of definition, and eye strain in her dominant eye (RE). The eye performance was impaired, while watching TV and reading. Eye strain was frequent even with the use of reading glasses. Her far UCVA was RE 20/25 (not improved with lens or pinhole), while near UCVA was RE J3; her near BCVA was RE J1-J2. The OCT scan revealed several deposits (drusen) beneath the macula in the RE (see Figure 3A).

The Amsler grid test showed irregular lines for the RE (Figure 4A). The contrast sensitivity was 1.8 in the RE (Figure 5A). The diet supplementation of antioxidants in the form of predetermined doses of nutraceuticals was administered. Supplementation consisted of 5g per day of 3 natural extracts in powder form: lutein from *Tagetes erecta*, resveratrol from *Polygonum cuspidatum* and *Vaccinium Myrtillus*.

No change in the dietary regimen of the patient occurred during the procedure.

The patient continued the supplementation with nutraceuticals for an overall period of 6 months. Supplements were administered for 20 days during each month.

The patient had been informed about the nature of the procedure, the alternative options for dry AMD, and signed an informed consent form.

Outcomes measurements

Disease outcomes were determined using the following measures from the methods reported above:

– Optical coherence tomography (OCT, SPECTRAL OCT SLOW OPTOS) was used for macular scanning. Imagine produced allows for real time, non-invasive evidence of basic retinal structure and external layers of the choroid.

– Amsler grid to measure the quality of the visual field (detection of blurred, wavy, broken or distorted lines).

– Pelli Robson chart for assessment of contrast sensitivity

– Snellen chart for far visual acuity (UCVA and BCVA)

– Jaeger chart for near visual acuity (UCVA and BCVA)

The clinical outcomes are reported at six months before administration and after six months of administration. All tests were repeated four times.

Statistical analysis

Measurements of visual variation are expressed as percentage. Values obtained in four trials are reported as mean \pm SEM and were compared by using paired Student's t-test. Null hypothesis was rejected for $p < 0.05$.

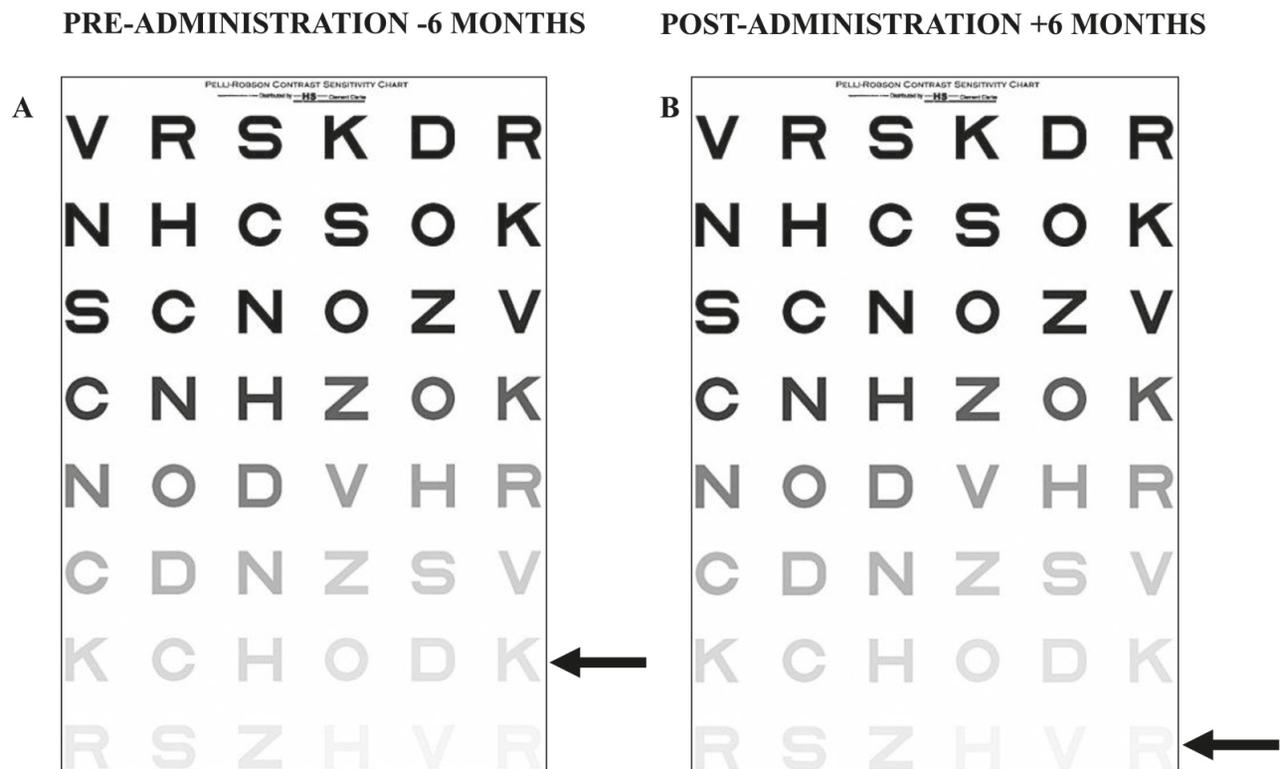


Fig. 5 - Contrast sensitivity before nutraceuticals (A) and after six months treatment (B). Arrows indicate the performance of the patient.

Results

OCT scan showed before starting diet indicated a number of drusen featuring a big size beneath the macular region (Figure 3A). The six months diet improved drusen volume and thickness in the central area of the macula and was associated with a more regular macular profile in the RE (see Figure 3B), after six months.

From a subjective point of view, the patient reported improved vision after treatment. Less eye strain, more colored contrast, higher definition, better far and near visual acuity were noticed.

The Amsler grid test revealed a less distorted grid with less wavy lines (Figure 4B). The contrast sensitivity test indicated an improvement from 1.8 to 2.0 in the RE (Figure 5B). The far UCVA was 20/20 in RE and near UCVA improved from J3 to J2 in the RE, near BCVA improved from J1-J2 to J1 in the RE (Figure 6).

The same clinical outcomes were measured at 6 months during and after the treatment.

The eye condition was stable and unchanged and no worsening of the outcomes was noticed after 6 months.

Discussion

This case report indicates a successful treatment whereby quality of vision was improved in a patient suffering from dry AMD; better visual acuity and contrast sensitivity were associated with a reduced anatomical damage in macular profile.

Overall, these results are encouraging. To confirm the efficacy of this approach, a study on a larger dry AMD population followed over a longer period is warranted.

To date, although there are no approved treatments for many retinal diseases, our results suggest antioxidants in the form of nutraceuticals could potentially facilitate a valid and promising approach for the dry form of AMD, which improves visual acuity and aids tissue repair, thereby preventing further progression of macular degeneration.

The morphological hallmark of AMD consist in the deposition of drusen, which alter the physiology of the external retinal layers. In the wet variant, drusen deposition occur in the context of production of novel blood vessels starting from the choroid and passing through breaks of Bruch's membrane

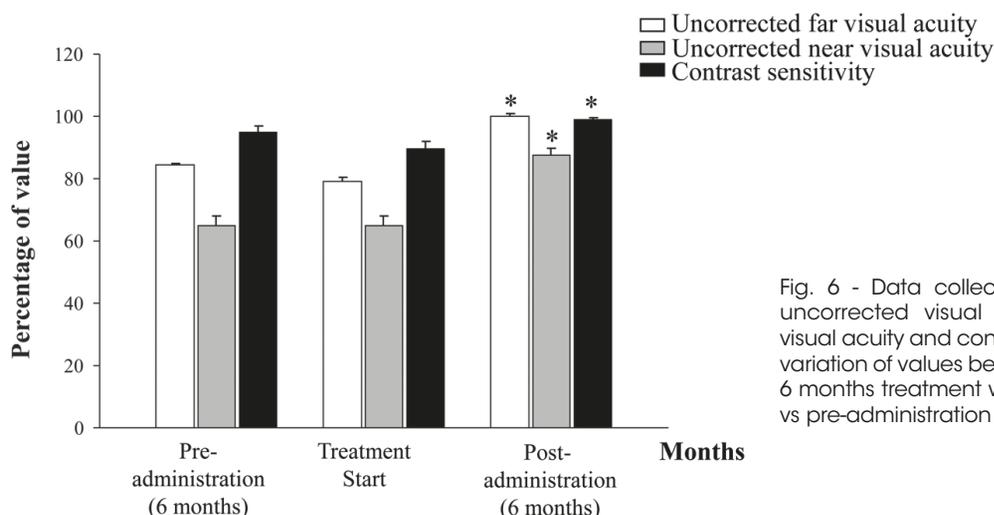


Fig. 6 - Data collection for the right eye (far uncorrected visual acuity, near uncorrected visual acuity and contrast sensitivity) showing the variation of values before the treatment and after 6 months treatment with nutraceuticals. * $p < 0.05$ vs pre-administration and treatment start.

into the external retinal layers, where bleeding and fluid accumulation increase interstitial pressure up to detachment of the pigment epithelium, which further worsens visual processing. The progressive organization of bleeding leads to fibrosis and lipid deposition which adds on drusen to accelerate the loss of visual acuity (Donoso et al., 2006; Jarrett and Boulton, 2012; Riva et al., 2017; Pawlowska et al., 2019). The dry AMD is morphologically defined by the accumulation of drusen without neovascularization. This makes it crucial to establish the origin and fine structure of drusen. Starting from the protein component, which is largely prevalent in drusen composition, various sources were postulated. These include (i) an origin from blood and cells in the choroid; (ii) undigested cell material from the outer segment of photoreceptors; (iii) protein clusters produced by the pigment epithelium. The proteomic analysis of drusen occurring in AMD reveals that most proteins derive from blood with a significant amount which is contributed by the pigment epithelium and a minimal contribution from photoreceptors (Bergen et al., 2019). Apart from their origin, the physical space filled by the drusen undoubtedly interferes with the process of visual processing since they are retained in site thereby enlarging the space between the basal lamina of the pigment epithelium and the inner collagenous tier of the Bruch's lamina (so called sub-RPE-BL space, Bergen et al., 2019). Apart from representing a mechanical source of derangement in the finely tuned architecture at the choroid-pigment border, drusen alter the planar distribution of photoreceptors

which may specifically induce the visual distortion. Nonetheless, the occurrence of drusen may be regarded as the consequence of a primary alterations of seminal functions exerted by the pigment epithelium. In fact, this external layer of the retina provides key functions to maintain the homeostasis of photoreceptors. The main biochemical pathways, which are activated within the pigment epithelium engage the autophagy flux, which is critical to prevent neurodegeneration. Thus, it is likely that a dysfunction in the clearance of misfolded proteins including complement molecules (Rudolf et al., 2008) may produce extracellular accumulation of polymorphic debris in the form of drusen. If this is the case, one may expect that compounds, which exert a stimulation of the autophagy machinery empower the pigment cells to metabolize properly such an excess of substrates. In fact, following administration of nutraceutical compounds acting as autophagy-promoter one may expect to restore the paracrine homeostasis bridging photoreceptors, with the pigment epithelium and the Bruch's lamina of the choroid. In fact, in the present case when a prolonged treatment with specific nutraceutical compounds was prolonged for several months to a patient suffering from dry AMD we did not merely occlude the formation and deposition of drusen, we rather observed the reabsorption of such a deleterious extracellular material. This morphological regression of the AMD hallmark was associated with a recovery in visual acuity, loss of distortion and a gain of visual contrast. This may rely on the mere antioxidant activity produced by

lutein and resveratrol. Nonetheless, both compounds possess a well-documented stimulation of protein clearing machinery and this effect was hypothesized to be involved in dry AMD (Bowes Rickman et al., 2013). In fact, as reported by these authors, autophagy dysfunction accompanied by lipofuscin accumulation and ROS activates inflammatory reactions, further promoting long-term and chronic inflammatory cascade thus accelerating cell senescence of the pigment epithelium (Kaarniranta et al., 2013). The effects of compounds administered to the patient involve all these pathogenic events which are presently known to produce retinal damage in AMD since resveratrol induces antioxidant effects in the retina (Munia et al., 2020; Neal et al., 2020) similarly to lutein and Vaccinum Myrtillus (Mares et al., 2016; Untea et al., 2020). All these compounds share anti-inflammatory effects (Bola et al., 2014; Wang et al., 2015; Mares et al., 2016; Buscemi et al., 2018; Schink et al., 2018; Bungau et al., 2019). Remarkably, the synergism between these compounds may extend to protein clearing pathways, which play a fundamental role in tuning the orchestration at the retinal-choroid border, where retinal pigment cells are the pivot. The occurrence of pathological regression joined with improvement of symptoms of visual acuity and contrast with the occlusion of distortion in this case of dry AMD may greatly rely on the multiple step synergism between these compounds to interfere with the biology of disease. The secretion of debris in AMD occurs on both sides of the pigment epithelium which suggests that physiological polarity (secretion towards the basal membrane) is lost and a bidirectional polarity of secretion occurs, which concerns proteins mainly non-related with photoreceptors such as un-esterified cholesterol, apoE, complement factor H, and vitronectin (Rudolf et al., 2008). This calls to consider a rather generalized defect in protein handling by the retina-choroid junction in AMD, which is best targeted by a pharmacological synergism at multiple levels.

Conclusions

To date, there are no approved treatments for dry AMD; here we report a case where nutraceutical compounds all sharing similar mechanisms of action on visual acuity and contrast sensitivity.

These effects were associated with a regression of morphological alterations (drusen) assessed at OCT. A novel insight in the pathogenesis of dry AMD allows to further improve therapeutic approaches aimed at restoring the altered communication and metabolic derangement occurring at the level of the choroid retina border. The present results indicate that nutraceuticals could potentially be the therapy of dry AMD, thereby preventing further progression of macular degeneration.

In fact, this case report indicates a successful treatment, which improves the quality of vision in a patient suffering from severe dry AMD who following 6 months of administration experienced better visual acuity and contrast sensitivity along with reduced anatomical damage in macular profile.

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