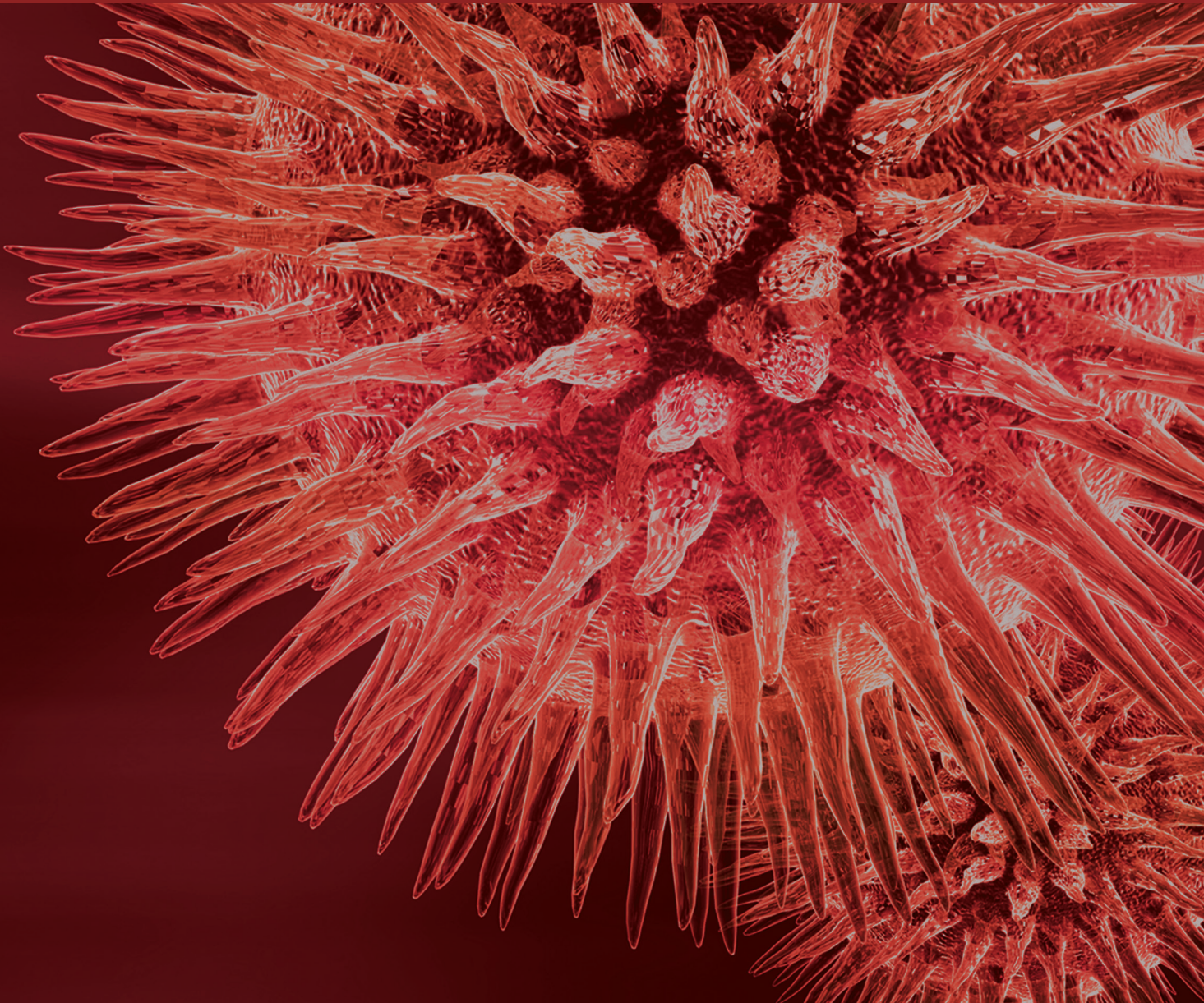# Ocular Comorbidities and the Relationship between Eye Diseases and Systemic Disorders

Guest Editors: Maria D. Pinazo-Durán, J. Fernando Arévalo, José J. García-Medina, Vicente Zanón-Moreno, Roberto Gallego-Pinazo, and Carlo Nucci

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Contents

Editorial

Ocular Comorbidities and the Relationship between Eye Diseases and Systemic Disorders

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Patients with ocular disorders may have additional ophthalmic problems that can have an impact on both morbidity and vision. Ocular comorbidities are commonly associated with vision-related disability and decreased quality of life related to visual impairment. The majority of studies on this topic deal with cataracts, glaucoma, uveitis, and/or retinopathies. It is important to summarize the available evidence to date on the association of one or several ocular diseases and the implications these comorbidities have on prognosis and therapy.

The relationship between eye disorders and systemic diseases has recently drawn special interest. The increasing prevalence of neurodegenerative disorders, diabetes mellitus, hypertension and cardiovascular pathologies, and osteoarticular processes has a great impact on the society. In order to appropriately manage eye disorders, it is pivotal to achieve an early and accurate diagnosis of concomitant systemic diseases. This type of integrated approach is essential to ensure a better knowledge of the comorbidities to prevent blindness.

In this issue, a variety of works have precisely addressed important ocular comorbidities, as well as the state of the art on the relationship between eye diseases and systemic disorders. A total of 6 reviews, 3 clinical studies, and 4 research articles have been joined herein to nicely draw the multidisciplinary scenario of this special issue.

Regarding the review articles, M. Figus et al. go over Adamantiades-Behçet's disease with the most serious clinical manifestation being bilateral panuveitis, which may lead to blindness. The authors show that the use of biological agents improves the visual prognosis of the affected patients. H. J. Chung et al. take a close look at the relationship between primary open-angle glaucoma (POAG) and blood pressure (BP), showing that the increase in the latter induced an increase of the intraocular pressure (IOP) leading to a higher risk of glaucoma progression. E. M. Vingolo et al. provided a complete guide to reduce the risk of ocular manifestations due to Ebola virus disease. F. J. Muñoz-Negrete et al. reviewed current knowledge on the diagnosis and management of

uveitic glaucoma. Since this type of glaucoma is related to very high IOP and more severe optic nerve damage than other types of glaucoma, it is mandatory to diagnose as soon as possible this comorbidity and the most appropriate management of both uveitis and glaucoma. A. C. Martins et al. revised the ocular manifestations associated with the familial amyloid polyneuropathy, with the amyloid deposition in the vitreous, dry eye, and secondary glaucoma being the most relevant. Further investigations are needed in this regard to develop new therapeutic strategies to avoid or treat these ocular disorders. Finally, M. D. Pinazo-Durán et al. had a quick look at selected ocular comorbidities, such as dry eyes, glaucoma, cataracts, and retinopathies, as well as the eye manifestations in systemic diseases with special attention to the aging eyes.

The clinical studies deal with interesting subjects. H.-C. Kau and C.-C. Tsai assessed the clinical features of nasopharyngeal carcinoma patients with new onset diplopia after concurrent chemoradiotherapy and they found that this kind of diplopia could be caused by tumor recurrence or treatment complications with different manifestations or prognosis. J. L. Alio et al. compared the results of femtosecond laser versus manual technique for deep anterior lamellar keratoplasty and they concluded that both methods had similar visual and refractive outcomes with more evident wound healing when using laser. Finally, A. Ribelles et al. investigated the influence of working with computers on ocular surface in a sample of older women. These authors also studied the effect of oral supplementation with antioxidants/omega 3 fatty acids on ocular surface features in this context. They demonstrated that computer use during the working time induced obvious ocular surface sign and symptoms. Otherwise, supplementation was demonstrated to positively ameliorate this situation.

Four research articles have addressed the role of ocular comorbidities and the relationship between the systemic disorders and eye diseases. F. J. Muñoz-Negrete et al. showed the results of a study for implementing the diagnosis of glaucoma in diabetics by means of a set of criteria based on nonmydriatic monoscopic fundus photography. E. Salobrar-Garcia et al. performed screening of the peripapillary and macular segmentation thickness by optical coherence tomography in patients with Alzheimer's disease as compared to age-matched control subjects. The authors concluded that the increase in peripapillary thickness in patients affected with mild disease could correspond to an early neurodegeneration stage closely related to inflammation. J.-C. Yen et al. have done a nationwide population-based study on the risk factors of retinal artery occlusion, concluding that atrial fibrillation and coronary artery disease are the most relevant causes that may induce retinal vascular alterations. M. J. Roig-Revert et al., on behalf of the Valencia Study Group of Diabetic Retinopathy (VSDR), presented a research article on the biomarkers for diabetic retinopathy. Data from this study showed that plasmatic oxidative stress biomarkers were significantly higher in diabetics than in the control subjects. This status significantly improved in the subgroup of participants daily taking oral supplements with antioxidants and omega 3 fatty acids during 18 months of follow-up.

To summarize, this collection of papers covering different topics can be useful for medical specialists and interdisciplinary researchers to improve our understanding of the mechanisms underlying the ocular comorbidities as well as the relationship between eye diseases and systemic disorders.

Being the editors of this special issue, we hope that the readers can appreciate all these works that may also contribute to moving this important topic forward by stimulating innovative diagnostic and therapeutic strategies for better eye care.

Maria D. Pinazo-Durán
J. Fernando Arévalo
José J. García-Medina
Vicente Zanón-Moreno
Roberto Gallego-Pinazo
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Review Article

Eclectic Ocular Comorbidities and Systemic Diseases with Eye Involvement: A Review

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Coexistence of several ocular diseases is more frequent than suspected. In spite of the refractive errors, one or more of the following can be detected simultaneously: glaucoma, cataracts, uveitis, age-related macular degeneration, and dry eyes. In addition, as people age, ocular comorbidities are much more usually seen. Specific diseases are openly acknowledged to affect the eyes and vision, such as diabetes mellitus, hypertension blood pressure, arthritis, hyperthyroidism, neurodegenerative disorders, hematologic malignancies, and/or systemic infections. Recent advances in early diagnosis and therapy of the ophthalmic pathologies have reinforced patient options to prevent visual impairment and blindness. Because of this, it is essential not to overlook sight-threatening conditions such as the ocular comorbidities and/or the eye involvement in the context of systemic disorders. Moreover, the important role of the multidisciplinary cooperation to improve and sustain management of patients affected with eclectic ocular comorbidities and/or systemic disorders with eye repercussion is specifically addressed. This review intends to shed light on these topics to help in making opportune diagnosis and appropriately managing the affected patients.

1. Introduction

Currently, "comorbid" is employed to define a medical process that simultaneously exists in a patient with one or more medical conditions that, in turn, are independent themselves. In ophthalmology, "ocular comorbidities" are the eye disorder combinations existing simultaneously regardless of their etiopathogenic relationship [1–3]. This condition requires an effort from the ophthalmologist to gather information about the patients' morbidity. In fact, clusters of eye diseases/disorders composing the comorbidity patterns have to be necessarily identified. As it is quite rightly recognized, the eye importantly contributes to the diagnosis of a wide variety of systemic disorders, many times being the first visible clinical manifestation of the general problem, as well [4–6]. Early diagnosis and therapy may help anticipate or avoid complications.

Because of the importance of these topics, in this work, we have looked at some particular ocular conditions and also

Eclectic ocular comorbidities and systemic diseases with eye involvement		
1	2	3
Ocular comorbid conditions	Systemic disorders and the eye	Genetic syndromes and the eye

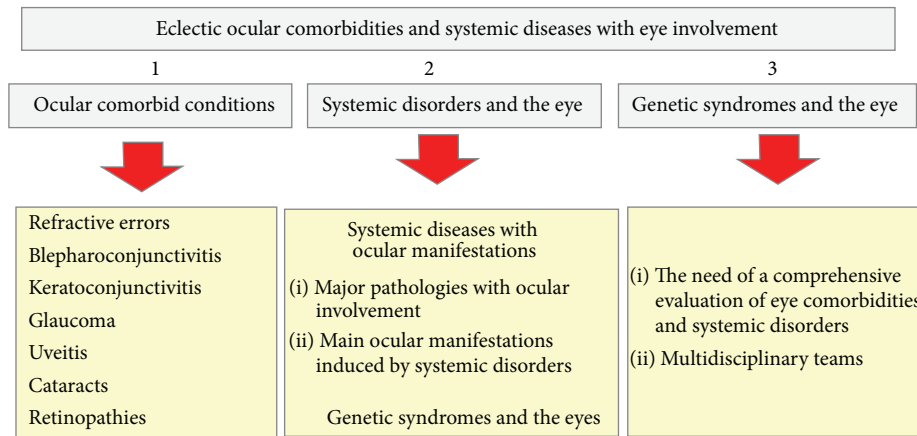| Refractive errors
Blepharoconjunctivitis
Keratoconjunctivitis
Glaucoma
Uveitis
Cataracts
Retinopathies | Systemic diseases with
ocular manifestations

(i) Major pathologies with ocular
  involvement
(ii) Main ocular manifestations
  induced by systemic disorders

Genetic syndromes and the eyes | (i) The need of a comprehensive
  evaluation of eye comorbidities
  and systemic disorders
(ii) Multidisciplinary teams |

FIGURE 1: Flow chart on the distinct sections included in this review.

at the most relevant systemic disorders that may affect the eye, and that has been also considered constituting a pose challenge to vision. Figure 1 is a flow chart of the distinct sections considered in this review.

2. Ocular Comorbid Conditions

The coincidence of two diseases is not the simple "arithmetic" addition of both processes, while this condition also creates a new status in the eye health that obviously warrants new consideration and outstanding strategies.

An overview of the most commonly seen associations in the clinical practice includes refractive errors and other eye diseases, anterior eye segment and adnexa processes such as conjunctivitis/keratitis/blepharitis, and ocular surface disorders with uveitis, cataracts, glaucoma, diabetic retinopathy (DR), or age-related macular degeneration (AMD), as reflected in Figure 1. Better knowledge of the ocular comorbidities is important to achieve a more accurate diagnosis and therapy of these disorders. Some of them are exposed in further detail below.

Searching the scientific literature, the most eclectic ocular comorbidities with high refractive errors have to be considered in the context of the three processes: (1) astigmatism, (2) hyperopia, and (3) myopia, all of them coexisting or not with anisometropia. A recent study carried out on 137 keratoconic patients concluded that 65% displayed with-the-rule anterior corneal astigmatism and 80% of eyes had against-the-rule posterior corneal astigmatism [7]. Fleischer ring, prominent corneal nerves, and corneal thinning have been recently described in association with typical keratoconus manifestations, like in asymptomatic individuals [8]. It is widely recognized that children with higher hyperopia are likely to display strabismus, amblyopia, and poor stereopsis, but other systemic disorders and/or developmental abnormalities have also been reported [9]. Higher myopia is usually associated with eye diseases such as glaucoma, cataracts, and choroidal neovascularization, significantly contributing to augmenting the risk of visual impairment and blindness in these patients [10]. Other eye comorbidities have been described in cases

of advanced surface ablation during laser refractive surgery that manifested themselves in the postoperative period with signs and symptoms such as burning/foreign body sensation, tearing, pain, and photophobia. As a consequence of this, instauration of a precise analgesic protocol has been recently suggested for patients subjected to these procedures [11].

Dry eye disease (DED) is a multifactorial disorder affecting the integrity of the lacrimal functional unit that frequently appears discordantly with the signs and symptoms [12]. Usually DEDs are linked to other eye diseases. Dry eyes have been found to be associated with vernal keratoconjunctivitis in children, probably affecting the ocular surface also during the quiescent phases of the disease. This finding contributes to our understanding of the very long-term consequences of this and other similar chronic mechanisms potentially damaging the ocular surface [13]. In analogous manner, DEDs of various degrees of severity have been reported in glaucoma patients chronically using hypotensive eye drops [14].

Uveitis is an important disease for the numerous eye complications that may occur in children and adults, many of which are vision threatening. It is known that these drawbacks increase with duration of disease. Specifically, noninfectious uveitis results in vision loss and a variety of ocular complications without adequate treatment. When comparing the risk of developing ocular complications between patients with noninfectious intermediate uveitis, posterior uveitis, or panuveitis, Dick et al. [15] reported that particular persistent cases are strongly associated with a higher risk of ocular comorbid complications (including band keratopathy, cataracts, glaucoma, and/or cystoid macular edema) than the matched healthy controls.

The number of people with cataracts has been estimated to increase (to 30.1 million and to reach 2.95 million, resp.) by 2020. Cataracts are the first evitable cause of blindness in the world. In general, cataracts are linked to aging. However, some cataracts can be related to genetic disorders, systemic diseases, long-term use of specific medications, or other eye conditions, such as uveitis, aniridia, glaucoma, ocular traumatisms, retinal detachment, retinopathy of prematurity, retinitis pigmentosa, DR, or AMD. Among the risk factors

for cataract development or progression, the following should be considered: DM, tobacco and alcohol habits, prolonged exposure to sunlight, corticosteroid systemic or local therapy, electric and heat injuries, nutritional facts, and so forth, some of them remaining controversial [16]. A study on the ocular comorbidities among 313 cataract-operated patients in rural China revealed that the leading comorbidities were the presence of refractive error (60%) followed by glaucoma (19%) [17]. And in this context, new surgical devices have been arising for implementing the results of the combined cataract and glaucoma interventions [18]. Higher demand of cataract surgery worldwide and the resulting complications need the instauration of outstanding strategies for avoiding vision loss. Different prophylactic measures have been recently reviewed to prevent macular edema after phacoemulsification surgery [19].

Glaucoma is the first cause of irreversible blindness worldwide. Major risk factor is the increased and sustained intraocular pressure (IOP) that induces optic nerve degeneration and atrophy [20]. There are two main glaucoma types, the open-angle (POAG) and the closed-angle (PCAG), the first being the most frequent clinical glaucoma form. It is well known that several hereditary conditions are associated with glaucoma, but other causes include prolonged use of corticosteroids, vascular abnormalities, and reduced blood flow to the eyeballs (as in the course of DR or retinal vascular occlusions) [21]. Correspondingly, ocular trauma or uveitis can induce secondary glaucoma. This latter is a common complication of uveitis affecting 20% of patients. Some ocular inflammations associated with secondary glaucoma that should be considered in the context of uveitic glaucoma are the herpetic keratouveitis, Fuch's heterochromic iridocyclitis, or the Posner-Schlossman syndrome [22]. It has also been reported that ocular comorbidity such as glaucoma or other surgery treatments following intraocular lens implantation may contribute to its opacification [23]. Additionally, higher prevalence of retinal diseases (DR, AMD) in glaucoma patients suggested a similar pathological process that needs further consideration [24].

Other retinopathies, such as the inherited retinal degenerative diseases, affect millions of people around the world, displaying several degrees of visual impairment and irreversible vision loss, with retinitis pigmentosa, choroideremia, juvenile retinoschisis, Stargardt disease, Usher disease, or Leber congenital amaurosis being the most frequent. Likewise, retinitis pigmentosa remains the leading cause of inherited blindness, with approximately 2 million people affected worldwide. Multiples genes have been identified and their mutation may result in the corresponding phenotype to retinitis pigmentosa. However, there is lack of knowledge on the molecular mechanisms involving the disease that exhibits a progressive and irreversible nature leading to continuous decline of the visual field and vision. Emerging treatments hopefully include gene therapy, stem cells, and electronic devices to restore vision [25]. Common eye comorbidities to retinitis pigmentosa are glaucoma and cataracts extremely contributing to the visual disability of the affected individuals [26].

Strategies to achieve a precocious diagnosis and to accurately plan the therapy of patients with ocular comorbidities may help in avoiding dangerous complications and visual loss.

3. Systemic Disorders and the Eye

Many diseases can directly or indirectly damage the eyes and vision, while other diseases possess associated ocular signs/symptoms and visual impairment. All of these have distinct mechanisms of action. For a better understanding, this section has been structured into two parts: systemic diseases with ocular manifestations and systemic syndromes and the eyes. Both sets of issues are exposed below.

3.1. Systemic Diseases with Ocular Manifestations. Main systemic disorders which may affect our eyes include endocrine and/or metabolic diseases, inflammatory and immune response processes, infections, hematological, cardiovascular, and cerebrovascular disorders, cancer, skin illnesses, and congenital/hereditary conditions. A summary of the processes that can affect the eyes and vision is reflected as follows.

Systemic diseases with eye involvement include the following:

Hematologic and lymphatic diseases.

Cardiovascular/cerebrovascular diseases.

Gastrointestinal/nutritional disorders.

Metabolic/endocrine disorders.

Musculoskeletal pathologies.

Pulmonary diseases.

Renal disorders.

Systemic viral infections.

Systemic bacterial infections.

Systemic protozoal infections.

Systemic fungal infections.

Systemic cestode and nematode infections.

Dermatologic pathologies.

Phacomatoses.

Collagen diseases.

Multisystemic autoimmune diseases.

Granulomatous diseases.

Immunosuppressive agents used in management of eye disease.

Ocular complications of certain systemically administered drugs.

Neoplastic diseases with ocular metastases.

Vitamins and eye diseases.

Miscellaneous systemic diseases with ocular manifestations.

Heritable connective tissue diseases.

Hereditary metabolic disorders.

Genetic syndromes.

3.1.1. Major Pathologies with Ocular Involvement. Some particular diseases are openly acknowledged to disturb the visual system, to a degree that the ocular manifestation may be used to accurately confirm the most complete diagnosis, as well as monitoring the appropriate therapy, such as in cases of diabetes mellitus (DM), hypertension blood pressure (HBP), hyperthyroidism, sarcoidosis, tuberculosis, arthritis, psoriasis, scleroderma, or systemic infections. The most relevant ones are explained in detail in this subsection.

Progression of DM of any type causes the diabetic eye disease that includes several sight-threatening ocular disorders, with the DR and diabetic macular edema (DME) being the most important that in the course of the disease may lead to visual impairment and blindness (Figure 1). In fact, both disorders, DR and DME, are leading causes of vision loss among working-aged adults (20–70 years) [27, 28]. Our understanding of the precise mechanisms by which DM induces DR and/or DME remains incomplete. A wide range of ocular pathologies are also associated with DM, such as cataracts, glaucoma, and optic neuropathy. Other ocular associations of DM distinct from DR are the anterior ischemic optic neuropathy, diabetic papillopathy, and extraocular muscles disorders [29]. Regarding these pathologies, studies suggest that up to 25% of patients with anterior ischemic optic neuropathy have a history of DM. Furthermore, diabetic papillopathy is characterized by acute disc edema and mild vision loss appearing suddenly in the course of diabetes. Also, extraocular motility disorders and diplopia occur in 25–30% of diabetic patients aged 45 years and older. Ocular diseases in which DM can be considered among the etiology also include the retinal vein/arterial occlusion or some corneal disorders. Furthermore, distinctive features have extensively been described during the ophthalmic surgery in diabetics. This topic has been reviewed by the Pan American Collaborative Retina Study Group (PACORES) in a study designed to evaluate the visual and anatomical outcomes after cataract surgery in diabetic patients with different intraoperative therapeutic strategies [30]. It is essential to promote early detection, timely and accurate treatment, and appropriate control of the clinical manifestations of the diabetic eye disease in order to prevent blindness in diabetics.

Retinal vascular changes can be the starting point of an asymptomatic HBP patient (Figure 2). However, in the course of the disease, both the acute and chronic hypertensive changes may display in the eyes severe abnormalities induced from the existence of a malignant HBP, or chronic changes resulting from long-lasting HBP. Retinal, choroidal, and optic nerve changes can be seen in different stages of the systemic disease widely known as the acute hypertensive retinopathy (or choroidopathy or neuropathy), as well as the chronic hypertensive retinopathy (or choroidopathy or neuropathy) [31]. All these processes are the result of adaptive changes and progressive degenerative damage to the arterial and arteriolar circulation caused by the HBP. It has been stated that major risk factors for the initiation or progression of hypertensive retinopathy are age, duration of hypertension, and systolic blood pressure levels. Severe degree of hypertensive retinopathy correlated with serious blood pressure concerns and the highest risk for stroke, as well as kidney and heart disease, while low levels of hypertensive retinopathy did not correlate with cardiovascular risks [32]. Moreover, the HBP predisposes patients to other eye disorders, such as retinal vascular diseases (central/branch retinal artery or vein occlusion, macroaneurysms, neovascularization, vitreous hemorrhage, epiretinal membrane formation, tractional retinal detachment, chronic papilledema, and optic atrophy) [33]. It is important to consider that HBP is an important contributing factor to DR leading to more advanced DR progression rates (see Figure 1) [34].

Retinal vascular changes appear in parallel with the pathological changes occurring in the coronary circulation. It has been described that retinal arteriolar narrowing was associated with reduced myocardial perfusion as determined by cardiac magnetic resonance imaging [35]. Other studies dealing with this topic reported that retinopathy signs positively correlated with coronary artery calcification (measured on cardiac computed tomography scanning) in a dose response manner, with more severe lesions associated with worse coronary artery disease on angiography [36, 37]. As a result of all these data, there is enough evidence to confirm that alterations in the retinal microvasculature may be pivotal indicators of the pathologies linked to vascular structure of the coronary microcirculation (Figure 1).

Patients suffering from scleritis and/or uveitis always have to be worked up for underlying systemic causes. Assessment for mucosa or skin lesions, arthritis, or infections may be carried out in each scleritic or uveitic patient. Uveitis associated diseases include syphilis, tuberculosis, ankylosing spondylitis (HLA-B27), or sarcoidosis. Rheumatoid polyarthritis, systemic lupus erythematosus, and systemic vasculitis were the most frequent associations with posterior scleritis that is commonly linked to other systemic diseases (40% of the cases) [38]. A multisystemic inflammatory process (*T-cell-mediated autoimmune disorder directed against melanocytic antigens*) known as the Vogt-Koyanagi-Harada syndrome, characterized by the panuveitis and serous retinal detachment, against a background of diverse neurologic and cutaneous manifestations, can be early detected and aggressively treated to prevent visual loss [39].

Respiratory disorders can also have an impact on the eyes. With this in mind, prevalence of obstructive sleep apnea syndrome has been found to be high in patients with nonarthritic anterior ischemic optic neuropathy and also in glaucomatous individuals [40]. Based on this and similar descriptions, performing polysomnographies in the affected patients has been recommended. Following this topic, inhaled corticosteroids (high doses/long-lasting treatments) are used by patients with chronic obstructive pulmonary diseases [41]. As a consequence of this therapy, a wide variety of ocular and systemic effects have been described such as cataracts, glaucoma, DM, HBP, pneumonia, and osteoporosis. Also, sleep apnea has been related to glaucoma progression [42]. Therefore, it is necessary to provide the most appropriate tools for monitoring these patients in order to prescribe proper treatment and preserve visual functions.

Regarding the kidney diseases, a special risk for specific ocular comorbidities such as dry eyes, uveitis, cataracts, and glaucoma is noticeably high in patients with chronic renal
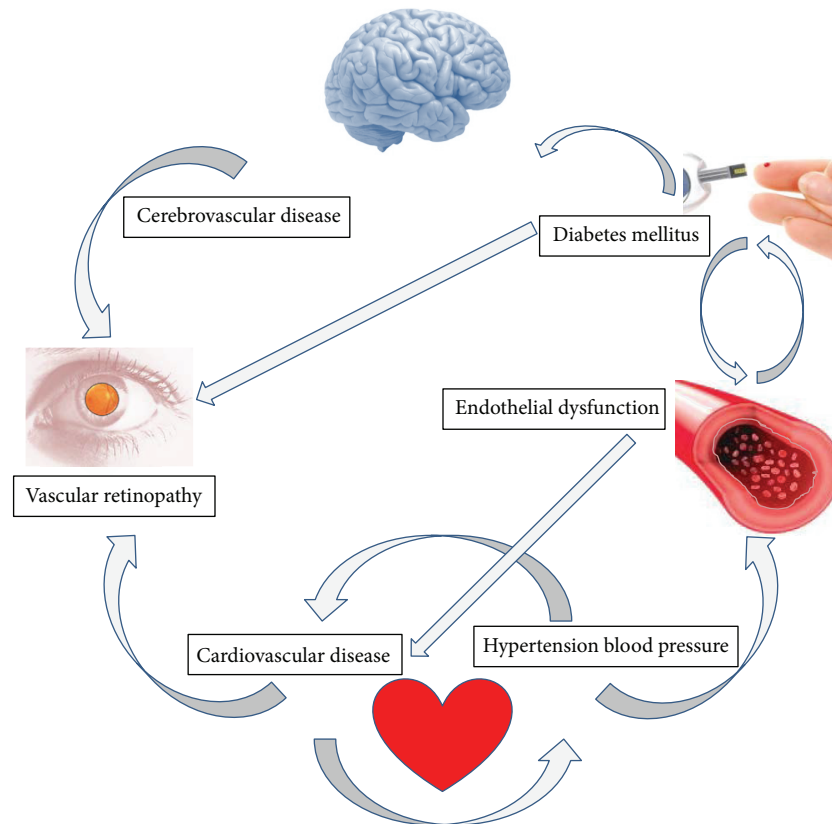
FIGURE 2: Pathogenic mechanisms of the vascular retinopathies.

failure, as reported in a recent study including 9,149 patients and 27,447 matched controls (age 40–98 years) from the Taiwan study group [43].

3.1.2. Main Ocular Manifestations Induced by Systemic Disorders. In many occasions, the eye signs and symptoms are the first visible or the most evident manifestation of other important systemic problems, including infections, traumatisms, neurodegenerative and mental disorders, thyroid dysfunction, autoimmune diseases, pharmacological drugs, and toxic substances.

Therefore, a systematized ophthalmic examination is essential for managing both the ocular pathology and the underlying systemic disorder.

In an infectious background, it has been reported that 60% of patients with acquired immunodeficiency syndrome display ocular disorders, which increases up to 90% in necropsies. Most common eye disorders in AIDS are cytomegalovirus retinitis and retinal microvasculopathies [44]. Precisely, emerging and resurging viral infections strongly represent a public health problem worldwide. Among them, dengue fever, chikungunya, Ebola virus, enterovirus, hantavirus, Henipavirus, influenza virus A (H1N1), Japanese encephalitis, Kyasanur forest disease, rickettsioses, Rift Valley fever, and West Nile fever may result in different ocular pathologies, such as chorioretinitis, vitreoretinitis, retinal vasculitis, optic neuropathy, retinal hemorrhages, or any other ocular inflammatory condition usually involving all the eye components [45]. Lyme neuroborreliosis is a disease caused by the tick-borne spirochaete *Borrelia burgdorferi* involving central nervous system and neurosensory organs, among others. The most frequent clinical symptoms observed are headaches (71%), vertigo (44%), meningeal symptoms (22%), and neurological paresis (27%) (including facial palsy, 23%). However, neuroretinitis has also recently been described in patients with Lyme disease [46].

Traumatic events are usually reflected in our eyes. Importantly, our eyeballs act as an open window reflecting obscure situations that may undoubtedly help physicians to detect silent damage to children and adults, such as in the cases of abusive head trauma in battered wives or in the shaken baby syndrome [47]. Furthermore, it has been reported that patients with posttraumatic stress disorder or depression have differences in dry eye symptoms and signs compared to a population without this condition [48].

It has been recently emphasized that psychological distress and depression are frequent comorbidities in glaucomatous individuals [49], as well as AMD patients [50]. Likewise, optic nerve degeneration in glaucoma patients has been found to frequently coexist with Alzheimer or Parkinson diseases and other neurodegenerative disorders [51, 52].

To keep on the prevalence of selected systemic comorbidities in patients with primary open-angle glaucoma (POAG), the most prevalent glaucoma type, Lin et al. performed

a nationwide, case-control study using an administrative database (76,673 POAG patients and 230,019 healthy subjects) including 31 medical comorbidities selected from the Elixhauser Comorbidity Index [53]. The authors reported that the prevalence difference of the glaucomatous patients with respect to the controls was 3% or higher for hypertension, hyperlipidemia, stroke, diabetes, liver disease, and peptic ulcer [54]. Often these glaucomatous individuals are completely full with other diseases, including DM, HBP, and CVD. Minor comorbidities with glaucoma are thyroid dysfunction, Alzheimer or Parkinson's disease, anxiety, depression, and stroke. It has to be considered that these patients have many more things going on simultaneously with glaucoma; because of this, the scope of concerns with these individuals seriously increases in comparison with others.

Thyroid orbitopathy also named Graves' orbitopathy is an autoimmune disorder associated with thyroid dysfunction that is manifested with typical self-limiting eye and adnexa signs and symptoms. Currently, the pathogenesis and effective treatment for this disease remain elusive. Manifestations of thyroid orbitopathy include eyelid retraction (affecting 90–98% of patients), lagophthalmos caused by incomplete eyelid closure, exophthalmos (eyeball proptosis and poor blinking), and extraocular muscles dysfunction which cause diplopia. Excessive eye exposure leads to increased tear evaporation and DEDs as well as superior limbic keratoconjunctivitis [55]. The coexistence of thyroid orbitopathy and myasthenia gravis with more severe ocular repercussion has also been reported [56]. The goal of managing patients with Graves' disease is the control of the thyroid state. Ophthalmologists may act in combination with endocrinologists, neurologists, and/or maxillofacial specialists to improve eye care.

Pursuing with the factors leading to the most relevant ocular manifestations induced by systemic disorders, the aging process has to occupy a preferential place. The World Health Organization estimated that the age-related eye disorders and visual impairment affect over 372 million older adults worldwide. Therefore, elder people suffering from comorbid conditions in the context of visual impairment constitute an additional problem because they importantly suffer impaired quality of life, a greater risk of falls (and the so-called "fear of falling"), faster cognitive decline, and a higher risk of accelerated aging and/or premature death as compared to individuals without visual affection [57]. There are four major age-related eye diseases: cataracts, glaucoma, DR, and AMD. Here, we will show the AMD facts related to the topic of this review, because the other diseases have yet been previously considered. The AMD is the leading cause of severe vision loss in people aged 60 years or more. Wong et al. have estimated the global prevalence of the disease and its burden projection for 2020 and 2040 and the results confirmed that the projected number of people with AMD (any clinical type) in 2020 is 196 million, increasing to 288 million in 2040 [58]. Having these data in mind, it is essential to review the systemic disorders that may worsen the vision-related quality of life of the affected patients. Several general pathologies have been associated with AMD such as HBP, CVD, cerebrovascular disease, dyslipidaemia, chronic kidney disease, and neurodegenerative disorders; some of them have

been reviewed above. Currently, increasing evidence points to the fact that AMD patients are at risk of stroke. Interestingly, it has been suggested that AMD is an ocular manifestation of systemic disease processes [59].

It is well known that every pharmacological substance (topically or/systemically administered) can induce unfavorable effects (eyelids, conjunctiva, cornea, lens, iris, ciliary body, retina, optic nerve, and the extraocular muscles), even when utilized according to standard protocols. Among the medicaments that may cause ocular toxicity and vision loss are chloroquine/hydroxychloroquine, thioridazine, chlorpromazine, tamoxifen, ethambutol, isoniazid, fluoroquinolones, and monoclonal antibody therapy [60]. It has also to be seriously considered that craniofacial and eye developmental abnormalities can be induced by the use of these drugs during pregnancy, as well as by the alcohol or psychostimulants abuse by the pregnant women [61, 62].

The ocular manifestations of the systemic diseases have to be managed by the ophthalmologists with the cooperation of the professionals involved in the related medical specialties. Also the utilization of new technological devices can help to achieve an early diagnosis of the affected patients. These new tools for ophthalmic examination require high-level technically skilled and knowledgeable users, as in the cases of the latest developments such as optical coherence tomography (*OCT angiography, en face OCT*), Scheimpflug imaging, scanning laser ophthalmoscope, ultra-wide-field imaging, microperimetry, multifocal electroretinogram, Doppler imaging, or the ocular ultrasound and magnetic resonance imaging. With these new techniques, an accurate diagnosis and proper therapy monitoring can be reached to better manage patients with systemic disorders and eye pathologies, mainly in cases of corneal or retinal damage [63–65].

3.2. Genetic Syndromes and the Eyes. Genetic syndromes are disorders caused by changes or mutations in the DNA which alter the synthesis or function of proteins. This usually involves major changes in the physical and behavioural development of the affected patient. In many cases, the alterations include eye disorders such as cataracts, glaucoma, myopia, or retinopathies, as well as craniofacial and ocular malformations.

Down syndrome (a trisomy of chromosome 21) and eye disorders coexist in 60% of all cases, with the following being the most frequent: strabismus, astigmatism, cataracts, or myopia [66]. The second genetic cause of mental retardation in the world, the fragile X syndrome (or Martin-Bell syndrome), is caused by a mutation in the regulatory region of the FMR1 gene on the X chromosome. Strabismus, myopia, and hypermetropia are commonly found in the affected patients [67, 68]. In cases of the Angelman and the Prader-Willi syndromes (caused by mutations in chromosome 15), strabismus is usually diagnosed. Bardet-Biedl syndrome is a rare autosomal recessive disease with very different manifestations, such as obesity, polydactyly, and mental retardation. Retinitis pigmentosa is one of the major features of this disorder [69]. Marfan syndrome is an autosomal dominant disease caused by a mutation in the FBN1 gene on chromosome 15, which causes changes in the function of fibrillin.

The classic Marfan is the most common clinical type, with the following being the accompanying eye disorders: retinal detachment, cataracts, lens displacement, and glaucoma [70]. Other rare GS also include ocular alterations, including Cri-du-chat syndrome (myopia, optic atrophy), Lowe syndrome (congenital cataracts, infantile glaucoma) [71], Marinesco-Sjögren syndrome (cataracts) [72], and Axenfeld-Rieger syndrome (50% develop glaucoma) [73].

In summary, the genetic syndromes are disorders affecting different organs that induce a high variety of symptoms and conditions. There are many ophthalmic features associated with these disorders, and very occasionally, suspicion of the genetic syndromes is raised by first presentation with ocular problems, with the following being the most frequent: strabismus, important refractive errors, and cataracts. The following have to be considered less frequently: myopia, retinal degeneration, and glaucoma.

The Need for a Comprehensive Evaluation of Eye Comorbidities and Systemic Disorders. A wider knowledge on the eye manifestations of systemic diseases as well as the ocular comorbidities can help to early diagnose a specific disorder, slow the progression, and/or prevent visual impairment or blindness in patients suffering serious eye complications.

Thus, a thorough ocular evaluation should include a precise anterior eye segment and media and dilated fundus examination, and when indicated, fluorescein angiography, OCT, visual field, and/or radiologic probes should be performed in patients suspected of being affected by an eye manifestation of systemic disease or an ocular comorbid condition.

It has to be considered that, in some cases, the eyes can show signs of an internal disorder before the disease progresses and becomes a more serious problem. The key is to understand what is happening as soon as possible to avoid severe complications for the health and vision. A wide spectrum of observable eye changes and visual variations can be recognized either by the patient himself/herself or by the physician. However, a handful of ophthalmological signs can certainly point to a systemic disorder. Among the external signs are the following: (1) specific conjunctival or scleral hyperemia or violet areas that do not respond to therapy, (2) bilateral palpebral eczema or swelling without secretion, (3) spots and pigmented (or depigmented) areas growing or changing, (4) episodes of partial or complete visual loss with/without aura, (5) external muscles intermittent of progressive alterations with ocular misalignment, eye strain, and diplopia, and (6) malposition of the upper eyelid with/without enophthalmos or protrusion of the eyeball. Most relevant internal signs include the pupillary abnormalities, aqueous humor or vitreous body changes, retinal arteries and veins alterations, and the presence of two or more of the following: retinal spots, pigmented zones, exudates, hemorrhages, atrophic areas, papillary swelling, and choroidal neovascularization.

The ocular comorbidities as well as the eye-related systemic disorders increasingly strain healthcare sectors and societies worldwide, especially within the aging population.

Most patients are primarily managed by general physicians and advanced practice nurses. A precise early diagnosis is needed with appropriate protocols in order to avoid severe complications, visual impairment, and blindness. For successful global and eye/vision care, outstanding new strategies on the basis of an interdisciplinary team have to be established with the main goal of introducing a variety of quality improvement interventions that can achieve better results in the clinical practice and health systems [74–77]. Appropriate training and effective communication among the primary care physicians, specialists and subspecialists, nursing, and other health care professionals, as well as the collaboration of patients and their family members and caregivers, are critical for ensuring the intervention effectiveness [78–80]. Practical elements for improving the most accurate diagnosis and management regarding the ocular comorbidities as well as systemic disorders with eye repercussion have to be highlighted, which can be implemented with relatively easy plans and little financial inputs [81, 82].

For the past fifteen years, our clinical and experimental research team has contributed to the knowledge and skills about the ocular comorbidities and the eye manifestations related to systemic disorders. The main challenge is to share the personal expertise with each other, to increase cooperativity in order to prevent blindness.

Competing Interests

The authors declare that they have no competing interests.

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Research Article

Enhanced Oxidative Stress and Other Potential Biomarkers for Retinopathy in Type 2 Diabetics: Beneficial Effects of the Nutraceutic Supplements

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We have studied the global risk of retinopathy in a Mediterranean population of type 2 diabetes mellitus (T2DM) patients, according to clinical, biochemical, and lifestyle biomarkers. The effects of the oral supplementation containing antioxidants/omega 3 fatty acids (A/ω3) were also evaluated. Suitable participants were distributed into two main groups: (1) T2DMG (with retinopathy (+DR) or without retinopathy (−DR)) and (2) controls (CG). Participants were randomly assigned (+A/ω3) or not (−A/ω3) to the oral supplementation with a daily pill of Nutrof Omega (R) for 18 months. Data collected including demographics, anthropometrics, characteristics/lifestyle, ophthalmic examination (best corrected visual acuity, ocular fundus photographs, and retinal thickness as assessed by optical coherence tomography), and blood parameters (glucose, glycosylated hemoglobin, triglycerides, malondialdehyde, and total antioxidant capacity) were registered, integrated, and statistically processed by the SPSS 15.0 program. Finally, 208 participants (130 diabetics (68 +DR/62 −DR) and 78 controls) completed the follow-up. Blood analyses

confirmed that the T2DMG+DR patients had significantly higher oxidative stress ($p < 0.05$), inflammatory ($p < 0.05$), and vascular ($p < 0.001$) risk markers than the T2DMG−DR and the CG. Furthermore, the A/ω3 oral supplementation positively changed the baseline parameters, presumptively by inducing metabolic activation and ameliorating the ocular health after 18 months of supplementation.

1. Introduction

Diabetes mellitus (DM), with a prevalence of logistic growth, rises pandemic proportions. It has been estimated that over 170 million people worldwide are currently affected by DM and it seems that these numbers will augment to over 360 million by 2030 [1]. A report on the incidence and prevalence of type 2 diabetes mellitus (T2DM) in 11 European countries showed that the age-adjusted/country-adjusted prevalence in 2004 was 10.2% in men and 8.5% in women and that these inequalities were highly related to the body mass index (BMI) [2]. In Spain, DM represents a big health problem, due to its prevalence of about 10% in the global population aged 30–89 years [3]. Number of diabetics among people aged 45 years or older in USA during 2012 was 24.6 million. Among them there were 2.1 million more diabetic men than women. The total (direct/indirect) estimated costs of DM in the same timeframe were $245 billion, and after adjusting for age/sex these data concluded that medical expenditures (in general) were double among diabetics compared with the no diabetics [4]. Other reports including large population collections also concluded that men had a higher T2DM prevalence than women [5, 6]. However, a more recent estimation of the risk factors for cardiovascular diseases described that diabetic women who smoke or are overweight develop cardiovascular conditions more frequently than men, with the same risk factors [7, 8].

Major diabetic complications are the result of a close interaction between genes and a wide variety of environmental factors. Diabetic retinopathy (DR), the microvascular complication of DM, is responsible for the decreased vision and blindness in young and middle-aged individuals. It has been estimated that about 80% of the type 2 diabetics (T2DM) and half of patients with type 1 DM (T1DM) develop DR in some point between the diagnosis and 15th year or more of the disease course [9, 10]. In this framework, it is well acknowledged that DR appear more frequently in T1DM than in T2DM patients and that DR prevalence end points positively correlate with the length of time afflicted by DM, as well as by the glycosilated hemoglobin (HbA1c), and hypertension blood pressure (HBP) levels [11, 12].

The retina is particularly sensitive to hyperglycemia. It has been shown that prolonged blood glucose elevation induces striking changes in oscillatory potentials in the electroretinogram (ERG), increased implicit times in the multifocal ERG (mtERG), and dyschromatopsia, whereas short-term hyperglycemia results in an overall decrease in the implicit times and increase in the amplitudes of the mtERG [13]. Chronic hyperglycemia induces retinal damage through different mechanisms, such as activation of protein kinase C, polyol, and hexosamine pathways, and/or generation of advanced glycation end products (AGEs). Nevertheless, hyperglycemia damages the endothelial cells and pericytes and increases vascular permeability with rupture of the blood-retinal barrier with the subsequent appearance of the retinal hypoxia processes, which, in turn, induces vascular endothelial growth factor (VEGF) expression, among other proangiogenic effectors [14, 15]. In this situation, independently of the presence or not of DR, main objectives for implementing eye care in diabetics have to include maximum nutritional and metabolic control.

Glycemic dysfunction has been widely associated with increased generation of reactive oxygen (O_2) and nitrogen species (ROS, RNS) [16, 17]. These are continuously produced in cells during metabolic processes. Eventually, the electrons are transferred to molecular O_2, by reducing this to H_2O in a reaction catalyzed by the cytochrome c oxidase, accounting for almost 98% of the molecular O_2 used by our body:

$$O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O \tag{1}$$
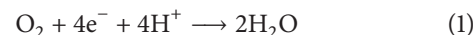
It is known that 1-2% of the O_2 undergoes an incomplete reduction by the mitochondria, generating the reactive oxygen species (ROS), very unstable forms such as the superoxide anion ($O_2^{\bullet-}$), formed when O_2 is reduced by an electron. However, this molecule rapidly dismutates to form the hydrogen peroxide (H_2O_2) that can itself undergo dismutation by a reaction that is catalyzed by the manganese superoxide dismutase. The rate of production of O_2/H_2O_2 depends on the Ca^{2+} availability. Importantly, the presence of transition metals, mainly iron, can induce H_2O_2 to generate the highly reactive hydroxyl radical $OH^{\bullet-}$:

$$O_2 + e^- = O_2^- + H^+ = HO^\bullet + O_2 \longrightarrow H_2O_2 \tag{2}$$

In fact, ROS/RNS originated from mitochondrial or nonmitochondrial sources have been related to DM, such as xanthine oxidase, NADPH oxidase, cyclooxygenase, lipoxygenase, cytochrome P450, and nitric oxide synthase. To counteract the effects of ROS/RNS, our body depends on the endogenous and exogenous antioxidants (AOX), including the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase [18].

Nevertheless, ROS overproduction or its deficient removal leads to vascular disorders that result in protein fragmentation, amino acids aggregation, membrane lipids alteration, and/or nucleic acids injury. These described changes may trigger cell damage and death. From the described oxidative background important biomarkers can emerge, and obviously these molecules can be determined in DM patients, especially in those suffering chronic diabetes-related complications [19, 20].

The retina is continuously exposed to light. Therefore retinal cells live under chronic oxidative stress conditions, also induced by the high O_2 requirements and the high O_2 partial pressure from the underlying vessels of the choriocapillaris. It has also to be considered that the photoreceptor outer segments are very rich in polyunsaturated fatty acids (PUFAs),

TABLE 1: Inclusion and exclusion criteria for the study participants. Criteria for eligibility in the Valencia Study on Diabetic Retinopathy (VSDR).

Inclusion	Males and females, aged >25 years and <80 years with type 2 diabetes for 12 months (at least)
	Insulin naïve
	Healthy individuals as controls
	No ocular or systemic diseases or aggressive treatments and no ocular surgery or laser for 12 months (at least); no other oral supplements with antioxidants and/or omega 3 fatty acids
	Provided written informed consent before any related activities commence
	Participants able to attend the visits and to follow the study guidelines
Exclusion	Males and females, aged <25 years and >80 years
	Insulin dependent patients
	Ocular or systemic diseases or aggressive treatments and/or previous ocular surgery or laser
	Antioxidant and/or omega 3 fatty acids supplements
	No acceptance for the study participation and/or not signing the informed consent
	Participants unable to attend the visits or to follow the study guidelines

mainly the omega 3 docosahexaenoic acids (DHA), which are extremely susceptible to lipid peroxidation and protein modifications that may be induced by the lipid peroxidation by-products [21]. Current knowledge on the physiological and pathological mechanisms of oxidative stress in DR has been collected from a wide spectrum of both the animal models research and the epidemiological studies [22, 23]. However, clinical trials have provided ambiguous results on the effects of the antioxidant [24, 25] and PUFAs [26, 27] supplements on the development and progression of DR.

Chronically elevated glucose/HbA1c levels altogether with a series of endogenous and exogenous risk factors lead to the retinopathy. The primary goal of the present study is to investigate the risk of retinopathy in a Mediterranean population of T2DM patients, according to clinical, biochemical, and lifestyle biomarkers, the oxidative stress status being among them. Secondary goal was to evaluate the effects of the oral administration of supplements with antioxidants and omega 3 fatty acids ($A/\omega3$) in the oxidative stress outcomes and the inflammation and vascular risk biomarkers, to better manage eye care and vision among diabetics.

2. Material and Methods

2.1. Community-Based Study Design.
The study was done in agreement with the Declaration of Helsinki for human studies (as revised in Edinburgh, 2000), and all records were treated according to the Spanish law for protecting personal data (LOPD: Organic Law 15/1999, de 13 Dic). Our study was validated by the Ethics Committee and Clinical Research of the University Hospital Dr. Peset of Valencia (Spain), the main center of the Research Project (number 12/33), and by the Ethics Committees of all participating institutions. Moreover, the study received the permission of the Agencia Española de Medicamentos y Productos Sanitarios (AEMPS).

We conducted a prospective multicenter study lasting for 18 months (Jan 2013–June 2014) that was carried out in 10 centers by 32 investigators, with the main goal of delineating the natural history of DM and eye disease as well as the risk factors for DR in T2DM patients and to evaluate whether $A/\omega3$ supplements could increase compliance with the health and vision care among diabetics.

The participant investigators were selected to obtain stratified ophthalmologists from main hospitals pertaining to the National Health System in the Valencia Community and ophthalmology clinics, as well as basic researchers involved in ophthalmology and vision sciences.

A total of 360 persons (25–80 years old) of both sexes were preinterviewed for eligibility for the study that was determined by the main investigator, according to the inclusion/exclusion criteria listed in Table 1. First point for recruitment was the diagnosis of T2DM and the duration of disease since diagnosis. In parallel, healthy individuals were also recruited. Therefore, from the initial sample, 265 suitable participants were enrolled, signed the informed consent, and were appointed to the first visit of this interventional study. Then, office visits were programmed every six months, including periodical telephone calls from the investigators throughout the study. Total study duration was 18 months and the characteristics of recruitment are enclosed in Figure 1.

2.2. Patients and Methods

2.2.1. Interview. Participants who completed the first enrollment interview ($n = 265$) were enclosed for demographics, patient characteristics, and lifestyle. The following variables were recorded: age, sex, ethnicity, DM duration, BMI (height, weight), familial background, current medication, smoking/drinking habits, and physical exercise. In the mid-twentieth century, Professor Ancel Keys observed the nutritional habits of people living in the European Mediterranean countries leading to the conclusion that a significant reduction in the incidence of chronic diseases and a higher lifespan were main characteristics of this area (*for further reading, Keys et al. The diet and 15-year death rate in the seven countries study. Am J Epidemiol. 1986; 124:903*). Major details of this type of diet are (1) the elevated consumption of fruits, vegetables, legumes, olive oil, bread, and cereals, (2) the moderate-to-high ingestion of fish and chicken meat, (3) the moderate intake of cheese, yogurt, and wine, and (4) the low levels of consumption of red meat. The UNESCO raised the Mediterranean diet in 2013 as a lifestyle in itself and a cultural heritage for the humanity. We used a 14-item
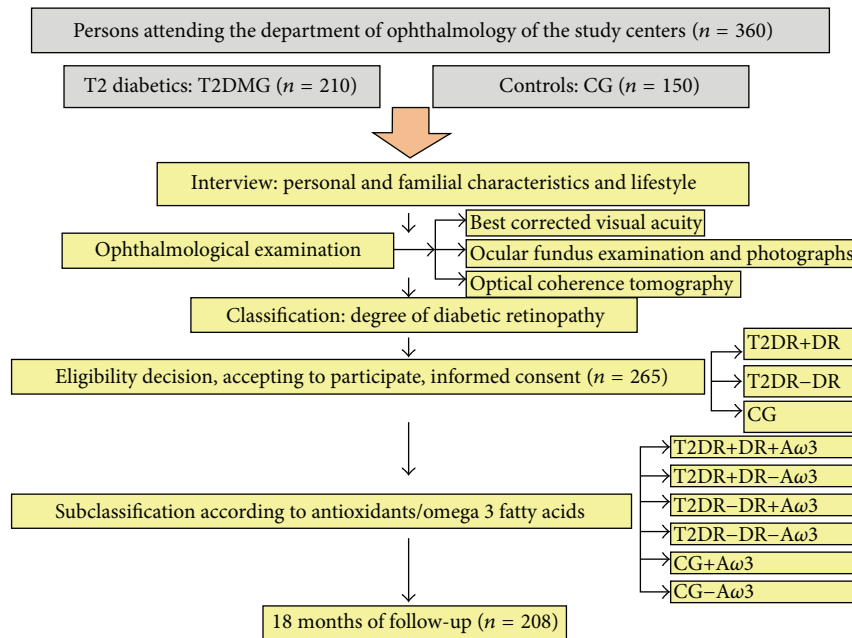
FIGURE 1: Flowchart of the challenges for the recruitment and screening methods of the study participants: characteristics of recruitment, sample size, and study design.

questionnaire to assess the adherence of the sample participants with the Mediterranean diet (AMD). The Mediterranean diet scores indicating compliance to the Mediterranean diet distinguished between the participants with a high intake of cereals, legumes, fruits, vegetables, olive oil, fish, bread, and red wine that were scored positive (1), while those with a low intake of these meals were scored negative (0). The results of this questionnaire were reflected as participants having either good adherence to the Mediterranean diet (GAMD) or poor adherence to the Mediterranean diet (PAMD).

The T2DMG (n = 165) was subclassified according to having or not having DR. Furthermore, each of these subgroups was homogeneously subdivided into those participants that were randomly assigned to take or not one pill per day of the A/ω3 supplementation (T2DMG+DR+A/ω3/ T2DMG+DR−A/ω3; and T2DMG−DR+A/ω3/T2DMG− DR−A/ω3). The CG (n = 100) was also randomly assigned to those taking (CG+A/ω3) or not (CG−A/ω3) the A/ω3 supplements. A flow chart with the number of patients pertaining to the groups and subgroups is enclosed in Figure 1. The nutraceutic formulation contains docosahexaenoic acid (DHA), vitamins (E, C, B1, B2, B3, B6, B9, and B12), lutein, zeaxanthin, glutathione, hydroxytyrosol, and trace elements (Se, Mn, Zn, and Cu), commercialized as Nutrof Omega, commercialized by Laboratorios Thea SA (Barcelona, Spain) that gently provided all the oral supplementation needed for the study participants. The study-related visits and the nutraceutic supplements (Nutrof Omega) were given to our participating volunteers (randomly chosen) without any charge.

2.2.2. Ophthalmic Examination. A systematized ophthalmological examination was performed to our 265 participants including best corrected visual acuity (BCVA) with each eye, ocular fundus examination and retinographs (ImageNet; Topcon, Barcelona, Spain), and optic coherence tomography (OCT) examination (Spectral Domain SD-OCT; Zeiss, Madrid, Spain).

The DR diagnosis was done from ocular fundus examination and photographs which were carried out in each eye of the T2DG (n = 165), based on the Early Treatment of Diabetic Retinopathy Study (ETDRS), and the DR severity was graded as described before [28]. The presence and number of microaneurysms, hemorrhages, venous beading, and/or intraretinal microvascular abnormalities were considered the most relevant factors for monitoring DR progression. In fact, retinopathy worsened basically by overall augment in counts of microaneurysms and haemorrhages. The ETDRS retinopathy severity scale divides the process into 13 levels ranging from absence of retinopathy to severe vitreous hemorrhage.

The OCT parameters were measured with the Cirrus SD-OCT direct cross-sectional imaging device of Zeiss Meditec (Dublin, CA, USA) in a total of 265 eyes with the clinical diagnosis of nonproliferative diabetic retinopathy (T2DG; (n = 165) with or without DR and/or clinically significant macular edema) and in the healthy participants (CG; n = 100). Prior to the OCT examination, each participant was administered a drop of tropicamide in both eyes to dilate the pupils. Each ophthalmologist visualized the representative A-scan and manually moved the measurement cursors (first at the signal that represents the internal limiting membrane and

second at the signal that denotes the retinal pigment epithelium). Then, the most profound zone of the foveal pit was taken as the center. Six consecutive scans were performed for each eye. Cross-sectional retinal images were optimized for each scan to obtain the highest intensity/definition; therefore these scans with signal strengths ≥ 7 and without artifacts were included in this study. Briefly, acquisition protocols were macular cube 200 × 200; HD 5 line raster/5 raster line; and HD one line. The raw OCT datasets were exported to a personal computer for analysis. The SD-OCT system analyzed retinal thickness, creating a topographic map and graphs for quantitatively and qualitatively documenting any changes in retinal thickness and edema. Main exclusion criteria for this technique were any retinal pathology other than DR or opaque media such as in cataracts and inability to undergo the OCT examination due to mobility or cognitive difficulties.

2.2.3. Sample Processing. At baseline and every 6 months of the follow-up, all participants were visited in the corresponding centers and the peripheral blood was collected from the antecubital vein under fasting conditions (between 8:00 and 9:00 a.m.). Part of the obtained blood tubes was analyzed by computerized systems to determine laboratory blood draws and analysis: glucose, glycosylated hemoglobin (HbA1c), cholesterol (HDL, LDL), and triglycerides. Two more EDTA tubes were obtained and transported in optimal conditions for preservation to the ophthalmic research centers. Each blood sample was centrifuged at 3000 rpm for 10 min and the plasma and erythrocytes were separated into Eppendorfs, labeled, and registered to be stored at −80°C until processing, as described below.

(1) Determination of Malondialdehyde- (MDA-) Thiobarbituric Acid Reactive Substances (TBARS). Thiobarbituric acid reactive substances (TBARS) including MDA were measured in plasma samples, as lipid peroxidation by-products. Briefly, at high temperature (90–100°C) under acidic conditions MDA-TBA complex was formed by the reaction between these molecules and extracted with butanol, according to previous descriptions [28, 29]. The fluorescence of these complexes was measured in duplicate at 544 nm excitation and 590 nm emission deep, relative to the fluorescence of the standard samples, and then the concentration of the samples was calculated by extrapolating the results in the standard curve.

(2) Total Antioxidant Activity (TAA). The antioxidant activity was measured in the plasma samples by means of the antioxidant assay (Cayman Chemical Company, Ann Arbor, MI) commercial kit. The kit is based on the antioxidant capacity of plasma to inhibit the oxidation of ABTS ($2,2'$-azino-di-[3-ethylbenzthiazoline sulphonate]) to $ABTS^+$ by the metmyoglobin, as previously reported [30, 31].

2.3. Statistical Procedures. For statistical analysis, the excel program and the SPSS 15.0 (SPSS Inc., Chicago, IL, USA) were used. All the programmed experiments were performed in duplicate for each sample. The continuous variables were

TABLE 2: Sociodemographic characteristics of diabetics with/without retinopathy.

	T2DM+DR	T2DM−DR	p^*
Age (years)	65.1 ± 8.6	62.3 ± 10.1	0.094
Gender (% of women)	52.7	48.6	0.645
DM duration (years)	19.2 ± 6.8	10.4 ± 7.1	<0.001*
Physical exercise (%)	27.3	45.7	0.035*
Smoking (%)	43.6	15.7	0.002*
Alcohol drinking (%)	38.2	8.6	<0.001*
BMI (kg/m^2)	28.8 ± 4.6	28.0 ± 4.0	0.345
GAMD (%)	18.2	44.3	0.002*

T2DM+DR: type 2 diabetes mellitus with diabetic retinopathy; T2DM−DR: type 2 diabetes mellitus without diabetic retinopathy; DM: diabetes mellitus; BMI: body mass index; GAMD: good adherence to Mediterranean diet. Data are shown as mean ± standard deviation.
*Statistically significant ($p < 0.05$).

expressed as mean ± standard deviation of the mean (SD). Categorical variables were expressed as percentages. The statistical test performed utilized the two/tailed test at 5% level of significance. Appropriate analysis of covariance models was also performed.

3. Results

From the initial suitable participants ($n = 265$), a total of 208 participants (130 T2DM patients (62 +DR versus 68 −DR) and 78 healthy controls) continued and completed altogether the 18 months of the study.

Demographics, patient characteristics, and lifestyle details of the study participants are listed in Table 2. It has to be emphasized that the HBP was twice frequent in the T2DMG+DR than in the diabetics −DR ($p < 0.001$).

The BCVA (each eye in separate) was lower in the T2DMG than in the CG [(CG at baseline: RE: 0.95 ± 0.10 and LE: 0.96 ± 0.08; $p < 0.001$/CG; at 18 months: RE: 0.95 ± 0.11 and LE: 0.95 ± 0.10; $p < 0.001$) (T2DMG at baseline: RE: 0.81 ± 0.30 and LE: 0.83 ± 0.22; $p < 0.001$/T2DMG; at 18 months: RE: 0.78 ± 0.22 and LE: 0.76 ± 0.22; $p < 0.001$)].

The baseline ocular fundus examination revealed that 62 patients of the T2DMG displayed DR with the following degrees of severity: 63.6%: mild DR, 29.1%: moderate DR, and 7.3%: severe DR. From baseline to the end of study, 11 patients remained without signs of DR progression, but the rest progressed as follows: 29.1% displayed mild DR, 36.4% moderate DR, and 14.5% severe DR. Furthermore, the 68 T2DMG patients that did not have DR showed progression of disease at the end of study in the following proportions: 14.3% of the patients without DR progressed to mild DR, 7.1% of the patients without DR progressed to moderate DR, and no progression to severe degree of DR was detected through the follow-up in any of these patients. In our study, the T2DMG displayed DME (clinically significant) at baseline 5.6% DME in the RE versus the 11.2% in the LE ($p = 0.037$, $p = 0.003$, resp.). Moreover, DME was more frequent in the T2DMG+DR than in the T2DMG−DR and the CG

Onset/progression of retinopathy in type-2
diabetic patients after 18 months of follow-up

- +onset/progression of DR
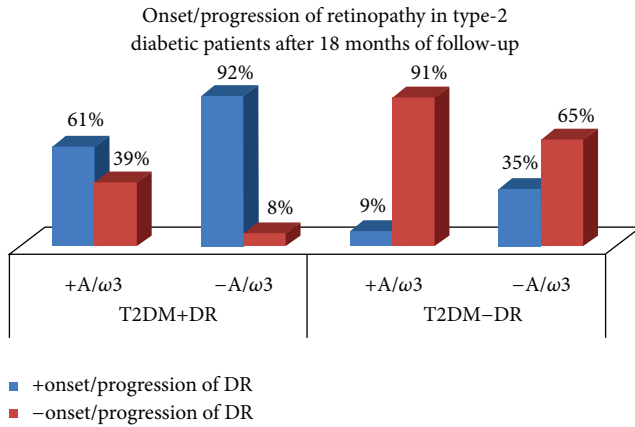- −onset/progression of DR

FIGURE 2: Percentages of development or progression of DR in the participants during the study course, according to being assigned or not to the oral supplementation. +A/ω3: oral supplementation with antioxidants and omega 3 fatty acids. −A/ω3: without taking the oral supplementation. T2DM+DR: type 2 diabetics with retinopathy. T2DM−DR: type 2 diabetics without retinopathy.

($p = 7.036^{E-005}$ for the RE and $p = 2.214^{E-006}$ for the LE). At the end of study, it was seen that DME was significantly more frequent in patients with severe DR (50%) than in those with mild or moderate DME ($p = 0.03$). A summary of the progression characteristics based on the ocular fundus and SD-OCT examination of the study participants is reflected in Figure 2.

All the results obtained from the ophthalmic examination of the study participants are enclosed in Table 3.

Classical and oxidative stress hematologic parameters from the T2DM participants are enclosed in Table 4. Parameters related to oxidative stress (lipid peroxidation (MDA/TBARS), total antioxidant activity (TAA)) showed that the plasmatic MDA/TBARS levels were significantly higher, and the TAC was significantly lower in diabetics as compared to the CG (Table 4). It was also observed that the MDA/TBARS displayed significantly higher concentrations and the TAA showed significantly lower activities in the T2DMG+DR with respect to those diabetics without DR and the CG (Table 4). Data from the oxidative stress parameters according to the degree of severity of DR (ETDRS international scale) are reflected in Table 5. The MDA/TBARS levels augmented with DR progression, but the TAA significantly decreased with DR progression.

The adherence to the Mediterranean diet nutritional facts of the study participants were reflected in the 14-item questionnaire. Specifically, average values for the 14-item score were significantly higher in the CG (9.8 ± 2.0) versus the T2DMG (6.4 ± 1) ($p < 0.05$). The comparison of the pro-oxidant and antioxidant markers analyzed in the present study in the T2DMG with poor and good adherence to the Mediterranean diet (PAMD/GAMD) is reflected in Table 6.

When assessing the oxidative stress status at the end of the eighteen months of the follow-up, it was seen that plasmatic MDA/TBARS levels significantly decreased in the T2DM+A/ω3 subgroup with respect to the T2DM−A/ω3

TABLE 3: Ophthalmic examination records of the study participants.

		T2DM
BCVA RE	Baseline	0.81 ± 0.296
	18 months	0.78 ± 0.220
	p value	0.176
BCVA LE	Baseline	0.83 ± 0.22
	18 months	0.78 ± 0.22
	p value	0.388
IOP RE (mmHg)	Baseline	15.2 ± 2.8
	18 months	15.6 ± 2.5
	p value	0.099
IOP LE (mmHg)	Baseline	15.6 ± 2.9
	18 months	16.1± 2.5
	p value	0.048*
RNFLT RE (μm)	Baseline	251.65 ± 22.79
	18 months	254.14 ± 31.60
	p value	0.001*
RNFLT LE (μm)	Baseline	258.53 ± 55.029
	18 months	262.22 ± 6.38
	p value	0.011*
MT RE (n)	Baseline	5.6% (13)
	18 months	7.2% (9)
MT LE (n)	Baseline	11.2% (14)
	18 months	10.4% (13)

DM − R: diabetes mellitus without retinopathy; DM + R: diabetes mellitus with retinopathy; BCVA: best corrected visual acuity; RE: right eye; LE: left eye; IOP: intraocular pressure; RNFL: retinal nerve fiber layer thickness; MT: macular thickness. Data are shown as mean ± standard deviation.
*Statistically significant ($p < 0.05$).

patients (Figure 3(a)). Furthermore, the TAC significantly increased in the T2DM+A/ω3 subgroup, while the nonsupplemented participants did not show any noticeable change (Figure 3(b)).

4. Discussion

The retinopathy is the major cause of blindness among adult diabetics (aged 20–75 years) in the developed world [1–6]. In this population-based multicenter study carried out in 265 participants from the Mediterranean area of Spain (T2DMG; $n = 165$ versus CG; $n = 100$), "*The Valencia Study on Diabetic Retinopathy (VSDR)*," we have identified significant risk factors of DR, such as the HBP, duration of the DM, physical exercise, the BMI, the smoking/drinking habits, the dyslipidemia, and the oxidative stress. In fact, plasmatic oxidative stress biomarkers increased and antioxidant activity decreased in the diabetics with DR, with respect to those without DR and the healthy controls, data consistent with previous reports from our group and other authors [18, 19, 22–25]. More precise information about each of these risk factors is provided below.

The HBP was present in 76.4% of our T2DM+DR versus 58.6% of the T2DM−DR and 9.3% of the CG ($p < 0.001$). Emdin et al. [32] studying patients with T2DM emphasized

TABLE 4: Haematologic parameters in diabetics with/without retinopathy at baseline and at 18 months of follow-up.

	T2DM−DR		p	T2DM+DR		p
	Baseline	End of study		Baseline	End of study	
MDA (μm/L)	2.37 ± 1.39	2.43 ± 0.98	0.724	3.63 ± 1.30	3.83 ± 1.81	0.482
TAS (μM)	2.64 ± 1.52	2.87 ± 1.31	0.203	1.96 ± 1.32	2.09 ± 1.39	0.020*
HbA1c (%)	7.12 ± 1.55	6.99 ± 1.30	0.307	7.87 ± 1.48	8.72 ± 1.45	0.046*
Total-c (mg/mL)	186.8 ± 39.4	184.3 ± 37.6	0.669	178.8 ± 32.6	181.3 ± 33.8	0.522
HDL-c (mg/mL)	50.4 ± 13.1	51.6 ± 14.2	0.479	51.5 ± 13.0	51.4 ± 13.0	0.959
LDL-c (mg/mL)	104.3 ± 28.6	102.3 ± 35.2	0.687	99.9 ± 29.4	102.6 ± 35.4	0.587

DM − R: diabetes mellitus without retinopathy; DM + R: diabetes mellitus with retinopathy; MDA: malondialdehyde; TAS: total antioxidant status; HbA1c: glycosilated hemoglobin; total-c: total-cholesterol; HDL-c: higher density lipoprotein-cholesterol; LDL-c: lower density lipoprotein-cholesterol. Data are shown as mean ± standard deviation.
* Statistically significant ($p < 0.05$).
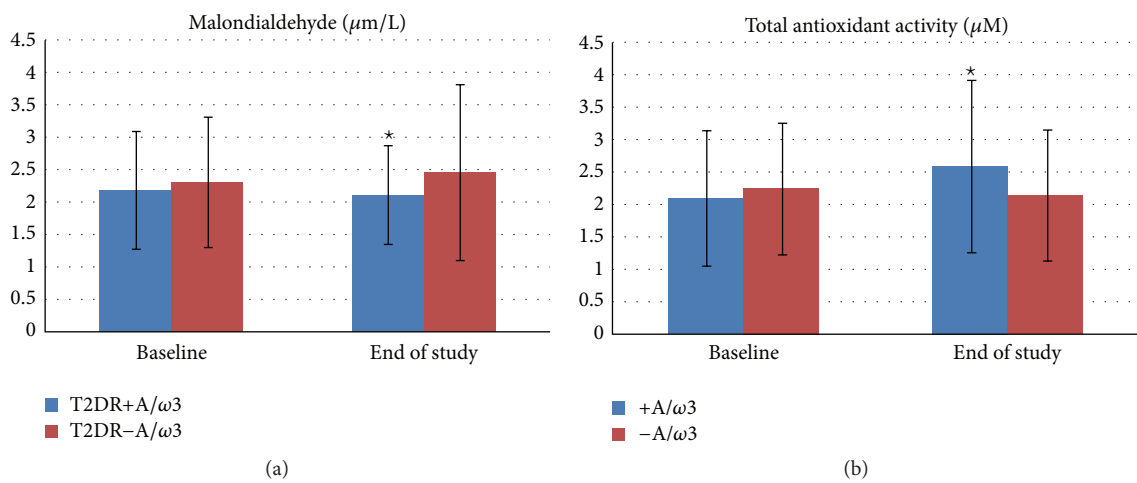

(a)

(b)

FIGURE 3: Oxidative and antioxidant status in the type 2 diabetics during the 18 months of follow-up, according to being assigned or not to the oral supplementation of A/ω3 supplements. T2DR+A/ω3: type 2 diabetics with retinopathy taking the oral supplementation; T2DR−A/ω3: type 2 diabetics with retinopathy not taking the oral supplementation; +A/ω3: assigned to the oral supplementation; −A/ω3: not assigned to the oral supplementation.

TABLE 5: Oxidative status in diabetics according to the degree of retinopathy (*ETDRS International scale*).

T2DMG	MDA/TBARS (μmol/L)	TAA (μmol/L)
−DR	1.19 ± 0.53	3.84 ± 1.24
+DR		
Mild DR	3.00 ± 1.74	2.38 ± 1.30
Moderate DR	4.94 ± 1.29	1.29 ± 0.59
Severe DR	5.36 ± 0.51	1.10 ± 0.34
p value*	$2.997E − 006$**	$4.857E − 005$**

−DR: diabetes mellitus without retinopathy; +DR: diabetes mellitus with retinopathy; MDA: malondialdehyde; TAA: total antioxidant activity. Data are shown as mean ± standard deviation.
* p value obtained from ANOVA analysis.
** Statistically significant ($p < 0.05$).

that appropriated HBP lowering was related to improved mortality and other positive clinical changes in the affected patients. Barrios and Escobar [33] reported that DM and

HBP are closely related disorders creating an optimum background for atherosclerosis. In fact, the strict control of both processes is pivotal to ameliorate macroangiopathy/microangiopathy prognosis in the affected patients.

The lead time between the diagnosis of the disease and the first appointment for the present study was considered as the duration of DM. Average DM durability in our participants was 14.2 ± 8.2 years. Furthermore, in the T2DMG+DR, it was 19.2 ± 6.8 years while in those without retinopathy the duration of DM was 10.4 ± 7.1 years. In our work this parameter was also related to the degree of severity of DR, as shown in Table 5. We demonstrated that the duration of DM was strongly associated with a higher risk of retinopathy (OR = 1.18 $p < 0.001$). Other authors also reported a strong relationship between DM duration and retinopathy, neuropathy, nephropathy, and peripheral vasculopathy in a sample of 1,157 individuals of Northwest India [34].

Healthy participants performing physical exercise accounted for almost double than the diabetics (CG: 68% versus T2DMG 37.6%; $p < 0.001$). Regarding the presence

TABLE 6: Comparison of the plasmatic prooxidant and antioxidant markers found in the diabetes mellitus patients according to having poor/good adherence to Mediterranean diet.

	T2DM−DR		T2DM+DR		p^*
	PAMD	GAMD	PAMD	GAMD	
MDA1 (μm/L)	2.30 ± 1.14	2.47 ± 1.67	3.77 ± 1.26	3.01 ± 1.32	<0.001[a,b]
MDA2 (μm/L)	2.32 ± 0.85	2.58 ± 1.11	4.12 ± 1.78	2.49 ± 1.31	<0.001[a,b,c]
TAA1 (μM)	2.57 ± 1.50	2.71 ± 1.58	1.86 ± 1.10	2.39 ± 2.06	0.051
TAA2 (μM)	3.11 ± 1.50	2.58 ± 0.99	1.91 ± 1.38	2.88 ± 1.17	0.001[a]

T2DM−RD: type 2 Diabetes mellitus patients without diabetic retinopathy; T2DM+DR: type 2 diabetes mellitus patients with diabetic retinopathy; PAMD: poor adherence to Mediterranean diet; GAMD: good adherence to Mediterranean diet; MDA1: malondialdehyde-baseline; MDA2: malondialdehyde-end of study; TAA1: total antioxidant activity-baseline; TAA2: total antioxidant activity-end of study. Data are shown as mean ± standard deviation.
* p value obtained from ANOVA analysis.
a: significant differences between DM − DR PAMD and DM + DR PAMD; b: significant differences between DM − DR GAMD and DM + DR PAMD; c: significant differences between DM + DR PAMD and DM + DR GAMD.

of retinopathy, significantly higher percentage of subjects performing physical exercise was observed in T2DMG−DR than in the T2DMG+DR (45% versus 27%; $p = 0.035$). Our results coincide with those reporting that physical exercise is beneficial with respect to both the glycemic control and the diabetes-related comorbidities, including the vitreoretinal disorders [35].

The importance of data regarding the BMI from our participants was notorious. Significant differences between the CG and the T2DMG (13.8 + 3.8 kg/m^2 versus 28.4 + 4.3 kg/m^2; $p < 0.001$) were detected. When the presence of retinopathy was analyzed, it was seen that the differences of BMI between the T2DMG+DR and T2DMG−DR were not significant (28.8 + 4.7 kg/m^2 versus 28.0 + 4.0 kg/m^2; $p = 0.345$). In agreement with these data, it has been reported that BMI in correlation with HbA1c, HBP, and cholesterol seems to be related to DR progression in T2DM patients [36]. Furthermore, it has been suggested that BMI may be considered as a predictive marker for the development and progression of DR [36, 37]. In addition to the carbohydrate restriction, dietary recommendations on this topic include saturated fat restriction (<7% of daily caloric intake) and cholesterol restriction (<200 mg/dL) among diabetics.

Tobacco use is a major cause of disease, disability, and premature death worldwide. Our results showed a higher frequency of smokers among the T2DMG than in the CG (34.4% versus 20%, resp.; $p = 0.03$). Diabetics also displayed higher alcohol consumption than the healthy participants (24.8% versus 17.3%) but the difference was not statistically significant ($p = 0.217$). Furthermore, the T2DMG+DR showed significant differences regarding tobacco and alcohol consumption with respect to the T2DMG−DR ($p = 0.001$ and $p < 0.001$, resp.). In fact, it has been described that drinking in excess has to be avoided because it induces ketoacidosis and hypertriglyceridemia. Moreover, it has to be emphasized that alcohol taken outside the meals can cause hypoglycemia crisis and a relevant increase of a host of health problems [38, 39].

Oxidative stress, affecting the cell and tissue morphology and functions, as well as leading to cell death, is a major player in the pathogenesis of a wide variety of retinal diseases. This fact called our attention regarding DR, because knowledge on the precise molecular mechanisms of DR development and progression is far from complete. Up to date, there is no definitive treatment for the disease. In the Mediterranean Spanish region of Valencia, 500.000 diabetics (from a total of 5.004.844 inhabitants) have been recorded in the last year. Blood samples from our diabetics were analyzed for the oxidative and antioxidant activities, as well as for determining the glycemia, HbA1, HDL/LDL cholesterol, and triglycerides. Despite the classical parameters that were significantly higher in diabetics (glycemia, HbA1C), as expected, it was quite surprising that the triglycerides levels were significantly lower in the CG than in the T2DMG at baseline (97.5 ± 59.8 mg/dL versus 149.6 ± 96.6 mg/dL; $p < 0.001$) and after 18 months (114.7 ± 69.3 mg/dL versus 142.6 ± 80.9 mg/dL; $p = 0.014$). However, this data was not statistically significant between the diabetics with/without DR at baseline (133.63 ± 65.16 mg/dL versus 156.86 ± 114.85 mg/dL; $p = 0.385$) neither after 18 months (134.0 ± 65.9 mg/dL versus 141.3 ± 91.0 mg/dL; $p = 0.812$). Other authors concluded that no significant association between DR, triglycerides, HDL, and total cholesterol was detected in a sample of Danish diabetics [40]. Miljanovic et al. did not find associations between lipid profile and DR progression [41]. In contrast, the global case-control study in 13 countries concluded that DR was associated with triglycerides and high-density lipoprotein cholesterol in matched analysis [42].

It has been widely recognized that MDA/TBARS are associated with the production of AGEs implicated in the DR development. This mechanism is mainly due to AGEs accumulation in the ocular tissues by increasing cell permeability, angiogenesis, and disruption of the internal blood-retinal barrier [43]. Plasmatic MDA/TBARS levels were significantly higher and the TAA levels were significantly lower in the T2DMG than in the CG. Furthermore, the oxidative status was significantly higher and the antioxidant activity was significantly lower in diabetics +RD with respect to diabetics −RD, data similar to previous reports demonstrating enhanced oxidative stress in T2DM patients [16–19, 23, 28]. Interesting results came from the relationship between the DR degree of severity and the plasmatic oxidative stress

markers, pointing to the fact that the prooxidants significantly increased and the antioxidants significantly decreased in plasma samples of the T2DMG according to the ETDRS severity scale, as shown in Table 5. Data strongly suggests that the hyperglycemia created a particular oxidative stress background in the retina of the participant diabetics that, in turns, influences the onset and progression of DR, as reflected throughout the 18 months of the follow-up.

We also observed that a nutraceutic formulation containing A/ω3 is capable of counteracting the oxidative stress detected in this diabetic Mediterranean population, as reflected in Figure 2 and Tables 4–6. In addition to the antioxidant enzymes, the robust antioxidant capacities of a high variety of nonenzymatic antioxidants—among them are vitamins (ascorbic acid (vit C), α-tocopherol (vit E)), transition metals (selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn)), glutathione, carotenoids (β-carotene, lutein, and lycopene), hormones (melatonin, oestrogen), phenols (catechin, quercetin), and so forth—and the added benefits of the antioxidant and anti-inflammatory properties of the PUFAs are well defined in the literature [21, 22, 24–27]. Therefore, possible protecting mechanisms against oxidative stress of the oral supplementation with a combination of A/ω3 in our diabetics may include a mixture of the strong antioxidant activity from vitamins C and E, the carotenoids lutein and zeaxanthin, the free radical scavenging glutathione, and the trace elements Se, Zn, Cu, and Mn, as well as the DHA and DHA-metabolic by-products, all of which are participating in scavenging excessive oxidants, preventing free radical generation, recycling other antioxidants, and probably ameliorating the mitochondrial functions (oxidative phosphorylation). In this framework, it has been reported that systemic interventions are highly required for controlling DR patients. Among these, early detection of hyperglycemia and intensive glycemic control together with hypertension and dyslipidemia therapy have to be programmed. As a consequence of the results raised during this study course, strategies for avoiding DR development and progression should therefore aim to prevent specifically the effects of glycation but also the oxidative stress consequences. Therefore, it is reasonable to consider that biotherapies with multiple targets can be more useful than unique drugs in DR treatment [43].

However, it is notorious that studies with oral supplements on DR have yielded conflicting findings. Among the studies on the role of A/ω3 in the prevention of DR, the San Luis Valley Diabetes Study did not find protective effect in DR and vitamin E on DR of the b-carotene, vitamin C, and vitamin E [44]. The Third National Health and Nutrition Examination study described that α-tocopherol and vitamin C serum levels were not associated with DR [45]. Moreover, the Diabetes Control and Complications Trial described that patients assigned to a low-fat diet decreased the rate of DR progression as compared with patients not following the low-fat diet [46]. In addition, antioxidant and antiangiogenic properties of edible berries were shown to be protective for DR [47]. García-Medina et al. [48] designed a follow-up study in the Mediterranean area of Spain in T2DM patients with nonproliferative diabetic retinopathy,

evaluating the effect of A/ω3 supplementation over a 5-year follow-up. The DR stage showed a retardation of progression in the subgroup with supplementation but worsened in the nonsupplemented subgroup. It was also described that the oral supplements maintained the antioxidant defenses activity as shown by the high plasmatic TAA levels, which was related to the decreased oxidative plasma activity. In several experimental studies using different strains, it was shown that increasing the diversity of antioxidants provides significantly more protection than using a single antioxidant for the supplementation. Moreover, when antioxidants were administered to diabetic rats with the main goal of assessing the ability of these agents to inhibit the development of DR, the authors concluded that long-term administration of antioxidants could inhibit the development of the early phases of DR [49]. Hence, the oral supplementation with A/ω3 represents an achievable adjunct to help preserve vision in diabetics.

It is recognized that proliferative diabetic retinopathy (PDR) is the most usual sight-threatening vitreoretinal damage in T1DM patients, while diabetic macular edema (DME) is considered the main cause of visual impairment and blindness in the T2DM patients [50, 51]. In our study, the T2DMG displayed DME (clinically significant) at baseline 5.6% DME in the RE versus the 11.2% in the LE ($p = 0.037$, $p = 0.003$, resp.). Moreover, DME was more frequent in the T2DMG+DR than in the T2DMG−DR and the CG ($p = 7.036^{E-005}$ for the RE, and $p = 2.214^{E-006}$ for the LE). Among the recognized risk factors are the hyperglycemia, HBP, dyslipidemia, DM duration, and oxidative stress status. However, our study supports the benefits of the A/ω3 supplements as well as the important modifiable component for the risk factors profile for better managing the diabetic eyes and vision.

One additional interesting aspect of our work is that compliance with the Mediterranean diet was assessed by a brief 14-item validated questionnaire which is faster and less complicated to the participants than other questionnaires, being considered very useful by the investigators [52, 53]. The Mediterranean diet is a nutritional and lifestyle pattern based on the traditional dietary habits of Greece, Italy, and Spain [52]. The term Mediterranean diet was set by Ancel Keys in 1960. In our study course, we have detected that the average values of the 14-item Mediterranean diet scores were significantly higher in the CG versus the T2DMG ($p < 0.05$). Moreover, comparison of the prooxidant and antioxidant markers in the T2DMG with GAMD or PAMD also reported significant differences (Table 6). These results point to those people with GAMD with the above described dietary habits who had a lesser risk of DR, which agree with previous reports regarding the lesser risk of chronic and cardiovascular diseases among the Mediterranean countries inhabitants [50–52]. At this point, we may suggest that this type of questionnaire is useful for evaluating the role of the Mediterranean diet in the ocular diseases.

A limitation of this study was the relatively small sample size of 265 participants at baseline (T2DMG; $n = 165$ versus CG; $n = 100$) and the important patients lost to follow-up (a total of 208 participants—among them are 130 patients

from the T2DMG (62 +DR versus 68 −DR) and 78 healthy individuals from the CG—continued and completed altogether the 18 months of the study). For this reason, these findings are helpful enough to understand the characteristics of the studied population, but we are trying to control these important variables for further research, to better generalize our results to the broader community.

5. Conclusions

Healthy living includes everything we have to plan for improving our wellbeing. Specifically, diabetics have to be able to handle life's challenges, including exercise, being aware of smoking and drinking in excess, and maintaining the body weight. All of these have to be considered key milestones for eye care in diabetics. The DM induced an oxidative background in T2DM participants of the Mediterranean study area that was enhanced in patients +DR. The A/ω3 formulation utilized herein provided the appropriate vitamins, minerals, and PUFAs, resulting in decreased plasmatic oxidative level and increased antioxidant activity in diabetics. Antioxidant supplementation may be useful to help manage diabetics at risk of retinopathy and vision loss.

Disclosure

Maria D. Pinazo-Durán is Coordinator of the VSDR. Rosa Dolz-Marco, Maria I. López-Gálvez, David Galarreta-Mira, Carmen Galbis-Estrada, Jose J. García-Medina, Roberto Gallego-Pinazo, and Maria D. Pinazo-Durán are OFTARED (Spanish Net of Ophthalmic Pathology) researchers.

Conflict of Interests

All authors of this work have disclosed that they have no significant financial relationships or financial interests in the commercial companies that are related to this study or paper.

Authors' Contribution

Maria J. Roig-Revert, Antonio Lleó-Pérez, Vicente Zanón-Moreno, and Bárbara Vivar-Llopis collaborated equally in the present work, sharing the first place.

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Review Article

Current Approach in the Diagnosis and Management of Uveitic Glaucoma

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Uveitic glaucoma (UG) typically is associated with very high intraocular pressure (IOP) and more intense optic nerve damage than other glaucoma types. This secondary glaucoma requires an early diagnosis and adequate management of both uveitis and glaucoma. It is mandatory to identify the mechanisms of IOP elevation that in many eyes have multiple combined mechanisms. Management of these patients commonly requires an interdisciplinary approach that includes a glaucoma specialist and rheumatologist to control the inflammation and IOP. Glaucoma surgery is required early in these patients due to the high IOP usually present and is less successful than in primary open-angle glaucoma. Recurrent uveitic episodes, multiple mechanism, and the complications associated with uveitis make surgical management of UG challenging. In this review, the management and treatment of UG are updated to clarify the pathogenesis and prevent optic nerve damage.

1. Introduction

Patients with uveitis have an increased risk of intraocular pressure (IOP) elevation not only because of the disease but also as a side effect of corticosteroid use [1].

Uveitic glaucoma (UG) includes a range of disorders whose common end result is glaucomatous optic nerve and visual field damage. Compared with primary open-angle glaucoma (POAG), patients with UG are younger and IOP values are higher with acute elevations and varying responses to antiglaucomatous drugs.

The mechanism of UG is complex (different open-angle and closed-angle mechanisms can coexist in the same patient), and management requires careful diagnosis and adequate control of both IOP and inflammation. A multidisciplinary approach is necessary in many cases to achieve a successful outcome.

The very high IOP and the complex interrelation with ocular inflammation explain why many patients with UG require glaucoma filtering surgery sometimes combined with phacoemulsification. Strict control of inflammation increases the chance of success, but surgery for UG historically has been considered refractory because of the increased risk of failure. Otherwise, ciliary body inflammation can result in prolonged postoperative hypotony, making the results of filtering procedures more unpredictable.

This update is intended to assist ophthalmologists who are managing patients with UG. We did not rate the quality of evidence cited but described the study design in many cases.

2. Methods

We searched the published peer-reviewed medical literature to identify studies that evaluated UG. Multiple databases were

searched, including MEDLINE, EMBASE, Cochrane, ERIC, and EBSCO. The search was limited to articles in English and foreign language publications with English abstracts. Several key words were used including uveitic glaucoma, inflammatory glaucoma, Posner-Schlossman syndrome (PSS), and Fuchs heterochromic cyclitis. All retrieved articles were cross-referenced and citations in the bibliography were retrieved if deemed relevant. Articles displayed in the "related articles" link on PubMed also were used when relevant.

3. Physiopathology

The mechanisms that determine an IOP increase in UG are diverse and complex; many are often present simultaneously in the same patient. Open-angle glaucoma (OAG) occurs as a result of mechanical obstruction of the trabecular meshwork by inflammatory cells, proteins, debris, fibrin, or inflammatory precipitates. Additionally, direct inflammation of the trabecular meshwork and/or the effect of corticosteroids on the trabecular meshwork may contribute to the open-angle mechanism of UG [1, 2]. Up to one-third of patients with uveitis treated with corticosteroids may have elevated IOP, and it may be difficult to distinguish between the side effects of the corticosteroids and the underlying inflammation. A family history of glaucoma, rheumatoid arthritis, diabetes, and younger age are considered risk factors of a steroid responder [1].

Secondary angle closure can result from synechial closure, neovascularization of the chamber angle, or seclusion pupillae with subsequent appositional angle closure. Less commonly, angle-closure glaucoma develops when inflammation and edema cause ciliary body forward rotation to close the angle, as in patients with Vogt-Koyanagi-Harada syndrome (VKHS).

These complex interactions cause patients with UG to have high IOP fluctuations and great variability in the therapeutic response.

4. Classification

Some authors have proposed differentiating between hypertensive uveitis and UG based on the absence or presence of optic nerve damage, but this distinction usually is not applied to secondary glaucomas [3]. Typical hypertensive uveitis, such as in PSS, can cause glaucomatous damage over time in relation to the number, duration, and intensity of the episodes (Figure 1) [4].

5. Diagnosis

Recent improvements in the clinical evaluation of the optic nerve and retinal nerve fiber layer (RNFL), such as scanning laser ophthalmoscopy and optical coherence tomography (OCT), and of the angle, such as ultrasound biomicroscopy (UBM) and anterior segment OCT, are as relevant to UG as to other glaucomas.

The higher IOP levels associated with UG may cause apparent structural damage detected by optic disc imaging that disappears when the IOP level returns to normal [3].

Optic disc imaging is a useful way to document the glaucoma status in this type of eye, provided that media opacification does not hamper image acquisition.

However, it is important to consider that uveitis is a major confounding factor in assessing the RNFL thickness. Moore et al. reported substantial RNFL thickening in patients with active uveitis and a thicker RNFL than expected in patients with UG [5], probably related to breakdown in the blood-retinal barriers and increased production of prostaglandin analogues (PGAs). After the inflammation improves, the retinal thickness decreases and thinning of the RNFL and increased cupping can be observed [6]. These changes raise concerns about the comparative value of RNFL scans as a method for detecting and monitoring glaucomatous damage in patients with uveitis.

Normal-appearing measurements of the RNFL thickness in patients with UG should be interpreted cautiously in those with elevated IOP. Physicians should recognize that continued thinning of the RNFL and increased cupping, despite good IOP control in such eyes, might be due to resolution of edema of the RNFL.

Screening for glaucomatous RNFL changes in uveitis must be performed during quiescent periods. Thinning of the inferior quadrant suggests that glaucomatous damage is in fact occurring [7]. Measurement of the RNFL may facilitate detection of signs of damage before disc or visual field changes and therefore identifies a subgroup that should receive more aggressive treatment [7]. In addition, OCT also has become a standard for confirming the diagnosis of macular edema [8].

If the cornea cannot be cleared adequately, UBM and OCT are useful for evaluating the angle [1]. UBM is valuable for evaluating different types of angle-closure glaucoma. This technique currently has an advantage over OCT in that the ciliary body can be visualized as the iridocorneal angle even in the presence of substantial corneal opacification. Visualization of the ciliary body is particularly useful for diagnosing chronic ocular hypotony, which paradoxically may be a late development in patients with chronic UG. It is also very important to evaluate closed-angle glaucoma secondary to anterior rotation of the ciliary body. Anterior-segment OCT also may be helpful to evaluate the length and position of glaucoma drainage device tubes and their relationship with the corneal endothelium [9] as well as to evaluate filtering bleb in eyes that have undergone filtration surgery [10, 11].

6. Epidemiology and Etiology

Glaucoma occurs in around 20% of all patients with chronic uveitis [2]. The incidence and clinical appearance of UG differ according to the disease etiology. The etiology of uveitis varies among different ethnicities and even among regions of the same country [12]. Higher rates are reported in those with rheumatoid arthritis-associated iridocyclitis, Fuchs heterochromic iridocyclitis (27%), sarcoidosis (34%), herpes simplex keratouveitis (54%), zoster uveitis (38%) [2], Lyme-associated uveitis, cancer-associated uveitis [13], juvenile idiopathic arthritis (JIA) (12–35%), Behçet's disease, pars
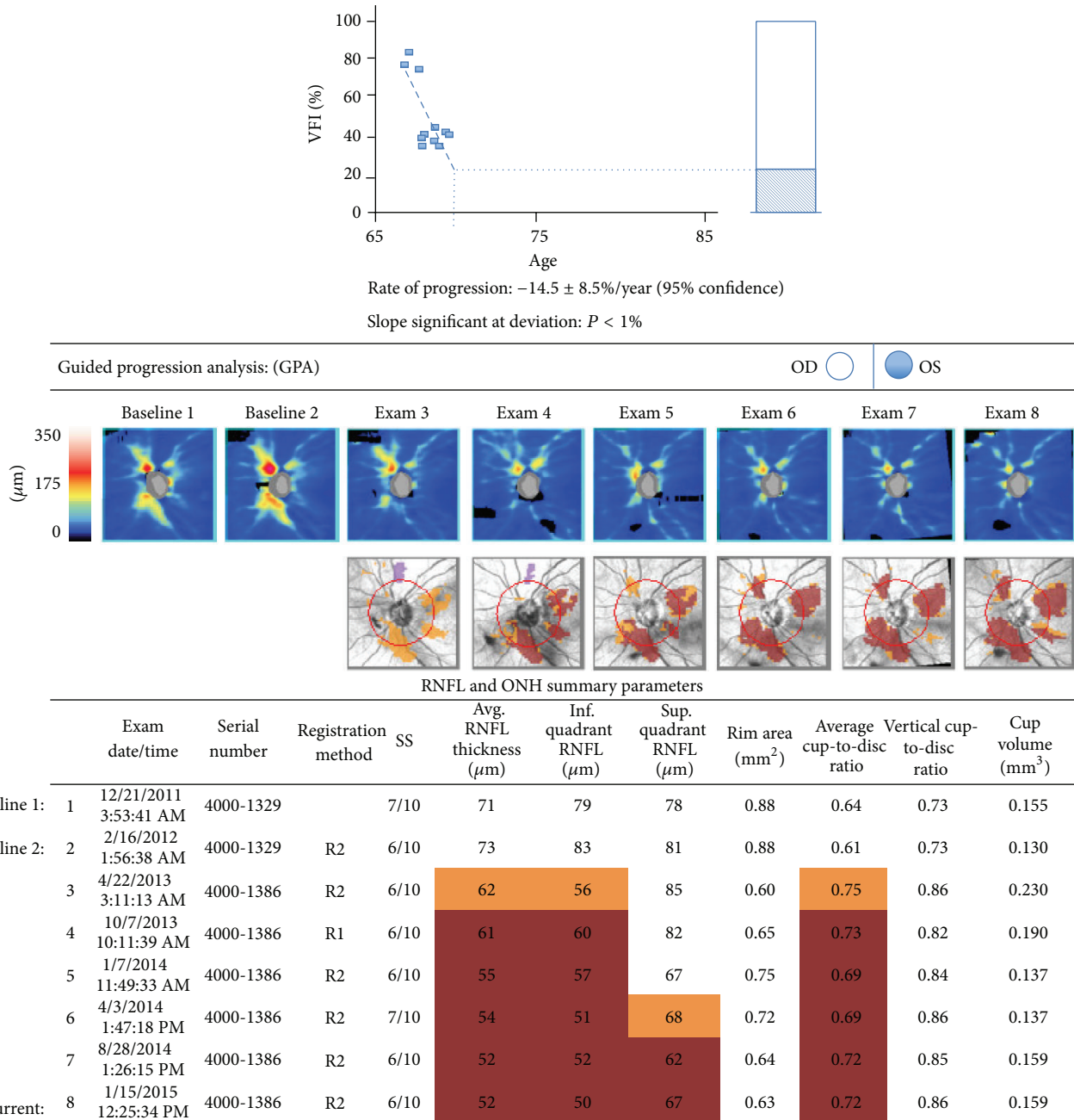
FIGURE 1: Visual field and OCT progression after recurrent episodes of UG (VFI: Visual Field Index).

planitis, sympathetic ophthalmia, and syphilis [2]. Acute IOP elevation is also typical in PSS. Some signs are characteristic of specific etiologies and may be helpful to establish a correct diagnosis (Figure 2).

6.1. *Fuchs Heterochromic Uveitis (FHU).* FHU was described as the triad of anterior uveitis, heterochromia, and cataract. It is unilateral in 90% of cases and the affected eye is the hypochromic one (Figure 3). The uveitis is chronic and low-grade, without synechiae and with typical small stellate keratic precipitates [1]. Microhyphema after paracentesis,

gonioscopy or tonometry (Amsler's sign) is typical of FHU and related to the anomalous vessels in the angle chamber. OAG is present in 13% to 59% of cases. Initially, it can respond to anti-inflammatory and medical treatment, but a filtering surgery is commonly needed to control the IOP. FHU is considered to have a higher risk of failure when associated with UG [14].

Chee and Jap reported that 41.7% of eyes with presumed FHU are cytomegalovirus- (CMV-) positive. Patients with CMV-positive presumed FHU are more likely to be men, be older at diagnosis, and have nodular endothelial lesions [15].
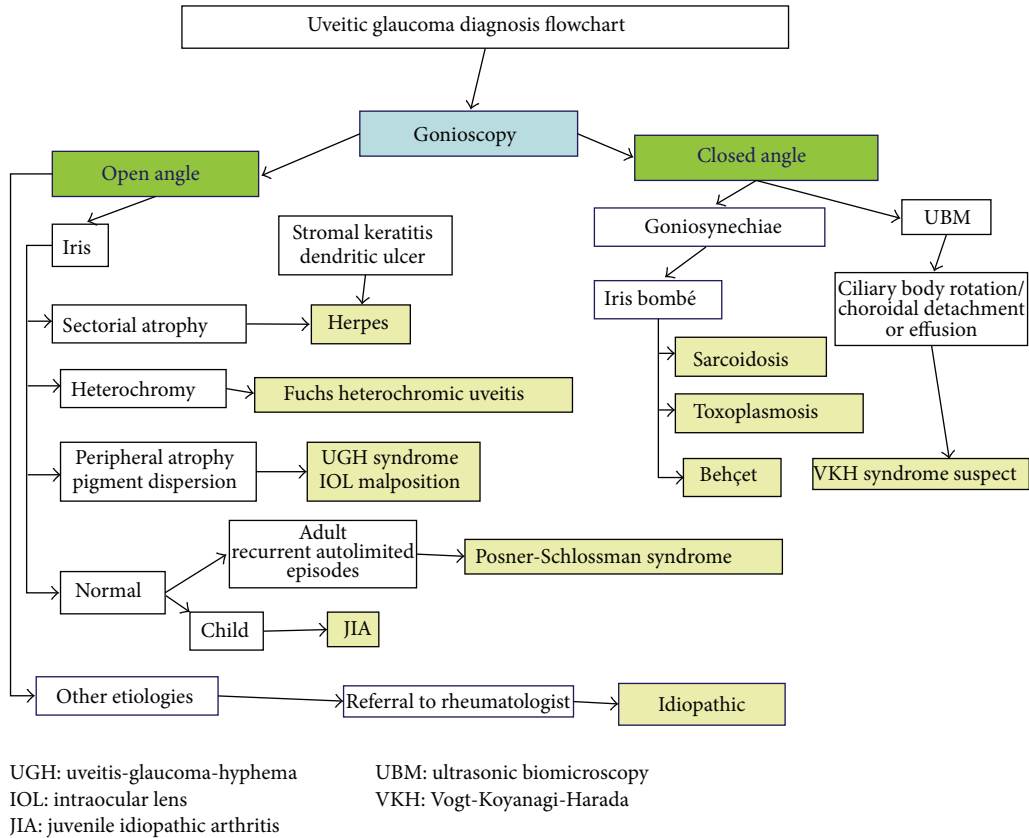
FIGURE 2: UG diagnosis flowchart.

(a)   (b)
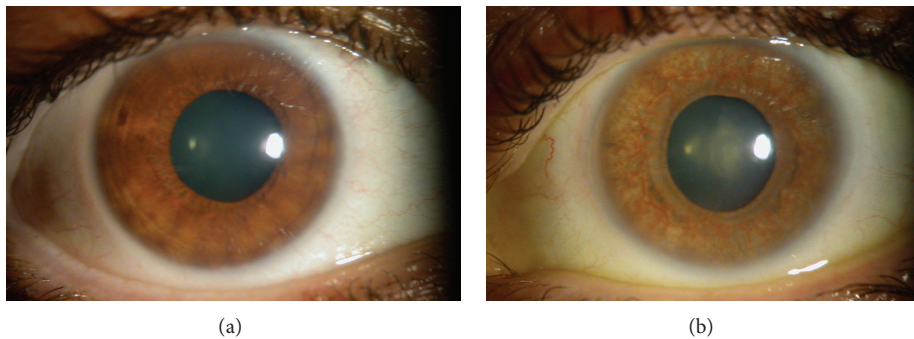
FIGURE 3: Fuchs heterochromic uveitis in one patient. The right eye is normal. The left hypochromic eye is affected.

6.2. PSS. Glaucomatocyclitic crisis, or PSS, presents typically with unilateral recurrent episodes of mild cyclitis with a few fine keratic precipitates and elevated IOP in the range of 40 to 60 mmHg during episodes that usually resolves spontaneously. The IOP is normal between attacks and the angle is open [15, 16]. The course is commonly benign, but about 25% of patients can develop glaucomatous damage if the number of episodes or the disease duration is sufficiently long (Figure 1) [4].

In two recent studies, more than 50% of aqueous humour samples from eyes with PSS were positive for CMV by polymerase chain reaction (PCR) analysis [15, 17]. Severe

endothelial cellular loss and a higher number of eyes requiring glaucoma filtering surgery were observed in patients with CMV-positive PSS [17].

6.3. Herpetic Uveitis. UG is the most common complication in patients with herpetic uveitis and it is typically unilateral. An acute increase in IOP in the presence of active iridocyclitis is the hallmark of a herpetic etiology, associated with herpes simplex virus or varicella zoster virus. Inflammation of the trabecular meshwork has been proposed as the cause of IOP elevations and is supported by normalization of the IOP after corticosteroid treatment [1]. Diffuse or sectorial iris atrophy
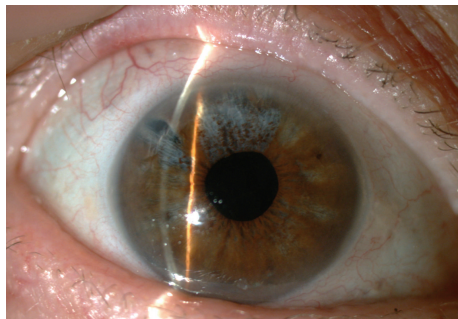
Figure 4: Sectorial iris atrophy is typical of herpetic uveitis.

is a characteristic of herpetic iritis (Figure 4). The presence of corneal stromal opacities is typical of herpetic stromal keratouveitis and can assist in the etiologic diagnosis. In some severe cases, posterior synechiae and fibrin deposition may be present [1].

6.4. JIA.
Most patients who develop uveitis have oligoarticular JIA [18]. About one-third of patients with JIA-associated uveitis develop secondary ocular complications such as posterior synechiae, cataract, band keratopathy, glaucoma, or macular edema [18]. The prevalence of glaucoma or ocular hypertension in JIA-associated uveitis has been reported to range from 14% to 42% [1]. Patients with persistent low-grade intraocular inflammation are at the greatest risk for developing glaucoma. OAG and secondary closed-angle glaucoma as a result of formation of posterior synechiae can be present in JIA uveitis. Immunomodulatory therapy such as methotrexate is often necessary to treat the chronic iridocyclitis associated with JIA [18, 19].

6.5. VKHS.
VKHS typically presents as bilateral panuveitis with dermatologic and central nervous system manifestations. Glaucoma can be present in 18% to 38% of cases. The management of the closed-angle mechanism is especially challenging because edema and anterior rotation of the ciliary body can be present, and these cases do not respond to iridotomy.

6.6. Postoperative UG.
UG can be present after complicated cataract surgery. Secondary glaucoma can develop because of retained nuclear or cortical lens fragments. Malposition or subluxation of an intraocular lens (IOL) can determine pigment dispersion and elevated IOP. The uveitis-glaucoma-hyphema syndrome is the typical clinical picture and IOL explantation may be required in some cases [20].

7. Management

Uveitis is a complex multifactorial ocular inflammatory disease process that often requires a multidisciplinary approach. Successful management requires simultaneous treatment of both uveitis and IOP elevation. Adequate control of inflammation is mandatory and a current mistake is to undertreat the uveitis to avoid the corticosteroid-induced IOP elevation.

This conservative approach can result in trabecular meshwork damage secondary to the inflammatory process.

Etiologic treatment may be helpful in some specific etiologies such as herpetic keratouveitis. When present, the angle-closure component must be managed [3].

7.1. Anti-Inflammatory Treatment.
The first step in UG management is controlling the inflammation, which minimizes the adverse effects of the inflammatory process. In some cases, controlling the uveitis may help reduce the IOP. Patients treated aggressively with anti-inflammatory therapy have a better clinical course of the UG [1].

Corticosteroids are the preferred anti-inflammatory drug used to treat uveitis. It is advisable to start with strong topical corticosteroids such as prednisolone acetate, but periocular or systemic corticosteroids may be required in refractory cases [1, 3]. Rimexolone and loteprednol induce the IOP steroid response less often; however, the anti-inflammatory effect is weaker and in UG it is necessary to use stronger corticosteroids. The chronic inflammation commonly present in FHU does not require continued anti-inflammatory treatment, but it could be useful to use corticosteroids in acute exacerbations of uveitis with transient IOP spikes [21].

Nonsteroidal anti-inflammatory drugs are not usually helpful for treating UG and can partially block the hypotensive effect of some glaucoma medications such as latanoprost and brimonidine [2, 22].

In corticosteroid responders, immunosuppression with drugs such as cyclosporine, azathioprine, methotrexate, or anti-tumor necrosis factor-alpha antibody therapy may be necessary. In these cases, coordination with a uveitis specialist or rheumatologist who is more comfortable in initiating or adjusting systemic immunomodulatory therapy is advised [2].

Cycloplegic agents must be used with anti-inflammatory treatment in some acute uveitic episodes, with the exceptions of PSS and FHU. In case of peripheral anterior synechiae with permanent angle closure, mydriatics and cycloplegics may be contraindicated.

7.2. Antiviral Treatment.
Antiviral treatment should be prescribed to treat specific etiologies such as herpes simplex or varicella zoster. Topical antiviral therapy is indicated in patients with keratouveitis to prevent viral replication during treatment with topical steroids, but it is considered ineffective in herpetic uveitis. Along with management of glaucoma, long-term antiviral prophylaxis such as oral acyclovir, valacyclovir, or famciclovir usually is required to prevent recurrences. Acyclovir 800 mg twice daily or valacyclovir prophylactically for patients with herpes simplex disease and double the dose for varicella zoster disease have been recommended [1].

Aqueous analysis by PCR recently has been positive for CMV in some patients with PSS and Fuchs heterochromic iridocyclitis [15]. Considering that more than 50% of patients with PSS will be positive for CMV after PCR analysis of the aqueous humor, ganciclovir and valganciclovir have been proposed as etiologic treatments.

Topical ganciclovir effectively clears the viral load, helps control IOP, and preserves the corneal endothelium of patients with CMV-positive PSS. The regimen used was topical 2% ganciclovir solution every 2 to 3 hours daily as induction therapy and every 4 hours for long-term maintenance therapy. All CMV-infected eyes treated with continuous topical 2% ganciclovir had undetectable CMV levels at subsequent analyses. During follow-up, the average number of antiglaucomatous agents decreased but a similar frequency of IOP spikes occurred in both groups.

Patients with CMV-positive eyes with a disease duration exceeding 5 years were likely to require glaucoma surgery. All patients undergoing surgery had CMV-negative PCR results during the IOP attack but had severe peripheral anterior synechiae and pigment clogging [17].

In the same way, 11 patients with PSS with positive CMV PCR analysis of the aqueous humor were treated with 900 mg of valganciclovir twice daily for 3 weeks followed by 450 mg twice daily for a mean period of 20 months. In the first week of treatment, the IOP decreased significantly and remained stable during the entire treatment period. However, two patients had a recurrence after the drug was discontinued. No side effects of therapy developed. Long-term oral therapy with valganciclovir seems to lower the recurrence rate in patients with clinically diagnosed PSS and positive CMV aqueous humor [23].

7.3. Antiglaucomatous Drugs.

In UG, the effectiveness of antiglaucomatous medical treatment may vary in the presence of inflammation or when combined with mandatory steroid treatment. Less topical medication may be absorbed in the presence of inflammation, and the IOP-lowering effect of most ocular hypotensive agents can vary markedly in uveitis, ranging from no response to profound reductions (70%–80%) with relatively small amounts of ocular hypotensive medication in the occasional uveitic eye with very labile IOP levels [2].

No clinical evidence supports a first-line therapy for UG. Traditionally, topical beta-blockers and CAIs have been considered the first-line agents to treat increased IOP associated with uveitis. PGAs can be used as first-line therapy in UG with controlled uveitis [24, 25]. Systemic CAIs should be considered if topical medications fail to achieve the desired effect [1].

7.3.1. Beta-Blockers.

Nonselective topical beta-adrenergic antagonists are considered the first-line agents used to decrease IOP in patients with UG without systemic contraindications [3]. Metipranolol, including the unpreserved preparation, should be avoided because of its association with anterior granulomatous uveitis [3, 26, 27].

7.3.2. PGAs.

Controversy exists concerning the use of PGAs in patients with uveitis due to the theoretically higher risk of anterior uveitis, blood-aqueous barrier disruption, cystoid macular edema (CME), and reactivation of herpes simplex keratitis. However, in a comparative study on the efficacy and safety of latanoprost against a fixed combination of brimonidine and timolol in patients with UG, latanoprost was at least as effective as the fixed combination and there were no differences in the rate of inflammatory recurrences and incidence rates of CME between the treatments. The authors concluded that latanoprost is as safe and effective as the fixed combination of brimonidine and timolol for treating UG [24].

A paradoxical reaction after treatment with latanoprost was reported in three patients with UG with increased IOP and recurrent inflammation 7 to 16 days after rechallenging with topical latanoprost. However, all patients had undergone a previous complicated intraocular surgery [28].

Another concern is related to the possible induction of chronic conjunctival inflammation that may have a negative effect in future filtering surgeries. After studying conjunctival cells by impression cytology for inflammatory markers by flow cytometry, Taylor et al. found that the use of topical PGAs does not induce conjunctival inflammation over that already present in patients with UG. This finding supports the use of topical PGAs in patients with UG, indicating that their use is unlikely to adversely affect subsequent glaucoma filtration surgery through induction of chronic conjunctival inflammation [29].

In summary, PGAs and prostamides may be first-line therapy choices in patients with UG, especially in cases of quiescent uveitis without previous complicated intraocular surgery or preexisting CME [1, 24, 25]. In eyes with a history of herpetic keratitis or keratouveitis, PGAs are best avoided [25].

7.3.3. CAIs.

The IOP-lowering effect of topical CAIs varies greatly in patients with uveitis [27]. A potential advantage is the possible positive effect in preventing and treating CME coexistent with UG.

Dorzolamide significantly inhibits CAI activity. Irreversible corneal decompensation has been described after topical administration of dorzolamide in patients with underlying corneal endothelial compromise. In patients with preexisting corneal endothelial injury, topical CAIs must be avoided [3].

Acetazolamide is used frequently to manage acute IOP elevations in combination with other antiglaucomatous drugs. It is especially helpful in preparing patients for filtering surgery.

Although an anecdotal case report has reported the additive effect on IOP reduction with the concomitant use of topical and systemic CAIs [30], the general trend is to consider that topical CAIs do not have an additive effect to maximal oral doses of acetazolamide.

7.3.4. Alpha-2 Adrenergic Agonists.

Currently, brimonidine, an alpha-2 adrenergic agonist, is considered a useful second-line therapy for patients with glaucoma and it is most often used in combined therapy.

Granulomatous anterior uveitis has been described after long-term use of apraclonidine and brimonidine. Most cases developed about 1 year after alpha-2 adrenergic treatment and typically an allergic reaction preceded the anterior uveitic episode and the patients had not stopped the treatment after this episode (Figure 5).
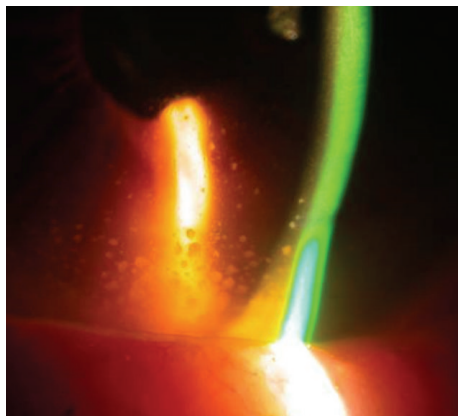
FIGURE 5: Granulomatous uveitis after long-term apraclonidine treatment.

Some cases recurred after rechallenging with brimonidine, confirming the causal relationship. Typically, the inflammation resolves rapidly after stopping the alpha-2 adrenergic treatment and with use of topical corticosteroids [31, 32].

When using an alpha-2 adrenergic agonist, it is important to be alert for the first signs of intolerance or allergic reaction and immediately stop treatment. Anterior uveitic reactivation is possible after brimonidine treatment in patients with UG.

7.3.5. Cholinergic Agents. Cholinergic agents or miotics generally are contraindicated for treating UG because of the potential exacerbation of inflammation via blood-aqueous barrier breakdown. In addition, miotics promote development of posterior synechiae, and in patients with synechial angle closure these drugs are generally ineffective given their mechanism of action of increasing trabecular aqueous outflow [27].

7.4. Laser Trabeculoplasty. The common angle-closure mechanism in many cases of UG may preclude the use of argon laser trabeculoplasty (ALT). There is also concern about the risk of exacerbating inflammation and trabecular meshwork damage after ALT. ALT currently is not recommended for treating UG [3].

Selective laser trabeculoplasty (SLT) has been suggested as an alternative treatment for UG. Siddique et al. reported a significant IOP reduction after SLT in naïve eyes with UG (19.8% after a 1-year follow-up). SLT was less effective in eyes that underwent a previous glaucoma surgery [1]. However, the complete results and complications have not been published, and currently there is insufficient clinical evidence to recommend SLT to treat UG.

7.5. Surgery. If medical management fails to control IOP, surgery is the next step. About 30% of eyes with UG may require surgery [33]. The surgical success rates in UG varies markedly (50%–100%) [13]. There is a consensus that the surgical success rate of filtering surgery is lower for eyes with UG compared with POAG.

As a rule, suppression of inflammation in the perioperative period significantly improves outcomes [12]. Regardless of the surgical modality chosen, all patients require meticulous control of inflammation preoperatively and vigilant monitoring for reactivation postoperatively. Otherwise, the ciliary body can be damaged by inflammation and/or mitomycin C (MMC) use during filtering surgery. Surgeons should exercise caution when recommending irreversible filtering glaucoma procedures and in concomitant use of antimetabolites to avoid prolonged hypotony and the risk of phthisis bulbi.

Preoperatively, although good control of intraocular inflammation for a number of months is ideal, filtration surgery rarely is an elective procedure, and a regimen of preoperative topical or systemic corticosteroid treatment (e.g., 0.5 to 1 mg/kg/day of oral prednisolone) is useful to reduce intraocular inflammation and the inflammatory cells in the conjunctiva [2].

Intraoperatively, antifibrotics during filtering procedures may retard postoperative wound healing. Alternatively, an infraorbital depot of 40 mg of methylprednisolone or intravitreal 4 mg of triamcinolone can be administered at the conclusion of surgery [2].

Postoperatively, a major challenge to successful filtration surgery for uveitis is the accelerated healing that occurs in the presence of postoperative inflammation. However, there is no way to completely eliminate postoperative inflammation and the severity of uveitis may increase postoperatively.

The significant risk factors for surgical failure are male sex, age younger than 45 years, nongranulomatous uveitis and prolonged postoperative inflammation [12].

The choice of the most appropriate surgery depends on patient age, inflammatory activity, previous ocular surgeries, conjunctival scarring, pathophysiology of the IOP elevation, surgeon experience, and postoperative IOP goal.

7.5.1. Trabeculectomy. Classically, trabeculectomy has been the procedure of choice for treating UG, with the exception of aphakic eyes, neovascularization, or poor visual function [3]. Success rates from 50% to 100% have been reported after trabeculectomy to treat UG [13]. Poor success rates with trabeculectomy performed without antiproliferatives have been reported in UG; the standard of care is adjunctive 5-fluorouracil (FU) or MMC in these patients [1]. In UG, Towler et al. reported that after 5 years follow-up, 50% of eyes that underwent trabeculectomy with 5-FU were controlled versus only 30% of eyes in which 5-FU was not applied [34].

Trabeculectomy with MMC is less effective in UG than in POAG. However, Kaburaki et al. did not found differences in the efficacy and safety of trabeculectomy with MMC as the initial ocular surgery in inactive uveitis and POAG, although hypotonic maculopathy was more common in UG [35].

Granulomatous uveitis and previous cataract surgery are considered risk factors for failure after trabeculectomy with MMC [36]. In granulomatous uveitis, fibrotic tissue and granuloma containing Langhans giant cells accumulate in the trabecular meshwork and Schlemm's canal and may obstruct the filtering pathway created by trabeculectomy.

Trabeculectomy with MMC in patients with UG has been associated with a higher risk of cataract progression. Regarding the cataractogenic effect of trabeculectomy [37],

chronic ocular inflammation, and continuous corticosteroid treatment also may contribute to more rapid progression of cataract.

The most common complications after trabeculectomy in patients with UG are recurrent inflammation (17.6%) and hypotony (11.8%). Meticulous control of inflammation preoperatively and vigilant monitoring for reactivation is mandatory. Considering the associated damage to the ciliary body in some patients with uveitis, prudent use of MMC is advisable to avoid prolonged postoperative hypotony.

Even though subconjunctival bevacizumab (Avastin, Genentech Inc., South San Francisco, CA) has been used successfully for controlling wound healing after glaucoma filtration surgery, no data have been published on the safety and efficacy of intraoperative use of bevacizumab as adjunct to trabeculectomy in UG [1].

7.5.2. Ex-PRESS Mini-Glaucoma Shunt.

The Ex-PRESS glaucoma filtration device (Alcon Laboratories, Fort Worth, Texas, USA) is a metallic implant that provides an artificial channel to drain aqueous into the subconjunctival space. This technology is less invasive than traditional trabeculectomy. The Ex-PRESS shunt does not require a sclerectomy or peripheral iridectomy; hence, there is less inflammation and risk of blockage of the inner window by fibrin, blood, or iris. All of these factors can be advantageous in UG.

In a small preliminary case series of five patients, Lee et al. reported the safety and efficacy of the Ex-PRESS glaucoma filtration device with intraoperative MMC for use in UG. The complete and qualified success rates were 80% and 100%, respectively, after 6-month follow-up. Postoperative hypotony due to ciliary shutdown occurred in 20% of cases, one of which was complicated by choroidal detachment and long-term hypotony maculopathy [38]. Larger trials are warranted to establish the long-term efficacy and safety of the Ex-PRESS for the treating UG.

7.5.3. Nonperforating Deep Sclerectomy (NPDS).

This is an attractive alternative for glaucoma surgery in UG and in steroid-induced IOP elevations with an open angle in that it avoids anterior chamber entry, iris manipulation, and prolonged hypotony. The absence of iris manipulation is of special importance in patients with uveitis and may reduce the risk of postoperative inflammation and hyphema. These complications have been associated with a greater risk of failure after filtering surgery.

The integrity of the trabeculodescemetic window allows controlled outflow of aqueous humor, which reduces the risk of profound and long-term hypotony, and it also has been postulated to prevent egress of cytokines and inflammatory mediators from the anterior chamber into the subconjunctival space, which reduces the risk of inflammation, scarring, and failure of filtering surgery [39–41].

There is increasing evidence that NPDS is probably more appropriate for UG [40–43]. Al Obeidan et al. published the largest prospective study of 33 consecutive eyes with uncontrolled UG treated with NPDS with MMC and implantation of either the T-Flux implant (Ioltech, La Rochelle, France) or SK gel (Corneal Laboratories, Paris, France). After a mean follow-up of 33.2 ± 19.8 months, the IOP decreased from a mean preoperative value of 37.2 mmHg to a mean postoperative value of 14.7 mmHg. Complete success was achieved in 72.7% of eyes and qualified success in 21.2% of eyes. Neodymium (Nd):YAG laser goniopuncture was performed in 36.4% of eyes, after which the iris adhered to the trabeculodescemetic window in one patient. Postoperative complications included cataract progression (27.3%), transient hypotony (18.2%), shallow choroidal effusions (12.1%), and hypotony with persistent maculopathy, hyphema, and decompression retinopathy (3%) [41]. These complications may be more prevalent in patients with UG than in those with POAG.

Regarding trabeculectomy, Dupas et al. showed in a retrospective study that similar midterm control of IOP was obtained by either trabeculectomy with MMC (0.4 mg/mL for 3 minutes) or NPDS with MMC (0.4 mg/mL for 3 minutes) and the T-Flux implant, with similar success rates at 12 months. No significant difference between the results of these procedures was found for postoperative complications or the need for reoperation. However, NPDS required many more postoperative adjustments than trabeculectomy (goniopuncture and needling) and trabeculectomy induced marked, though transient, worsening of intraocular inflammation. Visual acuity scores and postoperative cataract progression requiring phacoemulsification were similar in both groups [42].

Although randomized prospective comparative studies of these two procedures are still necessary, this study suggested that NPDS (with simultaneous use of an implant and MMC) and trabeculectomy with antiproliferative agents are both effective for managing UG. NPDS generates less inflammation during the early postoperative follow-up but requires close monitoring for appropriate adjustment of IOP-lowering interventions, such as goniopuncture or needling. However, trabeculectomy leads to high transient postoperative inflammation but facilitates direct IOP reduction with very few postoperative adjustments and might be indicated in cases in which close monitoring is difficult [42].

More controversial is the indication of filtering surgery in PSS. Campana et al. reported a patient with PSS who underwent NPDS with MMC and the T-Flux implant. Goniopuncture was required 9 months after NPDS. The IOP remained 15 to 16 mmHg without topical treatment and no subsequent episode of ocular inflammation 6 years after Nd:YAG laser goniopuncture [44]. In our personal experience, NPDS in PSS facilitates significant reductions in the number and severity of hypertensive peaks (unpublished data); one patient presented with persistent hypotony maculopathy after NPDS with MMC (Figure 6).

A modified NPDS has been described in JIA. The authors made two circumscribed punctures from Schlemm's channel into the anterior chamber, lateral to the sclerectomy. They used MMC (0.2 mg/mL) on the bare sclera for 1 minute in all cases without an implant and reported that IOP can be reduced sufficiently using standard trabeculectomy with MMC and NPDS with MMC, but trabeculectomy with MMC may be more effective. However, additional surgeries to adjust the IOP were common for both groups. In aphakic children,
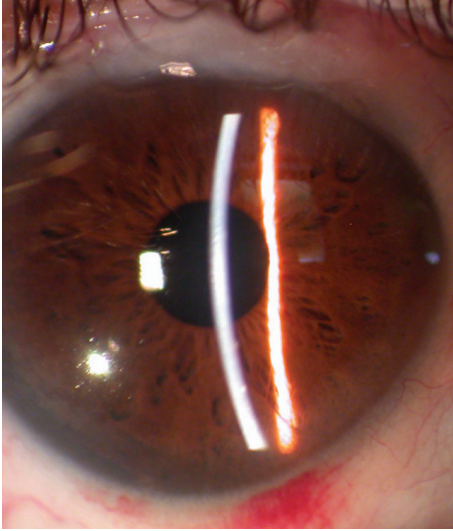
FIGURE 6: PSS. Two small endothelial precipitates are seen. A conjunctival filtering bleb after NPDS is seen.

FIGURE 7: Ahmed valve tube occlusion by fibrin exudation in a patient with UG.

the modified sclerectomy described earlier appears to be a better technique for avoiding vitreous prolapse [45].

7.5.4. Canaloplasty.

This procedure may be of special interest in UG surgery because it acts on an important source of outflow resistance in uveitic eyes exposed to steroids. Glucocorticoids increase IOP via deposition of extracellular matrix material in the juxtacanalicular tissue, leading to thickening of the trabecular meshwork beams, decreased intertrabecular spaces, and a subsequent increase in outflow resistance. Histologic analysis of UG eyes on topical steroids confirmed the trabecular meshwork beam thickening [46]. Canaloplasty expands and maintains a patent Schlemm's canal, increasing the previously reduced intertrabecular spaces.

In a retrospective pilot study of 19 uveitic eyes, canaloplasty with postoperative Nd:YAG goniopuncture was a safe and effective surgery for treating open-angle UG [28]. At the last follow-up visit (mean follow-up time, 2.6 ± 1.1 years), the complete success rate was 73.7% and the failure rate was 15.5%. A 55% reduction in IOP was achieved and the mean number of antiglaucoma drugs decreased from 3.7 ± 0.8 preoperatively to 0.4 ± 1.0 at the last follow-up. The postoperative complications were Prolene suture erosion into the anterior chamber (10.5%), transient hyphema (5.3%), prolonged hypotonous maculopathy after goniopuncture (5.3%), and rapid progression of cataract (5.3%).

The mean number of steroid drops was 0.5 ± 0.6 in the preoperative period and 0.7 ± 1.4 6 months postoperatively.

Canaloplasty is a promising technique for UG, because it expands the intertrabecular spaces, targeting an important source of outflow resistance in uveitic eyes exposed to glucocorticoids [33].

7.5.5. Glaucoma Drainage Devices (GDD).

These devices often are considered the first choice for UG surgery, especially in etiologies such as JIA [47]. In patients with extensive peripheral anterior synechiae, the tube should be placed in the sulcus rather in the anterior chamber to avoid endothelial trauma.
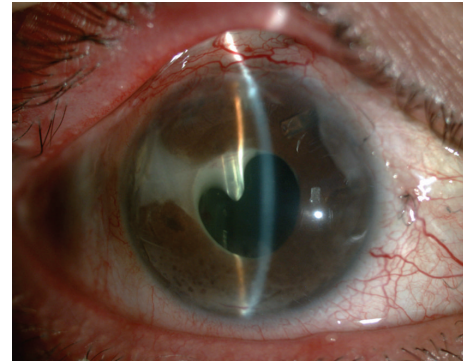
Owing to its unidirectional valve mechanism, implantation of Ahmed glaucoma valve (AGV) (AGV; New World Medical Inc., Rancho Cucamonga, CA, USA) may be more convenient because of the lower risk of immediate postoperative hypotony. Success rates of 77% and 50% have been reported with AGV in UG after 1 and 4 years of follow-up, respectively [48]. Encapsulated bleb (43%), transient hypotony (43%), and hyphema (21%) are the short-term complications most commonly described after AGV implantation in UG [48, 49]. Occlusion of the tube by inflammatory materials and corneal decompensation also has been associated with AGV implantation in eyes with UG (Figure 7) [48].

Preoperative use of corticosteroids may improve the surgical success of the AGV in UG. Mata et al. suggested prescribing 1 mg/kg/day prednisone preoperatively until the inflammation is controlled. In the postoperative period, oral corticosteroids are taped tapered over 4 weeks [50].

Nonvalved GDDs such as the Baerveldt implant (Abbott Laboratories Inc., Abbott Park, IL, USA) have been recommended. The cumulative probability of success was greater with a Baerveldt GDD than after trabeculectomy for UG. There was a significantly higher frequency of early complications in the trabeculectomy group compared with the GDD group; however, no significant differences were seen in the frequency of late postoperative complications between groups. The most common postoperative complications after implantation of a Baerveldt GDD were hypotony and CME, but there were no differences between the Baerveldt implant and trabeculectomy. The authors concluded that implantation of the Baerveldt GDD was more likely to maintain IOP control and avoid reoperation for glaucoma compared with trabeculectomy with antifibrotic therapy in eyes with chronic inflammatory glaucoma [13].

The Molteno aqueous shunt (Molteno Ophthalmic Ltd., Dunedin, New Zealand) also has been recommended for primary surgical treatment in UG. Vuori reported a qualified success rate of 85% after 4-year follow-up. The IOP decreased continuously during the first year postoperatively,

and the medication was slowly tapered even up to 3 years postoperatively. Therefore, the author suggested postponing further surgical interventions during the first postoperative year after Molteno implantation in UG, even if the IOP is not controlled. Persistent hypotony was present in 6.66% of cases and corneal decompensation in 3.33% of cases [51].

In summary, GDDs are one of the preferred first-line surgeries in UG. The AGV, Molteno, and Baerveldt GDDs have been used with good success rates, but no studies have compared if nonvalved or valved GDDs are preferable in UG.

7.5.6. iStent. In a mixed series of secondary glaucoma cases including four cases with steroid-induced glaucoma, Buchacra et al. reported that the Glaukos iStent (Glaukos iStent, Glaukos Corporation, Laguna Hills, CA) is a safe and effective surgical option for secondary OAG, but they did not present isolated data for steroid glaucoma [52]. Good results were achieved with Glaukos trabecular bypass in one case of IOP elevation induced by steroid treatment after laser in situ keratomileusis [53].

After preliminary studies, the Glaukos iStent may be an attractive alternative for steroid-induced glaucoma, considering the microinvasive and reversibility characteristics of the procedure, although larger well-designed studies are needed to confirm this conclusion.

7.5.7. Trabectome Surgery. Shimizu et al. performed trabectome surgery in a subgroup of patients with UG and reported a success rate of 75%, but more details about the safety and efficacy of trabectome in UG were unavailable [12].

7.5.8. Goniotomy. The procedure has been suggested for refractory glaucoma associated with chronic childhood uveitis [54–56]. The largest series included 54 goniotomies in 40 eyes, with the predominant diagnosis of juvenile rheumatoid arthritis (mean age at surgery, 10.3 years). Overall surgical success was achieved in 72% of cases (complete success, 55%). Phakic eyes, fewer peripheral anterior synechiae, age younger than 10 years, and eyes with no previous surgery had significantly better outcomes. The most common postoperative complication was mild and transient hyphema (80%) [55].

Goniosurgery is low risk and effective for refractory glaucoma complicating chronic childhood uveitis. For some authors, it should be considered the surgical procedure of choice for this condition, although almost half of patients will need glaucoma treatment postoperatively. The surgical outcome is affected adversely by increased age, peripheral anterior synechiae, previous surgeries, and aphakia [55]. However, goniotomy requires considerable skill and experience and is best avoided by specialists who do not perform it regularly [3].

Randomized comparative studies are needed to determine the efficacy and safety of goniotomy compared with trabeculectomy or GDD surgery.

7.5.9. Cyclophotocoagulation. Cycloablative techniques can be used to decrease aqueous production by destruction of the ciliary body using transscleral or intraocular diode or Nd:YAG laser cyclophotocoagulation. Unfortunately, cycloablative procedures can exacerbate inflammation and lead to postoperative hypotony and phthisis bulbi. The rate of hypotony after cyclodiode laser in uveitis (19%) is higher than in any other secondary glaucomas [2].

Schlote et al. reported a series of 22 patients who underwent transscleral diode laser cyclophotocoagulation (TDLC) for UG or scleritis-associated glaucoma. The IOP was controlled in 77.3% of eyes, although 63.6% of cases needed more than one treatment with TDLC. The investigators did not observe reactivation of inflammation, persistent hypotony, or phthisis bulbi in any case [57]. Good results also were reported in a case of UG secondary to JIA treated with TDLC [58]. Although preliminary studies have reported encouraging results with TDLC in UG, it should be the last resort for refractory glaucoma in eyes with poor visual potential in which conventional drainage surgery has failed or is impossible because of the ocular anatomic characteristics.

7.5.10. Cataract and UG Surgery. Cataract is very common in patients with uveitis. The optimal sequence of surgery with concomitant cataract and UG is controversial. Cataract surgery can compromise the success of trabeculectomy [59], but combined glaucoma and cataract surgery increases the risk of postoperative inflammation and may be less successful than isolated filtering procedures [3, 14].

If combined glaucoma and cataract surgery is indicated, good control of the inflammation is mandatory preoperatively and postoperatively. The use of antimetabolites at the time of combined surgery reduces the proliferative response [60]. A meticulous and minimally invasive surgical procedure also can help increase the surgical success, but the evidence is insufficient to recommend a specific filtering surgery for combined procedures in this kind of patient.

It is also essential to be alert for detecting and treating postoperative complications such as hypotony, athalamia, and choroidal detachment. Stronger and longer postoperative steroid treatment usually is required. Intensification of anti-inflammatory treatment may be necessary in case of recurrent uveitis.

7.5.11. Iridotomy. Nd:YAG laser peripheral iridotomies are indicated in cases of iris bombé and angle closure secondary to posterior synechiae (Figure 8). In UG, Nd:YAG laser iridotomy has an increased incidence of failure (61% in some retrospective studies) [61]. Spencer et al. reported that the median survival of Nd:YAG peripheral iridotomy was 85 days, with most failures occurring within the first 20 days. Those investigators recommended multiple (at least two) or large iridotomies (Figure 9), aggressive treatment with topical steroids and cycloplegics, and close monitoring of patients with frequent early review. If the iridotomy closes, there should be early consideration for a surgical peripheral iridectomy [61].

Recurrent herpetic keratouveitis has been described after argon laser iridotomy [62] and after Nd:YAG laser peripheral iridotomy [63]. The causal relationship is difficult to establish because patients were being treated with topical corticosteroids and in one case with latanoprost, previously

FIGURE 8: Iris bombé and pupillary seclusion in a patient with UG. Peripheral iris burns after argon laser iridoplasty are seen.
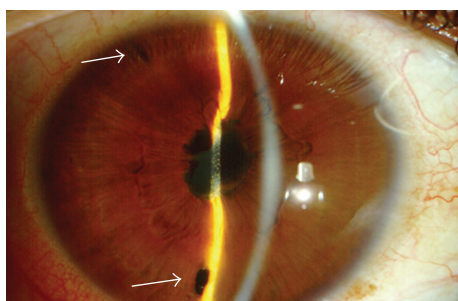

FIGURE 9: Iris bombé after two Nd:YAG laser iridotomies (arrows).

related to recurrent keratouveitis. Both cases resolved with oral acyclovir and discontinuation of latanoprost. Preventive treatment with oral acyclovir has been suggested if iridotomy is required in patients with UG associated with herpes virus.

Most studies that have reported the outcomes of trabeculectomy or GDD in uveitic eyes are not specific to uveitis-associated angle closure, and this warrants further investigation in more targeted studies. As in primary angle-closure glaucoma, phacoemulsification combined with goniosynechialysis may be an alternative in patients with uveitis with closed-angle glaucoma, although it is expected to be less successful when chronic peripheral anterior synechiae are present [47].

Other causes of secondary closed-angle glaucoma such as anterior ciliary body rotation, annular ciliary body detachment, or uveal effusion require specific surgical approaches.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

A Clinical Picture of the Visual Outcome in Adamantiades-Behçet's Disease

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Adamantiades-Behçet's disease is a multisystemic vasculitis with multiorgan involvement. Ocular disorders occur often in this syndrome typically in the form of a relapsing-remitting panuveitis and vasculitis and can lead to blindness as one of its most disabling complications if left untreated. There are known risk factors related with the worst visual prognosis, which require early and intensive treatment in order to obtain a rapid suppression of inflammation and to prevent future relapses. The management strategy to avoid vision loss and blindness currently involves the use of local and systemic drugs including steroids and immunosuppressive and biologic agents. This review aims to demonstrate how the introduction and the use of biologic agents improves the visual outcome of patients with Adamantiades-Behçet's disease.

1. Introduction

Adamantiades-Behçet's disease (ABD) is a chronic, multisystemic disorder characterized by recurrent inflammation that involves multiple organ systems throughout the body. It has a high prevalence along the ancient "Silk Road," but it is an important cause of morbidity throughout the world. The underlying pathology in ABD is a vasculitis that affects both the arteries and the veins in all organ systems. The involvement of major organs can cause permanent damage and severe complications that may be even life threatening [1]. Ocular involvement is present in around half of ABD patients with the percentage varying among 70% in young men with ABD and 30% in women and elderly patients [1–3]. Ocular manifestations usually manifest themselves within 5 years from the onset of the disease [2]. Further, bilateral involvement is frequent and is reported in 75–80% of ABD patients [2].

The ophthalmic findings described in ABD can involve either the anterior, posterior, or both segments of the eye and can be classified as suggested in the review by Ozyazgan et al. [4] as "reversible changes" or "irreversible changes." The reversible changes appear during the activation and completely disappear after the deactivation of disease; the irreversible changes develop slowly during the course of inflammation and do not disappear after remittance. The most sight-threatening complications often are consequences of both the reversible and the irreversible modifications to the anterior or the posterior segment of the eye. Complicated cataract, macular oedema, secondary glaucoma, epiretinal membrane, macular hole, and optic disc atrophy may cause vision loss and, if not treated, also blindness. The risk of blindness increases progressively reaching 25% at 10 years and remains constant thereafter [1]. Conventional treatment consists of prednisone, cyclosporine, azathioprine, and other immunosuppressive agents such as methotrexate and cyclophosphamide. Steroids are used usually for the rapid suppression of the inflammation but are quickly tapered to reduce the risks of secondary cataract and glaucoma. In patients with severe ocular involvement with vasculitis and relapses, immunosuppressive agents should be added to ameliorate the visual prognosis. Nonresponsive patients can also benefit from biologic agents. Interferon-alpha (INF-alpha), tumor necrosis factor-alpha (TNF-alpha) antagonists, and recently interleukin-1 (IL-1) blocking agents have been used with a significant improvement of visual acuity.

2. Steroid Treatment and Visual Outcome

In the early 1960s, the treatment of ocular manifestations of ABD was more dependent upon rheumatologist-prescribed corticosteroid therapy for extraocular manifestations of this disease, while corticosteroid monotherapy was the mainstay of treatment [5]. Currently if the inflammation is located predominantly in the anterior segment, topical treatment modalities are recommended together with mydriasis. Dexamethasone 0.1%, prednisolone 1%, and fluorometholone 0.1% have been employed topically or through subconjunctival injection (methylprednisolone acetate 20 mg) in severe anterior segment inflammation and for treating hypopyon [2]. Systemic steroid regime is necessary in case of posterior segment involvement. Initially, patients are treated with oral prednisone 1 to 2 mg/kg/daily for four days with gradual tapering of the dose according to the clinical signs [6], or with high-dose intravenous methylprednisolone [7]. Looking at a study of the National Eye Institute, comparing three decades of treatment [8], mean visual acuity was significantly worse in the 1960s than in the following decades, and accordingly the mean logarithm of the minimum angle of resolution (logMAR) score decreased with each decade: respectively, 0.91 logMAR in the 1960s, 0.82 logMAR in the 1980s, and 0.46 logMAR in the 1990s. This could be explained by the fact that the use of steroids as monotherapy fell significantly from the 1960s (96%) compared to the 1980s (8%) and the 1990s (16%) ($P < 0.001$). In the 1970s, it was reported that vision was lost after an average of 3.36 years from the onset of visual symptoms [9]. Mishima and associates found that more than 50% of the Japanese patients with ABD had a visual acuity of 0.1 decimal or less in 5 years [10].

3. Immunosuppressive Agents and Visual Outcome

3.1. Cyclosporine A. Cyclosporine A (CSA) is an 11-amino acid cyclic peptide. It is an alkylating agent that appears to affect preferentially immunocompetent T-lymphocytes [11]. CSA, in a dose of 5 mg/kg/day, was found to be effective in arresting the inflammatory activity in the eye of patients with Behçet's disease, resulting in a rapid improvement in visual acuity. The response rate to CSA in ABD patients varied between 80 and 91% [12–14]. In a first report published in 1987, visual acuity improved in 12 eyes, was unchanged in three eyes, and worsened in one eye of patients treated with CSA [12]. Ozyazgan in a single masked trial demonstrated that there was an initial improvement in visual acuity with 5 mg/kg/day of CSA versus monthly 1 gram of intravenous bolus of cyclophosphamide. However, this improvement disappeared during the follow-up time, and at the end of 24 months of observation, visual acuity remained approximately the same in both groups.

In the cyclophosphamide group, no significant change in visual acuity occurred; for this reason the author suggested CSA for short-term use [13].

According to a previous report, Masuda has demonstrated in a double masked trial that CSA 10 mg/kg per day was effective in treating ocular manifestation of Behçet's disease and he also observed that the efficacy did not weaken in long-term treatment [14, 15]. In these patients treated with CSA, caution is required regarding the potential for development of hypertension and renal failure.

3.2. Azathioprine. Azathioprine (AZA) is a purine nucleoside analogue. Immunologically, it decreases the number of peripheral T and B lymphocytes and further reduces mixed lymphocytes reactivity, interleukin-2 synthesis, and IgM production [11]. The azathioprine randomized controlled trial showed that AZA 2.5 mg/kg per day was superior to a placebo in preserving visual acuity in patients with established eye disease, but there was no evidence that AZA was useful in restoring compromised vision. It has been suggested also that AZA can protect against the development of second-eye disease [16]. According to the previous reports, another randomized controlled double blind study demonstrated that blindness and a 2-line drop in the visual acuity of the right eye occurred significantly more frequently among the patients originally allocated to the placebo group compared with patients who originally received AZA, despite posttrial treatment for patients in both groups when needed [17]. Tugal-Tutkun I described a cohort of 36 childhood-onset uveitides, treated with oral corticosteroids and immunosuppressive treatment if necessary. Twelve patients received AZA as initial line drugs; looking at final visual acuity, 50% of the patients showed a visual acuity of 0.6 decimals or better, but 22.7% had a visual acuity of 0.1 decimals and six patients (16.6%) were legally blind at the time of last visit [18]. In patients without eye involvement, the use of AZA seems to prevent the development of new eye disease even during follow-up and compared with the placebo group blindness occurred only in 13% of patients, which still remained a high percentage though [17]. Concomitant use of azathioprine and/or cyclosporine may improve the outcome.

3.3. Methotrexate. Methotrexate (MTX) is a folic acid analogue and an inhibitor of dihydrofolate reductase, the enzyme responsible for the conversion of dihydrofolate reductase in tetrahydrofolate reductase, essential for DNA replication [11]. Davatchi presented the results of a longitudinal study of up to 15 years, on 682 patients (5447 eye-years of follow-up) with Behçet's disease and ocular involvement. Patients were treated with MTX started at 7.5–15 mg/week and prednisolone was added at 0.5 mg/kg/daily and then adjusted as needed. At the end of the study, the visual acuity was improved in 46.5% of the eyes (20% recovered normal vision, 15,3% had useful vision and improved with the treatment, 2,4% were blind and recovered some vision, and 8,4% recovered at least one eye). In eyes with posterior uveitis, improvement was achieved in 75.4% of the eyes and in 53.7% of eyes with retinal vasculitis [19]. Nevertheless, a reduction of the visual acuity was still observed in 37.2% of eyes. Among them 4,5% became blind and 5.5% lost their useful vision. Obviously, the reduction of visual acuity was higher in eyes with posterior uveitis (11.1%) and retinal vasculitis (30.3%) [19]. It has been shown also that MTX is potent for anterior uveitis when used at a low dose of 7.5 to 25 mg weekly [20].

3.4. Cyclophosphamide. Cyclophosphamide is a nitrogen mustard-alkylating agent, the active metabolites of which are alkylate purines in DNA and RNA and result in cross-linking, aberrant base pairing, ring cleavage, and depurination [11]. In patients, it decreases the number of activated T lymphocytes, suppresses helper T lymphocytes functions, and decreases B-lymphocytes for months. Davatchi compared in a double blind controlled crossover study the short-term efficacy of pulse cyclophosphamide (PCP) plus prednisolone versus placebo over prednisolone alone. The mean visual acuity improved from 3.7 ± 3.2 to 4.9 ± 3.9 ($t = 3.309$, $P < 0.002$) in the PCP group and from 4.4 ± 3.6 to 4.5 ± 3.5 ($t = 0.317$, $P = 0.75$) in the placebo group. In the PCP group, VA improved in 57% of the eyes (95% CI: 44–60) and remained stable in 22% (95% CI: 11–33) but deteriorated in 21% (95% CI: 10–32). In the placebo group, 45% of the eyes improved (95% CI: 32–58), 14% remained stable (95% CI: 5–23), and 41% deteriorated (95% CI: 28–54). For this reason, the combination of PCP and prednisolone is superior compared to prednisolone alone in maintaining visual acuity [21]. Other parameters such as disease activity index improved more remarkably in the PCP group than in the placebo group, but differences were not statistically significant.

3.5. Biologic Agents. Looking at conventional treatment visual outcome, there are still high percentages (20–30%) of patients with a reduction of visual acuity and 10% of patients becoming blind from posterior eye involvement and uveitis complications.

In this scenario, biologic agents represent a valid alternative for patients nonresponsive to conventional treatments for achieving a stabilization of visual acuity and avoiding blindness. Interferon-alpha and TNF-alpha antagonists are the most frequently used biologic agents; recently, IL-1 blocking agents have been used with satisfactory results in terms of visual acuity maintenance.

Although the published literature consists of open and observational studies and while there are not yet available randomized controlled data on these drugs, the results available demonstrate a favourable response to biologic agents.

3.6. Interferon-Alpha. Interferons are a group of cytokines that include interferon-alpha-2a which is used to treat patients with severe ocular involvement and sight-threatening uveitis entities nonresponsive to conventional immunosuppressive agents. Although there is no controlled data, open and observational studies have shown the efficacy of interferon-alpha (IFN-alpha) in controlling uveitis attacks and reducing relapses [22–29]. However, there is no consensus about the ideal dose and duration of the treatment for ABD uveitis. For this reason, Onal investigated the long-term efficacy and safety of low-dose and dose-escalating therapy of IFN-alpha-2a in the treatment of uveitis in ABD.

This study included 37 patients receiving a daily dose of 3.0 million IU (MIU) subcutaneously for 14 days. Maintenance dose was achieved with 3.0 MIU 3 times per week given subcutaneously. The dosage was increased sequentially if uveitis relapses occurred. Total therapy duration was 24 months. Improvement in visual acuity was achieved in 41%

of patients with doubling of the visual angle associated with a decreased rate of uveitis' relapses [22]. Similar results with low dose of IFN-alpha-2a were obtained by Guedry that described stabilization or at least an improvement of visual acuity in 87.5% of eyes at two years of treatment [23]. In 2010, Sobaci presented the results of his prospective study; patients were treated with INF-alpha-2a 4.5 MIU 3 times per week for the first 3 months, followed by INF-alpha-2a 3 MIU for the next three months. Visual acuity improved in 28.3% of eyes and was maintained in 76.7% during the follow-up period [24].

Kötter treated patients with an initial higher dose of IFN-alpha-2a starting with 6 MIU subcutaneously daily for at least 14 days and reducing it until discontinuation. Visual acuity improved in 75.3% ($n = 55$) of eyes, remained stable in 22% ($n = 16$) of eyes, and worsened in 2.7% ($n = 2$) of eyes. The increase of visual acuity for the right eyes was 0.33, and for the left eyes 0.36 logMAR. In 7 eyes, the final visual acuity was inferior to 0.1 logMAR but remained unchanged from the beginning [25, 26]. Kötter obtained a response rate of 92% in a relatively short time of 2 to 4 weeks, and discontinuation of the treatment was possible in 40% of patients. After 5 years of follow-up in patients treated with IFN 2-alpha, 67% of eyes obtained an increase of two lines or more in visual acuity [27]. Similar results were obtained by Deuter with an improvement or at least maintenance of visual acuity in 94.8% of eyes treated with a dose of 6 MIU subcutaneously daily for at least 14 days that is then tapered to a maintenance dosage of 3 million IU twice per week and finally discontinued, if possible. Median visual acuity was 0.30 logMAR at the beginning and improved to 0.07 logMAR at the end of follow-up period. Only 12.5% of eyes had a final visual acuity of 1.0 logMAR or less, due to preexisting irreversible ocular damage [28]. Tugal-Tutkun I. observed in her retrospective analysis between September 2001 and May 2005 similar results in terms of visual acuity recovery. As a matter of fact, the best visual acuity was achieved after a median of 4 months of IFN-alpha therapy and was found to be 0.28 ± 0.34 logMAR units in the right eye and 0.45 ± 0.56 logMAR units in the left eye. The best level of visual acuity achieved by IFN-alpha therapy was preserved throughout follow-up in 95% patients [29]. In her series of patients, only 36.4% of patients remained relapse-free, and complete remission was achieved only in 20% of patients compared with the data reported by Kötter where 82% of patients were relapse-free and 40% of patients achieved complete remission [26]. Patients treated with IFN-alpha often experienced a flu-like syndrome, weight loss, and often depression. For these reasons, the use of this drug is quite limited.

The main advantage of IFN-alpha treatment seems to be the possibility of discontinuation of treatment without relapse in at least 50% of patients and the preservation of visual acuity in almost 90% of patients compared with standard immunosuppressive therapy and even when compared with anti-TNF [30]. However, as assessed by Bodaghi and colleagues [31], the efficiency of IFN-alpha in sight-threatening uveitis seems to act more to suspend rather than to cure and for this reason it may be proposed as a second-line therapy after failure of conventional immunosuppressive treatments

in nonresponsive patients. In this way, nonresponsive patients could avoid sight-threatening complications and blindness.

3.7. TNF-Alpha Antagonists.

Tumor necrosis factor-alpha is a pleiotropic cytokine that has been shown to be elevated in patients with Behçet's disease and other autoimmune diseases. Numerous cells, also lymphocytes, produce TNF-alpha and their targets are two receptors known as p55 (TNF-R1) and p75 (TNF-R2). When TNF-alpha is produced during inflammation, it activates T-cells and macrophages and determines upregulation and expression of endothelial adhesion molecules and proinflammatory cytokines [32].

The first available molecule targeting TNF-alpha was a chimeric IgG monoclonal antibody infliximab (Remicade, Schering-Plough Pharma Inc.); later etanercept (Enbrel, White Pharmaceuticals Inc.), a p75 TNF-alpha receptor fusion protein, was developed. The last anti-TNF-alpha agent produced was adalimumab (Humira, Abbott Pharmaceuticals Inc.), a recombinant human IgG1 monoclonal antibody [32].

3.8. Infliximab.

Infliximab infusion, used at a dose of 5 mg/kg every 6–8 weeks, is demonstrated to be effective in reducing ocular relapses and maintaining visual acuity [33–41]. Sfikakis described in a case series the effect of infliximab in 5 patients treated with standard immunosuppressive therapies. He observed a rapid and effective suppression of the ocular inflammation in these patients after seven days, confirmed also by an improvement and stabilization in visual acuity in all cases during the follow-up time [33]. These preliminary observations were confirmed also by Ohno and colleagues that described a significant reduction in the frequency of uveitis attacks during the efficacy-evaluation period of their study, and an improvement in visual acuity was noted in eyes in which uveitis remained in remission [34]. The visual acuity improvement obtained with the infusion of the anti-TNF-alpha agents could reach the sixth line of visual acuity as reported by Bodaghi and colleagues. This was a retrospective study of 12 patients (21 eyes) followed for a mean of 17.4 months (range: 8–30) [35]. Tugal-Tutkun described the long-term results of infliximab infusions of 5 mg/kg administered at weeks 0, 2, 6, and 14 and the patients were observed since enrolment for 54 weeks. The visual acuity improved significantly during the infusion period (weeks 0–22) but then decreased during the observational period (23–54 weeks). This was explained by the authors both for the frequency of uveitis attacks and for the need of corticosteroids treatment after the initial beneficial effects of infliximab infusions. For these reasons, treatment with anti-TNF-alpha agents should be continued in order to maintain the beneficial effects in cases of nonresponsive uveitis [36]. In a review paper in April 2007, Sfikakis et al. suggested that infliximab was the best available treatment in acute sight-threatening ocular inflammation in patients affected by Behçet's disease and should be used alone or as an add-on therapy in selected cases [37].

Compared with corticosteroids, high-dose methylprednisolone intravenously (1 g/day for 3 days), or intravitreal triamcinolone acetonide (4 mg) at the attack's onset, infliximab was equally effective in improving visual acuity from baseline but with less complications such as cataract or glaucoma and with a faster effect [38]. Yamada retrospectively compared the efficacy of infliximab versus CSA and described an improvement of visual acuity in 97% of eyes treated with infliximab versus the 93% of patients treated with CSA; the difference was not statistically significant. Nevertheless the study demonstrated that infliximab was more effective than CSA in the first 6 months [39]. Beside the rapid effect of action, a multicenter prospective study showed that uveoretinitis improved in 92% accompanied by visual acuity that improved from 0.736 logMAR at the first infliximab infusion to 0.616 logMAR after 1 year and remained unchanged in the other patients. Infliximab also decreased the frequency of recurrence and 44% of patients were attack-free after twelve months [40]. In addition, other studies demonstrated the efficacy of infliximab in improving visual acuity by reducing optic disc neovascularization and background retinal vascular leakage, as demonstrated with fluorescein angiography. The best corrected visual acuity was maintained or improved in 92.8% of eyes at 12 months, and in 80% of eyes at 24 months [41].

Finally we can conclude that almost all patients treated with infliximab, alone or as an add-on therapy, were nonresponsive to conventional treatments and achieved a fast suppression of the acute ocular inflammation. Less data is available on long-term outcomes, since it is also well known that the development of human antichimera antibodies (HACAs) has been implicated in the observed decline in therapeutic response to infliximab. The use of infliximab, as other anti-TNF-alpha agents, is not totally safe; Neri et al. in 2004 reported about the reactivation of tuberculosis under infliximab in a patient with ABD; remember that the endemic areas for ABD are also endemic for tuberculosis [32].

3.9. Etanercept.

Etanercept is a recombinant human p75 TNF-alpha receptor artificial fusion protein; in a double-blind, placebo-controlled study of 40 male patients with ABD, Melikoglu et al. [42] reported that etanercept (25 mg twice/week, for 4 weeks) was effective in suppressing most mucocutaneous lesions in Behçet's patients. Etanercept was also given to treat children with ABD-associated uveitis at a dose of 0.4–0.5 mg/kg administered twice weekly as subcutaneous injections. At study entry, 39/42 eyes had active uveitis with normal visual acuity in 59% of eyes, impaired visual acuity in 10% of the eyes, and legal blindness in 31% of the eyes. Legal blindness was bilateral in 14% of the eyes. Ten of the 21 patients (48%), with 19 affected eyes, had normal best corrected visual acuity in both eyes. The causes of legal blindness were cataract, cystoid macular edema, and retinal detachment. Patients were treated with etanercept or infliximab, and at the end of the study best corrected visual acuity improved in 5/11 patients (7/16 eyes, 43%). Four patients (5/16 eyes, 31%) improved as a result of cataract surgery. In two eyes (two patients, both on infliximab), improvement of visual acuity from 20/400 to 20/70 and from 20/400 to 20/20, respectively, was unrelated to cataract surgery. Vision decreased in 2/18 patients (2/27 eyes, 7%) from 20/100 to 20/200 and from

20/60 to 20/200, respectively, both on etanercept (13% of etanercept treated eyes) due to uncontrolled inflammation, and remained unchanged in 16 patients (25 eyes) during the study (difference between etanercept and infliximab treated group, P = 0.48). The difference in improvement of visual acuity between etanercept and infliximab treated patients was not statistically significant; however, the authors concluded at the end of the paper that infliximab seemed superior to etanercept not only because of decreasing the number of concomitant medications required to control the uveitis, but also due to a lower rate of new-onset glaucoma and cataract [43].

In conclusion, we can consider the soluble TNF receptor as an alternative for nonresponsive patients to the other anti-TNF-alpha agents.

3.10. Adalimumab. Adalimumab is a recombinant human IgG1 monoclonal antibody targeting the TNF-α; it also binds soluble and the membrane-bound form of TNF-α [32]. Adalimumab, as previous biologic agents, has been used to treat nonresponsive ocular Behçet syndrome as it has some advantages: first of all, the self-administration and then a lower risk of anti-drug antibody formation. In 2007, there was a first case series describing the effect of adalimumab in sight-threatening uveitis; in all three patients treated, the visual acuity remained stable; and in two eyes of two different patients, an improvement also has been observed [44]. In 2010, Bawazeer et al. described the improvement of visual acuity and the corticosteroid and immunosuppressive sparing effect of adalimumab in 11 patients with ocular Behçet's disease. Adalimumab was administered subcutaneously at a dose of 40 mg every 2 weeks; of the 21 eyes, 17 had an improvement of visual acuity by 4.3 (range: 0–8) lines; 4 eyes of 3 patients did not improve in visual acuity [45]. Takase et al. reported their experience describing the successful switching from infliximab to adalimumab in ABD patients. Changing to adalimumab induced clinical remission again, thus suggesting that adalimumab can be an effective alternative to infliximab for patients having a hypersensitivity to this drug [46]. The application of adalimumab can also be hypothesized beside nonresponsive uveitis in panuveitis and pediatric uveitis [47, 48]. Unfortunately, adalimumab treatment is not totally safe, as multiple side effects have been reported recently such as reactivation of latent tuberculosis, endogenous endophthalmitis, retrobulbar neuritis, bilateral optic neuropathy, precipitation of systemic lupus erythematosus, secondary malignancies, lymphoma, and cardiac failures [32].

Although the results provided in ABD can be promising, no controlled trial is available and it is not possible to compare the characteristics of different anti-TNF-α, so that they are limited to nonresponsive cases of the disease.

3.11. IL-1 Blocking Agents. IL-1-blocking agents have started to be used recently in sight-threatening and nonresponsive cases. A small study on 7 patients treated with a single infusion of XOMA 052 (gevokizumab) 0.3 mg/kg demonstrated a rapid and meaningful improvement of the visual acuity starting from day 1, except in two patients; resolution of the ocular inflammation was observed in 5 patients at day 28; and all these 5 patients remained attack-free for a median of 49 days. XOMA 052 was well tolerated and no drug-related adverse events were observed [49]. Another recombinant form of human IL-1 receptor antagonist is anakinra, which is approved for the treatment of Rheumatoid Arthritis in combination with methotrexate. Emmi et al. described the use of this drug in a patient with ABD and serious ocular involvement. The patient was treated with anakinra at a dose of 100 mg/day and after three months from the beginning of the treatment, ocular inflammation had disappeared and visual acuity from 20/50 in RE and 20/32 in LE was restored to 20/20 in both eyes [50]. Ugurlu published the favourable results obtained with canakinumab, another IL-1 receptor antagonist, treating a 16-year-old female with ABD and ocular involvement nonresponsive to other immunosuppressives. The young patient treated with a single dose of 150 mg of canakinumab obtained a resolution of the ocular inflammation in the right eye with a visual acuity of 0.4. The left eye was not evaluated because of the presence of a complicated cataract. The patient remained free of attacks during the 8 weeks of follow-up. Interestingly, this patient was nonresponsive to anakinra but obtained a remission of the ocular inflammation with canakinumab. Both agents are IL-1 blockers but may act in different ways and have different half-lives. While anakinra blocks both IL-1α and IL-1β, canakinumab specifically targets IL-1β. It was also noticed that, after the infusion of IL-1 blocking agents, the circulating white blood cells developed a less inflammatory phenotype compared with baseline [51].

Although these studies have limitations resulting from their design and the small number of patients, the results do support additional studies to evaluate the role of IL-1 blocking agents for the treatment of uveitis and retinal vasculitis and for nonocular ABD manifestations as well as other types of noninfectious inflammatory uveitis.

4. Changes in the Course of the Disease Over Time

ABD is a rare disease but has a relatively high prevalence along the ancient "Silk Road." Since 1950 until the present, the medical treatments options have been evolving to obtain a better control of the inflammation, a reduction of the sight-threatening complications, and a better final visual outcome.

The first drugs introduced to manage this syndrome were steroids, followed then by immunosuppressive agents (alkylating agents, nucleoside analogues, folic acid analogues, and nitrogen mustard-alkylating agents) and finally biologic agents (INF-α and TNF-α antagonists and IL-1 blocking agents).

In a large series from Turkey, including patients seen between 1980 and 1998, initial visual acuity was 0.1 or less in 647/1567 eyes (41.2%) [1]. A recent multicenter Turkish study conducted in 2004 showed that a lower percentage (21.7%) of eyes with Behçet's uveitis had initial visual acuity of 0.1 or less, suggesting a trend toward a milder disease [52]. Cingu et al., who compared patients who presented

in the period 2000–2004 with those who had presented a decade earlier, have confirmed this tendency also. The authors found a better initial, potential, and 3-year visual acuity in patients from the 2000s period and none of the patients became legally blind during the 3 years of follow-up in this group. This may be explained by a milder disease in this period and/or more frequent use of immunomodulatory treatment before referral [53]. Disease severity and visual outcomes of patients with ABD uveitis over the decades have been changing significantly also in other countries. In Japan, Yoshida A. et al. described between the 1980s and 1990s an increase in the percentage of eyes with good visual acuity (≥20/30) and a significant reduction in eyes with poor visual acuity (≤20/200); and they justified this change as a result of improvements in environmental factors (e.g., putative microorganisms, lifestyles, and hygienic situations) [54]. Similar to trends reported from Japan, Khairallah and associates found that Tunisian patients with ABD uveitis who presented after 2001 had a better final visual acuity than those who presented before 2001. Only 3.4% of patients suffered from blindness and 12.5% suffered from unilateral blindness. The authors justify this trend due to the use of immunomodulatory drugs as first-line therapy after 2001 but a milder disease could not be excluded because initial visual acuity was also better in the more recent study period [55]. The study by Kump and associates in the United States has shown a similar trend in a nonendemic population; mean visual acuity in the 1990s group was significantly better than in the previous decades ($P < 0.001$ for the 1960s group and $P = 0.019$ for the 1980s group) and it seems that newer immunomodulating and biologic agents may offer an improved prognosis in patients with ABD [8].

In a recent paper, Taylor reported improved visual prognosis in patients with ABD who presented at two referral centres in England and in Australia between 2000 and 2010. They estimated the risk of visual loss to be 39% and the risk of severe visual loss to be 24% at 10 years. Male sex, unilateral disease, and left eye involvement increased the risks of severe visual loss at 5 and 10 years in this series of patients. Patients who were treated with anti-TNF-α were less likely to have severe visual loss, respectively, at 5 and 10 years. Taken together, these results suggest that adequate immunosuppression can reduce the risk of severe visual loss in patients with ocular ABD but that azathioprine is not effective enough to achieve this goal [56]. Krause and associates in Berlin analyzed retrospectively the data of 140 patients with ABD and found that the risk of losing useful vision was 21% [57], less than 75% of Benezra and Cohen's study [6] and 72% less than described in another study published in 1995 in Germany [58]. Mean and median Snellen visual acuities were reported to be 0.3 and 0.6 at baseline and 0.4 and 0.8 after 3 years, respectively [57]. In Switzerland, another nonendemic country, mean Snellen visual acuity was 0.74 at baseline and 0.79 after a mean follow-up of 5.7 years [59]. ABD appears to be less severe in this area, but in presence of ocular clinical and angiographic involvement, the authors decided to treat patients even if the visual acuity was good; and the final visual outcome justified this aggressive treatment. In a recent study from China in a single center,

which included 437 Behçet's disease patients seen between 1995 and 2006, final visual acuity was less than 0.1 in 20.4% of eyes after a median follow-up of 4 years [60].

Muhaya and associates compared Behçet patients seen in Japan and the United Kingdom in a cross-sectional observational study conducted simultaneously at two centres. The duration of ocular disease was around 7 years in both cohorts. Even if the treatment schedules were very different and Japanese patients had more active disease, the visual results were comparable. Visual acuity was worse than 6/60 in 31% of patients in Japan and 21% of patients in the United Kingdom; oral steroids and azathioprine are widely used in London; on the contrary, colchicine was used instead in Japan. This cross-sectional study reveals differences in the clinical features and management of ocular disease in ABD patients, but this had no significant effect on the visual outcome at seven years [61].

5. Conclusion

In conclusion, in order to restore and maintain good visual acuity in ABD with ocular involvement, the importance of the early use of combined immunomodulatory regimens and use of biologic agents seems clear. This is much more effective in nonresponsive cases where the reduced severity and number of uveitis attacks can prevent early visual loss [62]. The evidence-based European League Against Rheumatism (EULAR) recommendations for the management of ABD, published in 2008 and still valid, suggest for posterior eye involvement a treatment regimen that includes systemic steroids and azathioprine [63]. Corticosteroids rapidly suppress the inflammation but potential side effects, including cataracts and glaucoma, cause concern. Azathioprine is widely accepted as the initial agent for ocular involvement of ABD. The EULAR committee discussed also a possible role of azathioprine as a prophylactic treatment in patients with ABD at high risk of developing eye involvement; but it was decided that more prospective data were needed.

In case of severe eye involvement, identified as a drop of two lines or more in visual acuity on a 10/10 scale and/or retinal disease (retinal vasculitis or macular involvement), another immunosuppressive needs to be added. Particularly, it is recommended that either cyclosporine A or TNF-alpha antagonists such as infliximab may be used in combination with azathioprine and corticosteroids; alternatively, INF-alpha with or without corticosteroids could be used.

Due caution for hypertension and nephrotoxicity is important when using cyclosporine A; instead reactivation of tuberculosis has to be considered when using TNF-alpha antagonists. IFN-alpha, alone or in combination with corticosteroids, appears to be a second choice in eye disease due to financial and safety concerns, mainly depression and cytopenias [63]. Recently, IL-1 blocking agents have been used in sight-threatening and nonresponsive cases with preliminary good results [49–51].

Another important issue to consider when using immunomodulatory drugs and biologic agents is the switching effect in the event of failure of desired response to one biologic therapy [64]; the efficacy of changing from a biologic

agent to another one suggests changing the drugs either for poor response (primary failure) or for progressive decrease of efficacy because of the production of patient antibody reaction to the nonhuman part of the chimeric molecule used for treatment (secondary failure).

Conflict of Interests

None of the authors has conflict of interests regarding the submission.

Authors' Contribution

Michele Figus and Chiara Posarelli contributed equally to this paper.

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Review Article

Ocular Manifestations and Therapeutic Options in Patients with Familial Amyloid Polyneuropathy: A Systematic Review

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Purpose. This paper aims to review the morphological and functional characteristics of patients affected by familial amyloid polyneuropathy (FAP), with greater focus on type I and its progression after liver transplantation. We also analyse therapeutic options for the ophthalmic manifestations. *Methods*. The literature from 2002 through 2015 was reviewed, with a total of 45 articles studied, using the key terms related to amyloidosis and its therapeutic approaches. Information was collated, evaluated, critically assessed, and then summarised in its present form. *Pathophysiology and Treatment*. FAP results from mutation of the transthyretin gene, with Val30Met being the most frequent substitution. The symptoms are those typical of a sensorimotor autonomic neuropathy and can be halted with liver transplantation. Nowadays there are new medical therapies that delay the progression of the systemic neuropathy. However, there are still no options to avoid ocular disease. *Conclusion*. The main ocular manifestations in patients with FAP type I are amyloid deposition in the vitreous, dry eye, and secondary glaucoma. Despite liver transplantation, eye synthesis of amyloid persists and is associated with progressive ocular manifestations, which require continued ophthalmologic follow-up. New therapeutic strategies are therefore needed, particularly to target the ocular synthesis of the abnormal protein.

1. Introduction

Amyloidosis is a group of diseases characterised by deposition of amyloid, consisting of clumps of insoluble proteins at the level of the peripheral or central nervous system [1]. Its phenotype varies depending on the affected organs [2].

This condition may be primary in origin, very often hereditary and leading to familial amyloid polyneuropathy (FAP), or secondary to chronic inflammatory diseases and causing a sporadic form of the disease, senile systemic amyloidosis (SSA) [3, 4]. The latter is age related and has a milder clinical picture, mainly affecting the heart [5, 6].

FAP is a progressive autosomal dominant neurodegenerative disease, characterised by the accumulation of amyloid in the peripheral nerves and other organs, including the eye. It shows high phenotypic and genotypic heterogeneity, with incomplete penetrance and variable age of onset [7, 8].

It can be classified into three main types, according to the amyloid-forming precursor protein. The most common is transthyretin (TTR), and the others are apolipoprotein A-1 (ApoA1 ratio) and gelsolin (AGel) [9].

FAP originates from mutations of the TTR gene [10]. Over 100 different mutations have been described [1, 9]. The substitution of valine for methionine at position 30 of the TTR gene (Val30Met) is the commonest and the most studied mutation worldwide [11]. This mutation is responsible for the high prevalence of the disease in endemic areas, particularly the north of Portugal, Sweden, and Japan [6].

FAP can thus be classified according to its clinical characteristics and geographical origin [4]:

(i) Type I/Portuguese Type/ATTR: it is the most common type and primarily affects the lower limbs, with severe autonomic dysfunction. Portugal, Sweden, and Japan are endemic areas.

(ii) Type II/ATTR: polyneuropathy starts in the upper limbs, with mild autonomic dysfunction; it is common in families in Switzerland and Germany.

(iii) Type III/ApoA1: polyneuropathy, renal failure, and cranial neuropathy are also characteristic.

(iv) Type IV/Type AGel: lattice corneal dystrophy type II is characteristic; it is more common in Finnish, Irish, American, and Japanese families; apart from polyneuropathy it is characterised by cutaneous hyperextensibility.

FAP has also been divided into three stages according to the progression of neuropathy [2]:

(i) Stage I: there is impairment of the lower limbs, without difficulty in walking.

(ii) Stage II (after 5-6 years): there is impairment of the upper limbs and there is need for aid in walking.

(iii) Stage III (after 10 years): there is total dependence.

2. Methods

We performed a systematic review of English- and Portuguese-language articles, restricted to studies published from 2002 through 2015, with a total of 45 articles related to ocular manifestations in FAP and the therapeutic options. We used terms such as amyloidosis, transthyretin, familial amyloid polyneuropathy, clinical trial, ocular manifestations, diflunisal, tafamidis, liver transplantation, small interfering RNA, antisense oligonucleotides, pharmaceutical company names, and other related terms, alone and in various combinations, as keywords. Incidence rates and availability of therapeutic approaches and their relative risks were assessed.

The materials were collated, evaluated, critically assessed, and then summarised in their present form.

3. Structure and Function of TTR

TTR is a protein circulating in the blood at a concentration of 20–40 mg/dL, forming a stable tetramer. It carries retinol and, in a lesser amount, thyroxine (T4) [1]. If not attached to TTR, retinol is filtered by the kidneys and excreted in urine [12]. The TTR gene is located on chromosome 18 (18q12.1) [6, 13]. When mutated, its tetramer conformation turns into monomers that aggregate and form amyloid deposits [1, 2]. TTR is also present in the cerebrospinal fluid and aqueous humour [5]. About 90% [11, 14] is synthesised and secreted by the liver and a small portion (<2%) may be synthesised by the brain's choroid plexus, the retinal pigment epithelium (RPE) [6, 11], and the small intestine [15].

4. Pathogenesis

The Val30Met substitution is the most frequent, and it is the only variant found in Portugal, Brazil, and Sweden [2]. So far, over 120 mutations in the TTR gene have been described [5]. Several in vitro studies have demonstrated that the TTR tetramer dissociation and consequent formation of amyloid fibrils are necessary processes to cause amyloidosis [6].

However, other mutations have been described, associated with milder phenotypes, such as Arg104His/Val30Met and Thr119Met/Val30Met, as well as mutations associated with more aggressive phenotypes, such as Leu55Pro [2]. TTR

Val122Ile mutation is the most common pathogenic variant in the United States [9].

It was found that, in patients with Val30Met mutation, the circulating monomers are mostly wild-type; that is, they do not result from the mutation and are identical to those produced in senile systemic amyloidosis. This suggests that the mutant molecules are more unstable than the wild-type ones and therefore more prone to aggregation. However, the fact that circulating monomers are mostly wild-type could also simply result from the faster elimination of the mutant molecules [6].

Some authors suggest that the loss of peripheral nerve fibres that occurs in FAP, resulting in local ischaemia, is caused by endoneurial amyloid deposits [15]. Another possible mechanism to explain the toxicity of amyloid deposits is direct toxicity to cells [2].

It has been suggested that plasma TTR does not cross the blood-retinal barrier [14], despite evidence of progression of ocular manifestations, after liver transplantation. This reaffirms the belief that intraocular amyloid fibrils are not synthesised by the liver but locally at the RPE [11]. It is surprising, however, that the retinal deposits are mainly located in the inner layers rather than the layers closer to the RPE [11].

Other studies have suggested that amyloid deposits in the anterior chamber of the eye are produced by the pigmented ciliary epithelium, while vitreous deposition originates from the RPE [16].

5. History

FAP associated with TTR (TTR-FAP) was described in Portugal in 1939 and published in 1952 by Corinho Andrade, a Portuguese neurologist who studied a population of 74 patients from Póvoa de Varzim, a city in the north of Portugal [17, 18].

New cases were identified in Japan in 1968 and Sweden in 1976, and the disease can now be found worldwide [2].

The first TTR mutation causing FAP was described in 1985 on chromosome 18q11.2-q [15].

Liver transplantation is currently the only therapy that can halt the natural course of the disease, although there are other medical alternatives that may delay its progression. The first liver transplant for TTR-FAP was performed in 1990 on a male Swedish patient with type I FAP [19].

In 1995, in Coimbra, Portugal, Linhares Furtado performed the first liver transplant from a donor with FAP to a patient with metastatic liver disease. This type of transplantation has been known since then as sequential or "domino" transplantation [20]. Nowadays, livers from patients with FAP are even transplanted to patients with cirrhosis or carcinoma [14].

6. Epidemiology

TTR-FAP is considered a rare disease, given its low incidence of <1/200; the prevalence of the genetic mutation is 1 in 1 million [21], with an incomplete penetrance [15].

Despite that fact that it occurs throughout Portugal, Póvoa de Varzim and Vila do Conde are the cities which have the largest clusters of people with TTR-FAP V30M [16], with a prevalence of 1/538 [6, 22] to 1/1000 people and more than 500 families diagnosed [2].

About half of all cases of TTR-FAP do not have a family history and are designated as sporadic cases [2].

Families originating from endemic areas of Portugal and Japan usually have earlier onset of the disease with higher penetrance [5].

Women have a later onset of the disease than men (33.7 + 5.8 versus 29 + 6.4) [23].

In the US Caucasian population, the prevalence of Val30Met mutation in FAP patients is about 1 in 100,000. In Sweden, the frequency of heterozygosity is about 1.5%, with very low penetrance.

In the Afro-American, West African, and Hispanic populations, it was observed that the most frequent mutation is Val122Ile, with a prevalence of 3.0–3.9%, 5.0%, and 0.44%, respectively. This mutation's largest clinical expression is hypertrophic restrictive cardiomyopathy [2, 6].

7. Diagnosis

The lag time between the onset of symptoms and FAP diagnosis is usually 2 to 6 years [24]. FAP requires a biopsy and pathological exam to demonstrate amyloid deposits. Nerves, heart, kidney, colorectal mucosa (sensitivity 70–80%), abdominal fat aspirate, or salivary glands can be biopsied [21]. Tissues are stained with hematoxylin and eosin to reveal homogeneous eosinophil extracellular areas [2]. Because of its beta-pleated configuration, the amyloid substance stains with Congo red and has green birefringence, when viewed under polarised light [6]. Amyloid deposits are also stained by thioflavin s. Under specular microscopy it is possible to see the unbranched, thick parallel margins of the amyloid fibrils [2].

For a faster and more reliable diagnosis, genetic testing can be done to check for the TTR Val30Met mutation [21].

To rule out the presence of eye disease, the initial approach should include a full ophthalmological examination, including measurement of visual acuity, biomicroscopy with pupil and anterior chamber examinations, fundoscopic exam, and analysis of visual fields [2].

8. Clinical Manifestations

The clinical manifestations of FAP are highly variable. The homozygous phenotype resembles the heterozygote one in Val30Met mutation patients and is also indistinguishable from amyloidosis acquired by deposition of immunoglobulin light chains [25]. The average age for the onset of symptoms is around 33 but can vary from age 17 to 78. Approximately 80% of cases occur before age 40 and 85% of patients have a positive family history [22].

Initial symptoms are typically autonomic sensorimotor neuropathy, including paraesthesia, decreased thermal and pain sensitivity in the extremities and in the cornea [8],

afferent ataxia, and autonomic dysfunction [1]. Neuropathy is usually symmetrical with focal distribution and centripetal progression [2, 16].

Extra central nervous system manifestations are the result of amyloid deposits in organs such as the eyes, heart, kidneys, and gastrointestinal system [6]. About ten years after FAP diagnosis and without treatment, the patient is at the final stage of the disease, suffering flaccid paralysis of the limbs, multiorgan dysfunction, and autonomic dysregulation [1].

Ocular manifestations are present in 10% of patients with TTR-FAP [9] and usually occur later during the course of the disease [22]. These symptoms correlate with neither the systemic symptoms nor the duration of the disease [11].

Kawaji et al. [26] described the case of a patient with ATTR Val30Met FAP without systemic manifestations and whose first and only manifestation was the amyloid deposit in the vitreous [26].

The main ocular manifestations present in FAP patients include [4, 7, 14]

  (i) vitreous opacities;

 (ii) chronic open-angle glaucoma (COAG);

(iii) abnormal conjunctival vessels (ACVs);

 (iv) keratoconjunctivitis sicca (KCS);

  (v) loss of corneal sensitivity and neurotrophic corneal ulcers;

 (vi) anterior capsule opacity of the lens;

(vii) retinal vascular changes;

(viii) pupillary light-near dissociation;

 (ix) irregular pupil;

  (x) optic neuropathy.

The amyloid deposition in the vitreous, with subsequent gradual decrease in visual acuity [2, 27], is almost pathognomonic of hereditary amyloidosis by mutation of the TTR gene, occurring either during the natural course of the disease or after hepatic transplantation [25]. This situation is more prevalent and occurs earlier in patients with Tir114Cis (100%) and Lys 54 mutations than in patients with variant Val30Met (24%) [28].

Figure 1 shows the eye of a patient with FAP I 15 years after liver transplantation. We can see the vitreous deposits adhering to the posterior lens capsule, which is referred to as *pseudopodia lentis* [4].

In these patients, COAG is the leading cause of irreversible blindness [29].

The pathophysiological mechanisms responsible for the elevation of intraocular pressure (IOP) include perivascular amyloid deposition in conjunctival and episcleral tissues, intratrabecular deposition, and deposition of amyloid on the pupillary edge, which may precede glaucoma by months or years [4].

In patients with glaucoma, erythropoietin (EPO) is increased in the aqueous humor, exerting a protective effect on the photoreceptors, RPE, and ganglion cells. But the same is not seen in patients with glaucoma and FAP [29]. Therefore,
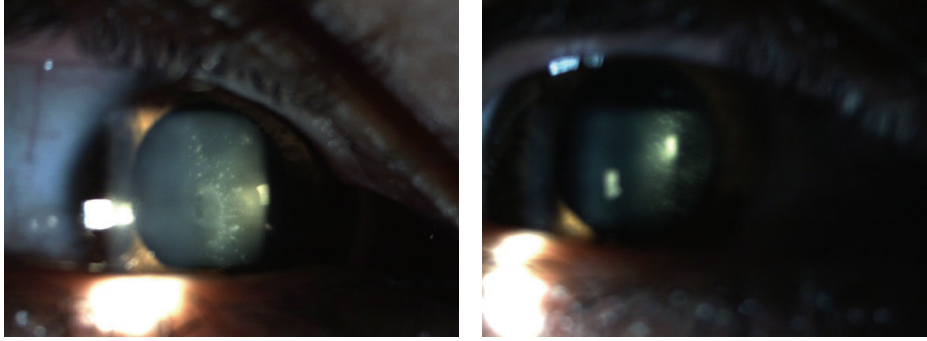
FIGURE 1: Man aged 42 underwent liver transplantation at age 27. Visual acuity decreased 3 years after pars plana posterior vitrectomy in 2005. There are amyloid deposits in the anterior vitreous, adhering to the lens posterior capsule [4].
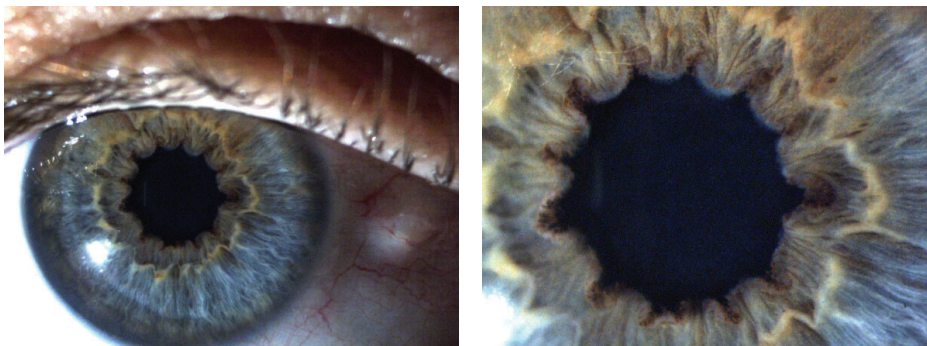

FIGURE 2: Multiple indentations of the pupillary edge and amyloid deposits in a 43-year-old patient with FAP 1, submitted to liver transplantation about 9 years ago [4].

in patients with FAP, substances with neuroprotective effect are scarce, which leads to the need for more aggressive treatments to preserve vision.

ACVs, described as red dots and segmental and fusiform dilatation of conjunctival vessels, afflict almost all patients during the disease. These changes result from liver synthesis of TTR, not from intraocular production, and consequently there is no progression after liver transplant, as expected [4].

Dry eye in FAP may be due to either autonomic neuropathy or amyloid deposition in the lacrimal gland [16], contributing to neurotrophic keratopathy and cornea perforation, which has been described in some cases [8]. Amyloid deposition in the cornea progressively lowers its sensitivity and damages the epithelium and stroma. Both situations contribute to the pathophysiology of dry eye, corneal epithelial injury, and parakeratosis [8].

Low or absent corneal sensitivity, spontaneous epithelial breakdown, and impairment of corneal healing characterise neurotrophic keratopathy (NK), a degenerative corneal disease that can threaten sight. Familial corneal hypoesthesia manifests itself by decreased corneal sensation, reflex tearing, blinking, and foreign body sensation [30].

Dosso and Rungger-Brändle [8] reported the case of a patient with FAP with bilateral corneal perforation who underwent bilateral penetrating keratoplasty (PK). Amyloid deposition in the cornea has a direct toxic effect by changing its sensory innervation and damaging the epithelium and stroma. Corneal amyloid deposition was also found after PK.

Intraocular production of mutated TTR leads to amyloid deposition in the anterior lens capsule that is often asymmetrical between the two eyes. This condition may impair spatial contrast sensitivity at all frequencies [18] and lead to early presbyopia in patients with FAP [16]. This is related on the one hand to a loss of lens elasticity and on the other to autonomic neuropathy, which affects the ciliary muscle accommodation [31].

Beirão et al. [31] found that 35 patients with FAP presented with presbyopia earlier than the normal population (32 versus 42 years) and required higher diopter addition. They also concluded that liver transplantation has no influence on the development of presbyopia.

Retinal changes occur in about 20% of FAP patients, normally as haemorrhages or cotton wool spots, and they are more prevalent in patients with Y114C mutation [32].

Kojima et al. [33] reported the case of a 59-year-old patient with FAP with choroidal vascular changes observed on indocyanine green angiography in the form of hyperfluorescent foci along the choroidal vessels.

Another ocular manifestation in patients with FAP is amyloid deposition at the pupillary edge, leading to peculiar indentations, as can be seen in Figure 2 [4].

There is also pupillary light-near dissociation, explained by the deposition of amyloid in the iris [11].

A rare cause of blindness in these patients is bilateral optic neuropathy. Hamann et al. [32] were pioneers in publishing a case of bilateral optic neuropathy after excluding other

diagnostic hypotheses, such as vitreous opacity or glaucoma. It concerned a Portuguese male patient with FAP TTR Val30Met who presented with visual impairment. It was possibly caused by ischaemia secondary to amyloid deposition in small vessels, as well as impairment of autonomic self-regulation.

A study conducted in Japan [11] analysed 9 autopsied eyes and confirmed the presence of the aforementioned ocular manifestations. During the study, all patients showed ACV and pupil changes. Retinal changes were detected in 8 patients (21.6%), including haemorrhages ($n = 4$), cotton wool spots ($n = 3$), and peripheral neovascularisation ($n = 1$).

In 1997, Ando et al. [34] analysed 37 patients with FAP I in Japan for a period between 1 and 12 years. Among the most important ocular manifestations, ACVs had a prevalence of 75.5%, pupillary changes 43.2%, KCS 40.5%, and glaucoma and vitreous opacities 5.4%. Ocular manifestations appeared after liver transplantation, probably due to the intraocular production of mutant TTR [16].

9. Phenotypic Variants

Areas where FAP resulting from Val30Met mutation is endemic have a higher number of patients with a positive family history and earlier onset of the disease. However, no endemic cases have a late onset of the disease, over age 50, and there is a higher prevalence in men and milder symptoms [1].

Type I FAP can be divided into early onset FAP (before age 50) and late onset. A 2006 Portuguese study [7] compared the clinical differences between 86 patients with similar gender and geographic distribution: 43 patients with early disease and 43 with late disease. The early onset cases were more commonly associated with positive family history and with autonomic dysfunction. In the late onset cases, however, organ involvement and neuropathic pain were more frequent.

Studies on Portuguese parents and children [24] have found that the age of onset of the disease is higher in women, and the mother is responsible for the transmission of the disease in 60% of the cases.

10. Therapeutic Options

Therapeutic options in a patient with FAP depend on the stage of the disease and the patient's age. In patients with a positive family history, presymptomatic medical assistance is of utmost importance, since there are treatments available that halt the progression of the disease but do not reverse the already existing nerve damage [1].

Thus the therapeutic options available are

 (i) orthotopic liver transplantation or

(ii) pharmacologic treatment [35]:

   (1) *tafamidis* and *diflunisal*: for stabilisation of the TTR tetrameric form;

   (2) gene therapy with antisense oligonucleotides and RNA interference: to block TTR hepatic synthesis;

   (3) doxycycline: to promote the clearance of amyloid fibrils.

Liver transplantation was initiated in 1990 and is currently the standard treatment for FAP and the only one able to change its natural history [36]. Liver transplantation removes the main source of mutated TTR, resulting in the fast decline of its concentration to levels around 1%. Thus it stops the progression of neurological symptoms [34] and promotes patient survival, as long as it is performed at an early stage [3, 6].

Before the era of liver transplantation, median survival of patients with type I FAP was about 10 years after disease onset. With liver transplantation it was possible to double this figure [34].

Liver transplantation replaces the mutant type with wild-type TTR. However, that does not apply to the cerebrospinal fluid or the eyes, which continue to produce the mutated form through the choroid plexus and the RPE, respectively [5]. Eye deposition of amyloid may occur from 4-5 years after the liver transplantation [14].

Rosa et al. [4] studied 20 eyes of 10 patients with Val30Met mutation and detected some major changes: dry eye (20%); vitreous opacities (20%), existing after transplantation and in some cases recurring after posterior vitrectomy; secondary glaucoma (20%); corneal nerve hyperplasia in 2 patients (20%); and pupillary abnormalities (10%).

Similar results were found in a clinical study [37] of disease progression in 22 patients, where the onset of glaucoma was reported in 3 (14%) patients, amyloid deposits on the pupil's edge were also found in 3 (14%) patients, and vitreous opacities were found in 1 (5%) patient.

In a Japanese observational study [34], 22 patients with TTR Val30Met mutation and 3 with Tir114Cis mutation (Tyr114Cys) were observed and it was found that 3 (12%) had glaucoma, 3 (12%) had amyloid deposits on the pupil edge, and 1 (4%) developed vitreous opacities.

Obayashi et al. [3] described the disease course in a 28-year-old patient, transplanted two years after the disease onset. The patient presented pupil amyloid deposition and vitreous opacities 10 years and 13 years, respectively, after the transplantation.

Beirão et al. [16] studied 2 groups, 32 transplanted patients and 32 nontransplanted ones. After 15 years, there was an increase of the ocular manifestations and an attenuation of the differences between the two groups. This study concluded that transplantation does not influence the deposition of amyloid in the iris, retinal amyloid angiopathy, or tear film instability. Schirmer's test, which evaluates aqueous tear film deficiency, was most commonly abnormal in the nontransplanted group (81% versus 56%). Greater prevalence of amyloid deposition in the lens, vitreous body, and glaucoma was observed in the transplanted patients. Chronologically, the first manifestation observed was dry eye, followed by deposition of amyloid in the iris and anterior capsule in the lens and VAC. The vitreous deposition and glaucoma appeared later. The last manifestation was retinal amyloid angiopathy.

Despite being a very useful therapeutic strategy, liver transplantation has all the limitations of an invasive procedure and requires immunosuppressive adjuvant therapy. It does not halt the progression of ocular, CNS, or cardiac clinical manifestations [6].

Not all patients are candidates for liver transplantation, and it is not recommended for patients over 65 years of age, those in an advanced stage of disease, or those with heart failure. Nor is it an option in patients with senile systemic amyloidosis due to the continuous deposition of wild-type TTR [6].

To date, over 1.500 liver transplants have been performed from living and dead donors in 19 countries [2, 6]. Portugal is the country with most transplanted FAP patients [16]. Each year, 120 liver transplants are performed in FAP patients worldwide [5].

10.1. Transplant in Sequence/in Domino. Sequence transplantation or "domino" is the use of a FAP donor liver for transplantation in patients with end stage liver disease to provide the receiver with a longer life free of symptoms and at the same time to reduce the shortage of organ donors [19, 38]. It assumes that FAP carrier liver is functionally and structurally normal despite the long-term production risk (8–10 years) of the mutated protein, which could theoretically lead to FAP in the recipient [17, 21].

The aim is to restore function in patients with hepatic failure, such as cirrhosis or liver cancer, and at the same time to solve the genetic defect in patients with FAP [15].

Although the development of amyloidosis and ocular manifestations has been described in the recipients of domino liver transplants, these manifestations appear at a late stage and do not constitute a contraindication for the procedure [20]. Tafamidis and diflunisal are recent pharmacological treatments [6] that stabilise the TTR tetramer and prevent its disassembly by protein denaturation, thus slowing the progression of the disease. They can change FAP's natural history, especially if used at an early stage [12].

Tafamidis meglumine is the first authorised and only available drug for TTR-FAP [2]. It binds selectively to TTR, stabilising its tetrameric structure and thereby reducing the amyloid monomers. Clinical trials tested a dose of 20 mg for 18 months [39] and have demonstrated its efficacy [12], with adverse effects similar to those in the placebo group. In 2011 tafamidis meglumine was approved by the European Medicines Agency for the treatment of FAP, being approved in Europe for the disease in its early stage [1, 6, 12] and in Japan at any stage [6].

Diflunisal is a nonsteroidal anti-inflammatory agent [12] that binds to TTR and prevents amyloid fibril formation. It acts on the wild variant of TTR [6]. According to clinical trials, the dose of 250 mg twice a day for 24 months stabilises FAP patients, reduces the neurological progression, and maintains the quality of life [39], without showing any adverse effects. This agent has however not been authorised yet.

New gene therapies revealed promising results in clinical trials [6]. Gene therapy has been developed to suppress the expression of variant TTR [6]. Its purpose is to silence the TTR gene with the use of antisense oligonucleotides and RNA interference. These components are in phase III.clinical trials [12, 24].

Various agents to increase the clearance of amyloid fibrils are currently under investigation. These studies are in the preclinical and clinical phases [36].

In mouse models, doxycycline has shown increased clearance of amyloid by disruption of the fibrils and by promoting its absorption. However, as yet there is no randomised trial to evaluate its role in TTR-FAP [35].

Thus, with the emergence of new treatment options, liver transplantation may be replaced by those less invasive strategies that have proven efficiency not only in FAP but also in senile systemic amyloidosis [6].

11. Treatment of Ocular Manifestations

11.1. Keratoconjunctivitis Sicca. For patients with severe dry eye disease, patients refractory to treatment with topical artificial tear, or patients with closure of the punctum [40], topical cyclosporine has been shown to be beneficial in patients with FAP after liver transplant, showing symptomatic improvement and improving the quality life. Cyclosporine reduces inflammation and improves the composition of the tear film through its inhibitory action on T lymphocytes and by increasing goblet cells in the conjunctival epithelium. Its topical use is not associated with adverse effects and it has a low systemic absorption [40].

Other therapeutic options for corneal epithelial injuries with vitamins, collagenase inhibitors, anti-inflammatory agents, prophylactic topical antibiotic, or bandage contact lenses are frequently inadequate or of transient efficacy. In severe cases, oral doxycycline, autologous serum, amniotic membrane transplantation, tarsorrhaphy, and a conjunctival flap are employed alone or in combination. However, successful modulation of the healing response is rarely accomplished [30].

Marta Guerra and João Quadrado [30] evaluated the response to treatment with *Cacicol*, a matrix regenerating agent (RGTA, polycarboxymethyl glucose sulphate), designed to mimic the heparan sulphates bound to corneal extracellular matrix proteins, protecting them from proteolysis and enabling growth factors and cytokines to act on the injured site.

They studied the response to treatment in 3 patients with FAP and refractory NK. After an average period of 33 days, there was full reepithelialisation, without recurrence of corneal ulcer in the follow-up of 2–7 months. The treatment regimen was 1-2 instillations a week after debridement of the edges of the ulcer. No systemic or local side effects were noticed and no pain or discomfort during drop instillation was reported.

11.2. Glaucoma. With liver transplantation, there was an increased survival of patients and consequently a higher prevalence of glaucoma and greater need for therapeutic intervention.

Continuous production of mutant TTR can explain the difficulty in reducing IOP.

In patients refractory to topical therapy, IOP is usually between 35.0 ± 9.0 mmHg, often with good control after surgery [41]. The most promising strategy seems to be trabeculectomy with mitomycin C, keeping IOP less than or equal to 20 mmHg [29].

A Portuguese study analyzed the progression of glaucoma in 44 patients with TTR-FAP, where 29.5% of the patients had IOP less than 20 mmHg with medical therapy and 69.2% required surgical treatment [42].

EPO, as mentioned earlier, was shown to have a protective effect on ganglion cells and has been proposed as a possible neuroprotective treatment strategy [29].

11.3. Vitreous Opacity. If there is severe loss of visual acuity or if the opacity does not allow a view of the fundus, vitrectomy should be performed. The 25-gauge vitrectomy has proved to be a safe therapeutic option [43], with improvement in visual acuity, and may be considered the treatment of choice in patients with glaucoma and who have already undergone trabeculectomy [44]. This induces less damage and inflammation due to the smaller conjunctival incision and is preferred in patients with KCS, since it does not affect mucin secretion [44]. Vitreous opacities may recur after vitrectomy as described by Rosa et al. [4] due to local production of amyloid fibrils. This intervention can induce open-angle glaucoma through several mechanisms. On the one hand, vitrectomy increases oxidative stress in the trabecular meshwork and, on the other hand, in the absence of vitreous, the amyloid aggregates reach the trabecular meshwork and Schlemm's channel more easily and are deposited in both structures.

Thus, studies suggest that the vitreous acts like a filter which retains the amyloid fibrils and prevents their progression to the trabecular meshwork [43]. Therefore, incomplete vitrectomy is a viable option, to delay the progression of glaucoma.

12. Discussion and Conclusion

TTR is primarily synthesised in the liver; however, it is doubtful whether liver transplantation is a factor in the resolution of eye disease given that intraocular amyloid formation is independent of liver synthesis, as shown by RPE autonomy in amyloid production. Furthermore, TTR is unable to cross the blood-retinal barrier [16, 34].

Still, liver transplantation is now the only therapeutic option able to alter the natural course of the disease, at least until gene therapies are approved [10, 37]. It dramatically increases FAP patients' survival and thereby increases the prevalence of ocular manifestations over time [26]. Genetic and environmental factors, as well as immunosuppression, may influence the presence of posttransplant clinical manifestations [5].

The main ocular manifestations reported in studies of patients with type I FAP are the deposition of amyloid in the vitreous, dry eye, and glaucoma. Although ocular manifestations rarely arise as a first symptom, they are often part of the clinical course and should lead to a suspected diagnosis in patients from endemic areas [26].

New therapeutic approaches plus a rigorous follow-up are now needed to ensure quality of life for these patients whose ocular manifestations limit their daily life [16, 34].

A new substance, RGTA, polycarboxymethyl glucose sulphate, was experimented and showed compelling effectiveness in corneal wound healing; it was well tolerated by all patients, showing that this approach might be an excellent solution to treat NK, even in this particular group of patients [30].

Presymptomatic genetic testing may be of value in increasing survival, since patients will be able to access treatment in useful time, before the disease progresses [45].

When the genetic diagnosis is done, it is advisable to carry out the first eye exam too. In asymptomatic patients, follow-up should be every two years and in symptomatic ones it should be annual [16]. When there are ocular manifestations, the frequency of consultation varies depending on disease stage: it should be annual for ACV, every six months for KCS, for indentations of the pupillary edge, and for anterior lens deposition, and every three months for glaucoma, vitreous deposition, and retinal angiopathy [16].

In conclusion, since the liver transplant does not treat the eye disease, new therapeutic strategies, possibly gene therapy, are required if we are to give these patients quality of life, so as to avoid invasive treatments and their adverse effects and also address the ocular symptoms.

Disclaimer

The authors alone are responsible for the content and writing of the paper.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Atrial Fibrillation and Coronary Artery Disease as Risk Factors of Retinal Artery Occlusion: A Nationwide Population-Based Study

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We use Taiwanese national health insurance research database (NHIRD) to investigate whether thrombolism (carotid artery disease (CAD) as a surrogate) or embolism (atrial fibrillation (AF) as a surrogate) plays roles in later retinal artery occlusion (RAO) development and examine their relative weights. The relative risks of RAO between AF and CAD patients and controls were compared by estimating the crude hazard ratio with logistic regression. Kaplan-Meier analysis was used to calculate the cumulative incidence rates of developing RAO, and a log-rank test was used to analyze the differences between the survival curves. Separate Cox proportional hazard regressions were done to compute the RAO-free rate after adjusting for possible confounding factors such as age and sex. The crude hazard ratios were 7.98 for the AF group and 5.27 for the CAD group, and the adjusted hazard ratios were 8.32 and 5.34 for the AF and CAD groups, respectively. The observation time with RAO-free was shorter for AF compared with CAD group (1490 versus 1819 days). AF and CAD were both risk factors for RAO with different hazard ratios. To tackle both AF and CAD is crucial for curbing RAO.

1. Introduction

Central retinal artery occlusion was first described by Dr. Von Graefe in 1859 [1–3]. The extent to which thrombosis or embolism plays a role in causing retinal artery occlusion is a perennial question of ophthalmologists. Hayreh proposed that the etiology of emboli is the most common cause of retinal artery occlusion [2, 4]. We used the national health insurance database in Taiwan to delve into the possible linkage of etiologies for retinal artery occlusion to understand whether embolism or thrombosis plays a role in causing retinal artery occlusion or both of them play a role. In this study, atrial fibrillation was used as a surrogate of embolism and coronary artery disease as a representative of thrombosis. Based on this hypothesis, there then should be statistically significant results for the atrial fibrillation and coronary artery disease study group patients to develop retinal artery occlusion later. And if embolism is the major cause of RAO, then the hazard ratio for atrial fibrillation patients to develop RAO would be higher than coronary artery disease patients and vice versa.

In Taiwan, the government launched the national health insurance as a mandate on 1 March 1995; and the coverage rate is around 99% [5]. A nationwide population study using a longitudinal case-controlled cohort study was conducted to examine whether atrial fibrillation and/or coronary artery disease was or were risk factor(s) for retinal artery occlusion and to compare the relative hazard ratios between these two groups of patients. The Longitudinal Health Insurance Database 2000 (LHID2000) is a subdataset of the national

health insurance research database (NHIRD), which includes all claims data (from 1996 to 2008) of one million beneficiaries who were randomly selected from the system in 2000. There was no significant difference in age, sex, or average insured payroll-related premiums between the sample group and all enrollees.

2. Materials and Methods

2.1. Selection of Patients and Variables. This cohort comparison study consisted of all patients diagnosed with atrial fibrillation (AF) (n = 9,756) (ICD-9-CM codes 427.31) and coronary artery disease (CAD) (n = 95421) (ICD-9-CM codes 410, 411, 412, 413, and 414) from ambulatory (including emergency) care and inpatient care, from 1 January 2000 through 31 December 2008 in the LHID 2000. The control patients are four patients for every atrial fibrillation (AF) patient and one patient for each coronary artery disease (CAD) patient as patients not diagnosed with AF or CAD, which were randomly selected from the dataset. The reason the coronary artery disease cohort group could only be a one to one ratio is that the sample size of the CAD study group was too large, so we could not obtain enough control patients to be greater than the rate of 1 : 1. The patients included in the study and control group patients were matched by sex, age, and the index date of ambulatory care visit (including outpatient clinic and emergency department) or hospitalization for the initial diagnosis of AF or CAD patients. And among these datasets, we further examined all patients who had ever developed retinal artery occlusion after a diagnosis of atrial fibrillation or coronary artery disease as RAO (ICD-9-CM codes 362. 31 (central retinal artery occlusion (CRAO)), 362.32 (branch retinal artery occlusion (BRAO)), and 362.33 (partial retinal artery occlusion (PRAO)). Demographic data, such as sex and age, were recorded.

2.2. Statistical Analysis. SAS for Windows 9.3 (SAS Institute, Inc., Cary, North Carolina, USA) was used for this study. Descriptive statistical analyses were done to compare the characteristics of the two cohorts in terms of demographic characteristics and the risk of developing retinal artery occlusion. The risk of retinal artery occlusion between AF and CAD patients and controls was compared by estimating the crude hazard ratio with logistic regression. Logistic regression is widely used in analysis of categorical data especially data with variables that have binary responses. It can predict a dichotomous outcome using independent variables. This dichotomous outcome is presence or absence of RAO in this study. Kaplan-Meier analysis was used to calculate the cumulative incidence rates of developing retinal artery occlusion between the two different cohorts, and the log-rank test was used to analyze the differences between the survival curves. Thereafter, separate Cox proportional hazard regressions were done to compute the RAO-free rate after adjusting for possible confounding factors such as age and sex. Cox regression is method for investigating the effect of variables upon the time a specified event takes to happen. The coefficients in a Cox regression relate to hazard; a positive coefficient indicates a worse prognosis and a negative

coefficient indicates a protective effect of the variable with which it is associated. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Demographic Data. Between 2000 and 2008, 9,756 AF patients and 38,872 AF control patients and 95421 CAD patients and 95419 CAD control patients, with age-, sex-matched controls, were recruited, after excluding ineligible subjects. The median age of the AF patients was 68.9 years old and for the controls it was 68.3 years old. The median age of the CAD patients was 57.4 years old and for the controls it was 56.9 years old. The sex ratios between the two groups were as follows: M/F 54.6%/45.4% in the AF study group and 54.8%/45.2% in the AF control group; M/F 49.2/50.8 in the CAD study group and 49.1/50.9 in the CAD control group (Tables 1 and 2).

For the atrial fibrillation study group, there were 9,756 patients, and, among them, 18 patients (0.18%) developed retinal artery occlusion later, while, for the atrial fibrillation control group, there were 38,872 patients and 9 patients that (0.02%) developed retinal artery occlusion later. Therefore, there were 27 AF patients (0.05%) of 48,628 cases that developed RAO. On the other hand, for CAD study group, there were 95,421 patients and 79 cases that developed RAO later; and for CAD controls, there were 95,419 patients and, among them, 12 cases (0.02%) developed RAO later in the observation period. The differences in the risks of developing RAO in both groups were statistically significant ($p < 0.0001$). The median observation period and interquartile range between the two groups to develop the RAO were 1490 (666–1384) days in the AF study group, 1606 (728–1451) days in the AF control group, 1819 (1009–1882) days in the CAD study group, and 1854 (1016–1889) days for the CAD control group, respectively. The observation periods for the AF and CAD groups to develop RAO later differed in length. For the AF study group, it was around four years (4.08 years), while for the CAD study group it was around five years (5.07), meaning it will be around one year earlier to develop RAO later in AF patients than that of CAD patients. The differences in the survival analysis between the two groups, that is, the AF study group and AF control group and CAD study group and CAD control group, are statistically significant as well. The p value is <0.0001 (Tables 3 and 4).

Among the AF cohorts, the crude hazard ratio by logistic regression with a 95% confidence interval for the AF patients to develop RAO is 7.98 (3.59–17.77); and the adjusted hazard ratio by Cox proportional regression model is 8.32 (3.70–18.32). The adjusted factors included age and sex. For the CAD cohorts, the crude hazard ratio by logistic regression with 95% confidence interval for the CAD patients to develop RAO is 5.27 (3.03–9.15); and the adjusted hazard ratio by the Cox proportional regression model is 5.34 (3.27–9.26). The adjusted factors included age and sex. Kaplan-Meier survival analysis was conducted to examine the cumulative incidence rates of developing retinal artery occlusion between the two different cohorts, and a log-rank test was used to probe the differences between the survival curves. The results

TABLE 1: Demographics of AF group and control group.

Variable	AF patients ($n = 9,756$)		Control group ($n = 38,872$)		p value
	n	%	n	%	
Age, median (IQR[a])	68.9 (10)		68.3 (10)		0.99
Gender					0.99
Male	5,324	54.6	21,288	54.8	
Female	4,432	45.4	17,584	45.2	
RAO	18	0.2	9	0.02	<0.0001***
Observation time without developing RAO (days, median, IQR[a])	1,490	(666–1384)	1,606	(728–1,451)	<0.001***

[a]IQR: interquartile range, * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicated $p < 0.001$.

TABLE 2: Demographics of CAD group and control group.

Variable	CAD patients ($n = 95,421$)		Control group ($n = 95,419$)		p value
	n	%	n	%	
Age, median (IQR[a])	57.4 (10)		56.9 (10)		0.99
Gender					0.99
Male	46,879	49.2	46,879	49.1	
Female	48,542	50.8	48,540	50.9	
RAO	79	0.08	12	0.02	<0.0001***
Observation time without developing RAO (days, median, IQR[a])	1819	(1009–1882)	1854	(1016–1889)	<0.001***

[a]IQR: interquartile range and *** indicated $p < 0.001$.

TABLE 3: Crude and adjusted hazard ratios for developing retinal artery occlusion among patients with atrial fibrillation and the control group during the ten-year follow-up ($n = 48,628$).

Development of RAO	Total		Patients with AF		Control group	
	Number	%	Number	%	Number	%
Nine-year follow-up period						
Yes	27	0.05	18	0.18	9	0.02
No	48,601	99.95	9,738	99.82	38,863	99.98
Crude HR (95% CI)	—		7.98 (3.59–17.77)		1.00	
Adjusted[a] HR (95% CI)	—		8.32 (3.70–18.32)		1.00	

[a]Adjustments were made for sex and age.

TABLE 4: Crude and adjusted hazard ratios for developing retinal artery occlusion among patients with coronary artery disease and the control group during the ten-year follow-up ($n = 190,840$).

Development of RAO	Total		Patients with CAD		Control group	
	Number	%	Number	%	Number	%
Ten-year follow-up period						
Yes	91	0.5	79	0.1	12	0.02
No	190,749	99.95	95,342	99.9	95,407	99.98
Crude HR (95% CI)	—		5.27 (3.03–9.15)		1.00	
Adjusted[a] HR (95% CI)	—		5.34 (3.27–9.26)		1.00	

[a]Adjustments were made for sex and age.

(Figures 1 and 2) revealed statistically significant differences between the two cohorts, that is, the AF cohorts and CAD cohorts, $p < 0.0001$. In AF and CAD cases, to develop RAO, the sex did not increase the hazard ratios, yet the hazard ratios increased 1.03 times for every 10 years of age in atrial fibrillation patients and 1.376 times for every 10 years of age in coronary artery disease patients.

4. Discussion

The results showed that atrial fibrillation (AF) and coronary artery disease (CAD) will both increase the risk of developing retinal artery occlusion (RAO) later, as the crude hazard ratios with 95% confidence interval were 7.98 (3.59–17.77) and 5.27 (3.03–9.15); and the adjusted hazard ratios with 95% confidence interval were 8.32 (3.70–18.32) and 5.34 (3..27–9.26) for AF patients and CAD cohorts, respectively. This means the hazard ratios, regardless of whether the crude or adjusted ones were adjusted for age and sex, are still both quite high for both AF and CAD group patients to develop RAO later; and the hazard ratio for the AF group is around 1.5 times higher than that of CAD group. This result seems to echo Hayreh's point of view that embolism is by far the most common etiology of RAO. Wong et al. suggested the etiology of RAO was from thrombus, which is a different point of view [6]. This reason that arteriosclerosis causes thrombus in the internal carotid artery or heart and further influences and/or simultaneously attacks the ocular vascular system such as the retinal arteries is plausible, as the hypertension and hyperlipidemia would cause artery wall atheroma formation or artery sclerosis, that is, resistance to changes in feasibility during stress and the alternating relaxing condition later on. In this way, the thrombus would form in the arterioles or arteries and result in the retinal artery occlusion event as the
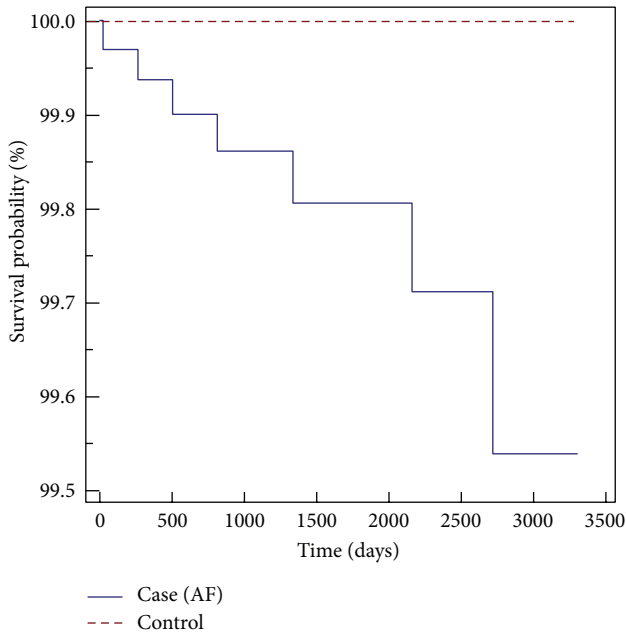
Figure 1: Kaplan-Meier survival analysis of AF patients with RAO-free time.

Figure 2: Kaplan-Meier survival analysis of CAD patients with RAO-free time.

one in four adults older than forty years old might develop AF later in their life, and many AF patients do not have conspicuous symptoms, so understanding the possible risk of developing RAO is critical because it might help prevent later visual tragedy and possible systemic misery. Clearly, detecting AF patients and subjecting them to anticoagulant treatment to prevent possible vascular events like the retinal artery occlusion are especially crucial for saving vision in this regard.

In clinical settings, ophthalmologists might not be convinced an embolism is by far the most common etiology for RAO because we could not see the emboli in the fundus in most RAO cases. However, as Hayreh proposed, this might be because the emboli in RAO were usually tiny and located in the microvascular bed. They could be dislodged later and therefore not show in the retinae. From our study, this viewpoint is also verified. For the RAO emboli sources, from the study of Arruga and Sanders in 1982, 74% were made of cholesterol, 10.5% of calcific material, and only 15.5% of platelet-fibrin. Some ophthalmologists have tried surgical embolectomy [8, 9] to treat RAO. In these studies, after removal of a calcified material embolism, some patients' visual acuity improved from no light perception to counting fingers. The reason some failed through surgical intervention might have been due to the delay in initiating treatment. As the clinical reality goes, most RAO patients do not visit ophthalmologists or physicians in the golden four hours. Most patients visit ophthalmologists several days later. Therefore, understanding the possible causes, appropriate management, and proper expectations are pivotal for both physicians and patients in preventing grave outcome of retinal artery occlusion events and reducing the disease burdens. Kirwan et al. revealed a case from Ireland where multiple amaurosis fugax attacks developed RAO later and with very poor visual acuity outcome as counting fingers was indeed a case of paroxysmal atrial fibrillation [10]. And so the authors highlighted the importance of Holter monitoring for the patients and in this way warfarin or other anticoagulant treatments are very critical in helping physicians including ophthalmologists and patients to deal with the disorders. A study conducted by Johns Hopkins Hospital [11] showed 42 CRAO patients between 1999 and 2006 with intra-arterial delivering of tPA in aliquots with the timeline up to 15 hours after attack of CRAO and found a statistically significant improvement in three lines of visual acuity or more of vision as compared with control subjects who did not receive thrombolysis. Yet the European Assessment Group for Lysis in the Eye (EAGLE) [12] conducted a multicentered, prospective randomized controlled trial of 84 patients with CRAO within 20 h of symptom onset and did not show a statistically significant difference in clinical improvement between the lysis and standard therapy groups (60.0 versus 57.1%). Thrombolysis can also be administered by other routes as intravenous tPA delivery. Better outcomes resulted from the starting treatment within 6.5 hours of CRAO attack in one case series [13]. There is no consensus on fibrinolytic thrombolysis treatment.

The cohort for AF was older (median age 68 years old) than that of CAD (median age 57 years old) in the Taiwanese

progression of atheroma plaques continues and the severity of arterial stenosis worsens.

We also found, between the two groups, it took one year less to develop RAO for the AF group (around four years) than that of the CAD group (around five years). In this respect, embolism raises our alertness to be a stronger promoting factor in RAO development. Therefore, for AF patients, as denoted by the cardiologist's perspective [7],

population. Slight sex differences existed in the AF cohorts, with males in the AF group comprising 54.8%, while there is almost no difference in the CAD cohorts (F/M 49.8%/50.2%). There is no statistically significant risk difference in sex towards the later development of RAO, yet the increase of ten-year age rank would increase the risk 1.34-fold.

This study was conducted based on a single-payer longitudinal national health insurance database in Taiwan to probe the hazard ratios of AF and CAD as risk factors for retinal artery occlusion development and verify the hypothesis mentioned previously. Were it not for this NHIRD longitudinal dataset, it would be very difficult to collect enough cases to conduct the research because of the time-consumption and cost issues. And since it is a national health insurance database, it is a population-based study, so the results are more robust and convincing without selection bias.

On the other hand, the claims data of national insurance health database could not show important clinical features such as the retinal images and stereotactic fundus pictures of possible emboli and/or fluorescein angiography. It could also not show personal health records like the body mass index and exercise or food intake habits, which are important in interpreting the etiology of RAO.

And there are flaws in the claims data such as the fact that our inclusion criteria were based on ICD-9-CM codes, but there was no way to verify the accuracy of coding by physicians, and so there should be some expected defects in this respect by using national health insurance research database.

5. Conclusion

Taiwanese national health insurance database was used to examine the risk of atrial fibrillation and coronary artery disease in developing retinal artery occlusion later. The results revealed both AF and CAD will increase the risk of later developing RAO as the crude HRs with 95% confidence interval for both groups were 7.98 (3.59–17.77) and 5.27 (3.03–9.15), and the adjusted HRs with 95% confidence interval were 8.32 (3.70–18.32) and 5.34 (3.27–9.26). The embolism factor (surrogate as AF) is more important for the etiology of RAO than that of thrombolism (surrogate as CAD).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

New Onset Diplopia in Patients with Nasopharyngeal Carcinoma following Concurrent Chemoradiotherapy: Clinical Features and Etiology

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Purpose. To investigate the clinical features and etiology of nasopharyngeal carcinoma (NPC) patients with new onset diplopia after concurrent chemoradiotherapy. *Methods*. We retrospectively reviewed the medical records of NPC patients with new onset diplopia after concurrent chemoradiotherapy from 1998 to 2012 in a cancer center. Their clinical manifestations of ocular motor dysfunction in relation to etiology were investigated. *Results*. Twenty-three NPC patients with diplopia after concurrent chemoradiotherapy were enrolled in this study. Unilateral cranial VI palsy (91%) was the most common ocular motor dysfunction in these patients. The new onset diplopia in these patients was secondary to tumor recurrence in 12 cases (52%), radiation neuropathy in 8 cases (35%), and skull base osteoradionecrosis in 3 cases (13%). Patients with tumor recurrence and skull base osteoradionecrosis tended to present a rapid progression of the nerve palsy or severe ocular duction deficit. Patients with radiation neuropathy were often manifested by incomplete nerve palsy with insidious onset and slow progression. Patients with osteoradionecrosis were associated with poor prognosis. *Conclusions*. A new onset diplopia in NPC patients could be caused by tumor recurrence or treatment complications such as radiation neuropathy and osteoradionecrosis, and they show diverse clinical symptoms, course, and outcome.

1. Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy with high prevalence in Southeast Asia, and radiotherapy or concurrent chemoradiotherapy (CCRT) is the mainstay treatment for this disease. The proximity of nasopharyngeal carcinoma to the skull base and cavernous sinus could cause nearby cranial nerve damage, among which cranial nerve III, IV, or VI dysfunction would result in limited ocular movement leading to diplopia. However, diplopic symptoms in NPC patients raise suspicion of not only tumor recurrence but also radiation-related cranial neuropathy. In particular, the diagnosis of radiation-induced cranial neuropathy is usually by exclusion, and a 3–6-month observation is often required to exclude tumor recurrence as the cause of nerve palsy. Therefore, how to make an early and accurate diagnosis

of the etiology for NPC patients with diplopia after radiotherapy or CCRT remains a clinical challenge. In the current study, we investigated the clinical characteristics and etiology of NPC patients who presented with new onset diplopia after CCRT.

2. Materials and Methods

We retrospectively reviewed the medical records of patients with new onset diplopia who were previously treated with CCRT for NPC from January 1998 to December 2012 at the Koo Foundation Sun Yat-Sen Cancer Center. The CCRT regimen was the same for these patients: 7000 cGy to the main tumor with three-dimensional conformal technique before November 2003 and intensity modulation radiation technique after December 2003, followed by chemotherapy

with cisplatin and 5-FU. The exclusion criteria were as follows: (1) follow-up period under 6 months, (2) diplopic symptom before CCRT completion, and (3) known persisted tumor under active treatment. When patients report a new onset diplopia, they are sent for ocular examination in the ophthalmology department and also for image study of the head and neck regions, by either magnetic resonance imaging (MRI) or computed tomography (CT). If there is any suspicious mass lesion in MRI or CT, patients would receive examination of ^{18}F-2-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) and nasopharyngoscopy with biopsy and microbiological culture. If the initial image study excludes suspicious mass lesion, patients would receive clinical examination regularly and follow-up image study if the diplopic symptom gets worse or other symptoms appear.

Collected data included age, gender, initial cancer stage, the latency between completion of CCRT and diplopic onset, the characteristics of ocular motor nerve palsy (type of cranial nerve, degree of ocular duction deficit, and progression of nerve palsy), the patient outcome, and the etiology of cranial nerve palsy. Ocular duction deficit was recorded on the scale described by Scott and Kraft [1]: zero (normal), −1 (to 75% full rotation), −2 (to 50% full rotation), −3 (to 25% full rotation), −4 (to midline), and −5 (inability to the midline). A complete palsy was defined as −4 or −5 duction. Rapid progression was defined as a change in scale of duction deficit ≥1 within two months. Slow progression was defined as a change in scale of duction deficit ≥1 during the follow-up period, but not significant within the initial two months. Stable condition was defined as no change of the duction deficit during the follow-up period. This study followed the tenets of the Declaration of Helsinki and was approved by the institutional review board.

3. Results

We enrolled 23 patients with new onset diplopia who were previously treated with CCRT for NPC in this study. There were 14 male and 9 female patients with a median age of 47 years (range 27 to 70) and a median follow-up time of 21 months (range 6 to 120 months). Three different etiologies were concluded in our series. The diagnosis of tumor recurrence is based on positive findings on MRI, CT, FDG-PET, and biopsy. The diagnosis of skull base osteo-radionecrosis (ORN) is confirmed by pathological examination and positive bacterial culture of the tissues from nasopharyngoscopy. If the results of MRI, CT, FDG-PET, and nasopharyngoscopy are all negative, radiation neuropathy is diagnosed which requires a regular follow-up of at least 6 months to exclude tumor recurrence as the cause of nerve palsy. In our series, the new onset diplopia was secondary to tumor recurrence in 12 cases (52%), skull base ORN in 3 cases (13%), and radiation neuropathy in 8 cases (35%). Table 1 shows their clinical manifestations and outcome according to the etiologies, respectively. The median latency between CCRT completion and diplopic onset was 44 months in tumor recurrence group, 48 months in ORN, and 70 months in the radiation neuropathy group (Table 2). Most patients presented with unilateral VI palsy (91%). Seven patients (30%) initially presented complete nerve palsy, in

TABLE 1: Clinical characteristics and outcome in NPC patients with new onset diplopia in relation to etiology.

	Tumor recurrence n = 12 (52%)	Skull base ORN n = 3 (13%)	Radiation neuropathy n = 8 (35%)
Mean age (range)	44 (27–59)	61 (47–70)	46 (40–63)
Gender			
Male	6	3	5
Female	6	0	3
Initial cancer stage			
I	0	0	0
II	1	0	0
III	4	0	4
IV	7	3	4
Outcome			
Alive	8	0	8
Died	4	3	0

n = number of patients; ORN = osteoradionecrosis.

which 4 cases were caused by tumor recurrence and 3 cases were due to skull base ORN. Sixteen patients (70%) initially presented incomplete palsy with −1 or −2 ocular duction deficit, in which 8 cases had tumor recurrence and 8 cases were diagnosed as radiation neuropathy. The former 8 cases with tumor recurrence showed rapid progression of the nerve palsy, with half of them deteriorated to complete palsy. The latter 8 cases with radiation neuropathy showed either stable condition (5 cases) or slow progression (3 cases) of their nerve palsy.

Four of the 12 patients in the tumor recurrence group died during the follow-up (Table 1). The cause of death included tumor bleeding, tumor invasion of the central nervous system, and pneumonia. The 3 patients in the ORN group were all dead during the follow-up. They died of carotid artery rupture, sepsis, and liver failure, respectively. All the 8 patients in the radiation neuropathy group were alive during the follow-up.

4. Discussion

We demonstrated in this study that new onset diplopia in posttreated NPC patients could be caused by tumor recurrence or radiation-induced complications. Furthermore, they presented with distinct clinical characteristics, course, and outcome.

Early diagnosis of recurrent NPC is a clinical challenge. The soft tissue change after radiotherapy, such as edema, fibrosis, scarring, and loss of tissue planes, may interfere with the detection of recurrent tumor. Cranial nerves III, IV, and VI palsy as the first symptom of NPC recurrence are common with an incidence rate of 20% to 38% [2, 3]. Our current study revealed that the majority of the new onset diplopia in posttreated NPC patients was a result of tumor recurrence (52%). Patients in this group tended to have rapid

TABLE 2: Clinical manifestations of NPC patients with new onset diplopia in relation to different etiologies.

	Tumor recurrence $n = 12$ (52%)	Skull base ORN $n = 3$ (13%)	Radiation neuropathy $n = 8$ (35%)
Latency between CCRT completion and diplopic onset (range, months)	44 (16–60)	48 (7–96)	70 (40–144)
Involvement of cranial nerve			
Unilateral nerve III	0	1	0
Unilateral nerve VI	11	2	8
Bilateral nerve VI	1	0	0
Severity of ocular duction deficit at diagnosis	Complete palsy: 4 Incomplete palsy: 8	Complete palsy: 3 Incomplete palsy: 0	Complete palsy: 0 Incomplete palsy: 8
Progression of ocular duction deficit	Persisted complete palsy: 4 Rapid progression: 8 Slow progression: 0 Stable condition: 0 Recovery: 0	Persisted complete palsy: 2 Rapid progression: 0 Slow progression: 0 Stable condition: 0 Recovery: 1	Persisted complete palsy: 0 Rapid progression: 0 Slow progression: 3 Stable condition: 5 Recovery: 0

n = number of patients; ORN = osteoradionecrosis.

progression of the nerve palsy and severe ocular duction deficit. Most of them had complete nerve palsy either as the initial presentation or during the follow-up. In a case series of 337 patients with recurrent NPC [4], Li et al. demonstrated that the common sites of tumor recurrence were in those regions not directly adjacent to the nasopharynx, for example, skull base, cavernous sinus, paranasal sinus, and orbital apex. They proposed that the radiation dose to these regions is usually low or none, so the tumor cells of subclinical lesions could survive and lead to tumor recurrence in these regions. The skull base and cavernous sinus are important aisles of multiple cranial nerves, including nerves III, IV, and VI. The limited space in these regions, coupled with the growth of recurrent tumor, probably explains the acute onset, rapid progression, and worse severity of the cranial nerve palsy seen in our series.

Despite great improvement in radiotherapy technique, skull base ORN remains one of the most serious complications for NPC radiotherapy. An estimated 2% of head- and neck-irradiated patients are at risk of developing ORN [5]. ORN results from the radiation-induced deficient cellular turnover and collagen synthesis in a hypoxic, hypocellular, and hypovascular environment, in which tissue breakdown exceeds the repair capabilities of the irradiated tissue [6]. For the first time, we reported 3 cases of skull base ORN in posttreated NPC patients presenting diplopia as the first symptom. All these three patients are male, with a relatively older age than the other two groups. In addition, they all presented acute complete nerve palsy (cranial nerve III or VI). Two of them had persisted complete nerve palsy through the follow-up. One of them recovered from ocular motor deficit after parental antibiotics; however, he died of liver failure from liver metastasis 3 years later. It has been reported that extensive ORN accompanied by radiation brain injury or cranial nerve damage had poor prognosis [7]. Despite aggressive treatment, all of the three patients in our series died during the follow-up, and one of them died of internal carotid artery rupture due to the extensive necrosis.

The incidence of radiation-induced cranial neuropathy in NPC patients was estimated to range from 1% to 5% [8, 9]. Adjuvant chemotherapy could result in increased risk of radiation neuropathy [10]. Lower cranial nerves were found to be more vulnerable. Upper cranial nerve palsy was seldom addressed in previous studies [11]. Besides, the severity and characteristic of radiation-induced upper cranial nerve palsy had never been reported in the literatures. Among the upper cranial neuropathy, the VI nerve palsy is relatively common. The vulnerability of the VI nerve is probably due to its small size [12] and its location near the skull base. In our study, all the patients in the radiation neuropathy group presented incomplete VI nerve palsy. As compared to the tumor recurrence and the ORN groups, the patients in this group had a longer latency between CCRT completion and diplopic onset, and they presented the neuropathy with an insidious onset and either slow progression or stable condition. Similar clinical presentations were also seen in radiation-induced brachial plexopathy. Harper et al. had analyzed the distinction between neoplastic and radiation-induced brachial plexopathy [13]. They found that 60% of patients in radiation neuropathy group reported little or no change in symptoms.

Accurate diagnosis of the etiology for new onset diplopia in posttreated NPC patients is difficult on occasion. MRI, CT, PET, nasopharyngoscopy, and biopsy are useful diagnostic tools to differentiate tumor recurrence from treatment sequelae. However, these examinations are either expensive or invasive, and there is limitation in all of them. For patients who have received radiotherapy, CT and MRI may have low sensitivity and specificity for distinguishing the recurrent skull base tumor from the postradiated soft tissue change [14–16]. FDG-PET is more sensitive in detecting tumor recurrence, especially for those who have inconclusive CT/MRI findings, but it is more expensive and may show false-positive in patients with ORN [17]. The final confirmation falls on pathologic examinations; however, it is often difficult to get a tissue proof from a doubtful skull base lesion. Therefore,

a long-term follow-up, repeated image studies, and probably repeated biopsies are sometimes required to get a correct diagnosis. In this study, we found that the clinical presentation of ocular motor nerve palsy varied in different etiologies. Patients with recurrent tumor and skull base ORN seemed to have a rapid progression of the nerve palsy and severe ocular duction deficit. Patients with radiation neuropathy usually presented insidious onset with either slow progression or stable condition of their nerve palsy. These clinical variations cannot replace the role of image study in detecting tumor recurrence. However, the follow-up records of these clinical manifestations might give a clue to the etiology of nerve palsy when image study and nasopharyngoscopy cannot provide a definite diagnosis.

In conclusion, a new onset diplopia in posttreated NPC patients could be secondary to tumor recurrence or treatment complications. They may show diverse clinical symptoms, course, and outcome. Detailed clinical history, close observation, and follow-up of the progression in cranial neuropathy, combined with image studies and nasopharyngoscopy with biopsy, are keys to make an early and correct diagnosis for these patients.

Conflict of Interests

None of the authors have any commercial interests in the material mentioned herein.

Acknowledgments

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Clinical Study

Ocular Surface and Tear Film Changes in Older Women Working with Computers

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The aim of this work is to investigate changes in the ocular surface (OS) and tear film (TF) by means of questionnaire-based subjective symptoms, TF break-up time, Schirmer test, and TF analysis in women working with computers and to analyze the effects of the oral supplementation with antioxidants/omega 3 fatty acids (A/ω3) in the OS outcomes. Women aged 40–65 years ($n = 148$) were recruited at the Administrative Offices of Valencia (Spain) and distributed into two age groups, 40–52 years (AGE1; $n = 87$) and 53–65 years (AGE2; $n = 61$), and then subdivided according to being (or not) computer users (CUG; NCUG) during the workday. Homogeneous subgroups were randomly assigned (or not) to the daily intake of three pills of A/ω3 for three months. At baseline and at the end of follow-up, personalized interviews and ocular examination were done. Reflex tear samples were collected from the inferior meniscus and processed for a multiplexed particle-based flow cytometry assay to measure proinflammatory molecules. Statistics were performed using the SPSS 15.0 program. The OS pathology was clinically evident in the AGE1-CUG (33%) versus the AGE2-CUG (64%) of women. Significantly higher interleukins-1β and -6 tear levels were found in the AGE1 versus the AGE2 women employees ($P = 0.006$ and $P = 0.001$, resp.), as well as in the CUG versus the NCUG ($P = 0.001$ and $P = 0.000$, resp.). Supplementation with A/ω3 positively influenced the OS pathology as manifested by the amelioration of the clinical signs/symptoms related to computer uses. Strategies involving a safe environment and oral micronutrient supplements may be managed within eye-care standards in older women.

1. Introduction

The ocular surface (OS) constituents (cornea, conjunctiva, eyelids, and tear film) and the lacrimal and accessory glands with the corresponding drainage system are essential for vision. When they fail in preserving the integrity of the ocular surface, tear film impairment and ocular surface pathologies appear as dry eyes (DEs) [1–4]. This disorder usually affects elder people aged 50 years or more [5–7] particularly women, with an estimated 3.23 million American women experiencing DEs [8]. Strong evidence suggests that having DEs is a multifactorial process in which multiple risk factors such as genetic, age, sex, nutrition, environmental conditions, lifestyle, working characteristics, immune situation, hormonal status, and medications contribute to

alter the morphology and function of the ocular surface constituents, leading to the pathological manifestations of DEs [1, 3, 9]. Environmental conditions play a relevant role in the apparition and progression of disease. Among the causes related to DEs are the following: temperature, humidity, wind, fumes, pollution, air speed, CO_2 concentration, and light intensity [3, 9]. Currently, there are two principal types, the clinical form that results from gland dysfunction (aqueous deficient) and the clinical form induced by the meibomian gland disorder (evaporative). The affected patients, mainly women, commonly display a mixture of both DEs types, independently of the etiology, with the most frequent symptoms being dryness, burning, stinging, grittiness, and foreign body sensation that usually are accompanied by visual impairment and loss of quality of life [1–6, 9].

What are the most important causes for eye and vision changes with aging? Firstly, in our 40s, the presbyopia appears and some refractive errors worsen. This is the time for being aware of increased risk for hypertension eye pressure, DEs, and/or computer use-derived problems. Next, in the 50s, there is an increased risk for cataracts, macular degeneration, DEs in women after menopause, and vascular disorders affecting the eyes and vision. Then, in the 60s and more, there is an increased risk for the most common age-related eye diseases including glaucoma, cataracts, DEs, retinal vascular occlusion, neurodegenerative disorders, and/or systemic diseases and comorbidities, with the age-related macular degeneration being the major ophthalmic problem in this decade.

It has been well documented that DEs prevalence rises exponentially with aging, and as older populations grow the disease becomes a much more important health issue and socioeconomic problem [1, 10, 11]. Furthermore, it has been reported that 1/10 women in their seventies and 1/20 women over the age of 50 complain of one or more dry eye symptoms in the United States [8]. A recent report revisited the impact of androgens on the morphology and function of the meibomian and lacrimal glands suggesting that androgen deficiency is associated with the etiopathogenic mechanisms of DEs [12]. However, other reports regarding the influence of estrogen and progesterone on the OS are contradictory. It has been described that testosterone regulates the expression of multitude of genes in the lacrimal/meibomian glands in the ovariectomized mice model [13]. Oprea et al. [14] suggested that optimal androgen levels are essential for lacrimal gland function and that prolactin and estrogens also play relevant roles on this activity.

Office employees often work in front of a computer. Adverse effects of such exposure have been referred to as computer-vision syndrome (CVS) that is a disorder resulting from focusing the eyes on a computer during noninterrupted time periods [15–18]. In this context, computers (Cs) users complain of a variety of ocular signs and symptoms such as itchiness, soreness, foreign body sensation, irritation, photophobia, redness, eye strain, tired eyes, blurred vision, double vision, and headache [19–22]. Ergonomic and ophthalmologic characteristics in the Cs-exposed individuals, including ameliorating symptoms by changing the computer location and/or the display features, the body position, and the light incidence as well as using eye solutions to improve spontaneous blinking and ocular good feeling (tested during work at video display terminals and during inactivity), were reported [19, 22].

Several etiopathogenic theories for DEs have arisen, with the autoimmune, caloric restriction or oxidative stress processes among them [23–27]. In this scenario, immune system involvement in OS pathologies has been documented in humans and animal models [28–31]. The OS alterations induce inflammation that leads in part to the development of an epithelial disorder and sensations of irritation [30–32]. Numerous immune response biomarkers, including proinflammatory cytokines and chemokines, have been identified in tears and the conjunctival and corneal epithelia of DEs patients [33–36]. These immune response mediators can trigger an inflammatory cascade on the ocular surface and subsequent signs and symptoms. However, the switch clock and specific mechanisms by which dryness and irritation stimulate chronic inflammation in the ocular surface have not yet been elucidated. Moreover, the pathogenic processes for OSDs in adults exposed to visualization screens in the office have not been fully evaluated.

Age-related ophthalmic pathologies are major causes of vision impairment and blindness worldwide. The antioxidant vitamins and essential polyunsaturated fatty acids (including $\omega 3$ and $\omega 6$) must be taken in the diet daily to meet physiological needs. However, epidemiological studies in well-nourished western populations suggested a role for nutritional supplements in delaying the onset of these disorders, but it is not yet possible to conclude that oral supplements can prevent cataracts, glaucoma, or macular degeneration [37–39]. In the case of $\omega 3$ fatty acids, these cannot be synthesized in sufficient amounts in the body, and deficiencies (that can worsen with aging) may cause well-defined symptoms [40, 41]. A recent report on dietary intake of $\omega 3/\omega 6$ from the International Society for the Study of Fatty Acids and Lipids specifically recommended the following proportions: linoleic acid intake (2 energy %) and α-linolenic acid intake (0.7 energy %), but a minimum intake of eicosapentaenoic acid and docosahexaenoic acid combined (500 mg/d) for optimum cardiovascular health [42]. The latest evidence has shown that the appropriate $\omega 3$ intake reduces the expression of inflammatory biomarkers in humans [43]. These findings propose a possible protective function of $\omega 3$ supplementation against inflammation.

To our knowledge, in addition to the aging process, the role of risk factors involved in the development/progression of DEs in women employees, particularly the exposure to Cs and the implications of the inflammatory mediators in this process, has not been fully investigated. In the present study we also deal with a more definitive evaluation of the effects of the oral supplementation with A/$\omega 3$ in the aging women employees suffering from DEs.

2. Material and Methods

2.1. Study Design. The present study is a prospective, randomized, open-label work that was approved by the Institutional Review Board of the University Hospital Dr. Peset (Valencia, Spain) (Ref: CEIC 2013). The authors carefully observed all tenets of the Declaration of Helsinki for the protection of human subjects in medical research.

Our main hypothesis was that older women employees working with Cs during the workday may be vulnerable to DEs compared to the nonuser women. We also hypothesized that exacerbation of inflammation in the OS constituents may be involved in the pathogenic mechanisms of DEs. Moreover, we wanted to test whether or not an appropriate supplementation with A/$\omega 3$ may ameliorate the clinical manifestations and personal impressions related to DEs and also to reduce the tear expression of inflammation biomarkers in the aging women employees. The present study was designed to provide information on these issues.
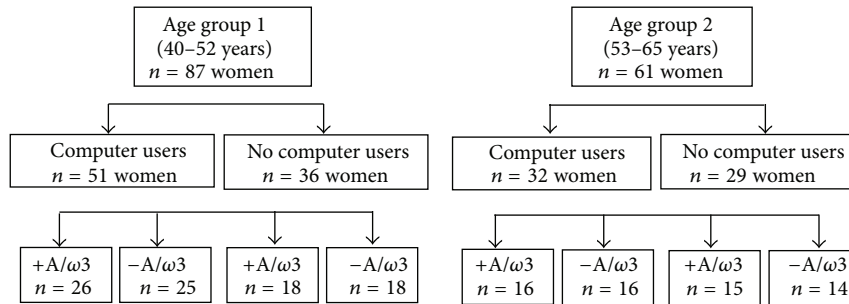
Figure 1: Classification of participants according to the main groups and subgroups.

Table 1: Inclusion and exclusion criteria for the study participants.

Inclusion criteria	Exclusion criteria
Women	Man
40–65 years	<40 or >65 years
Employees usually working with computers (CUG)	No computer user employees (NCUG)
With or without previous diagnosis of mild DEs	Eye disease, contact lenses, laser therapy, or ophthalmic surgery within the previous six months
Agreement to participate and collaborate with the study	Neurodegenerative disease or aggressive treatments that may interfere with the study
	Taking supplemental antioxidants or essential fatty acid supplements
	Declined collaboration in the study or had a disabling disorder (established or being monitored)

to the daily intake of three pills containing A/ω3 for three months. Thus, these latter participants were alternatively classified as +A/ω3 ($n = 75$) and −A/ω3 ($n = 73$). The A-ω3 formulation used was Brudysec 1.5 (Brudylab, Barcelona, Spain), each pill containing vitamin A (133 μg), vitamin C (26.7 mg), vitamin E (4 mg), tyrosine (10.8 mg), cysteine (5.83 mg), glutathione (2 mg), zinc (1.6 mg), copper (0.16 mg), manganese (0.33 mg), selenium (9.17 μg), docosahexaenoic acid (350 mg), eicosapentaenoic acid (42.5 mg), and docosapentaenoic acid (30 mg). The A-ω3 formulation (Brudysec 1.5) was produced by Brudylab (Barcelona, Spain) who gently donated the capsules for this study. Compliance with the oral supplement by the participants was one important point of this study, necessary to emphasize the effectiveness of the components and the reliability of the study data. After the basal appointment, women participants were visited every month during the study course (3 months) for recording incidences and feelings regarding the eyes, the job, and the A/ω3 intake. Each patient underwent 1 basal screening and 3 visits in this study.

2.2. Participants and Study Groups. Under the main criteria for inclusion and exclusion (Table 1), we recruited 148 women aged 40–65 years. Participants were selected consecutively from those attending the occupational medical services of the general Treasury Administration Offices of the Spanish government in Valencia (Spain), between the years 2012 and 2013. All suitable participants signed an informed consent.

Women participants were advised to discontinue for at least 3 months the use of nutritional supplements, systemic antihistaminics, and any treatment related to DEs. Those participants using contact lenses or suffering obvious eye and adnexa infection or notable eyelid inflammation were excluded from this study.

In parallel to supplementation, a dietary control of all participant women was performed to assess possible interferences of diet on the results of this study.

Participants were assigned to one of the following main groups as reflected in Figure 1: participants aged 40–52 years (AGE1 group; $n = 87$) and participants aged 53–65 years (AGE2 group; $n = 61$). Moreover, in each of these groups the women employees were classified as Cs users (CUG; $n = 83$) and nonusers (NCUG; $n = 65$). Homogeneously, employees from each group were randomly assigned (or not)

2.3. Proceedings. Interviews and ophthalmological examination were performed for all study subjects; especial importance was placed on the signs and symptoms of DEs and the participant subjective sensations. The OS disorder index (OSDI (Allergan Inc., Irvine, California, has the copyright)) questionnaire was carried out for all participants for differentiating those normal, mild, moderate, or severe DEs, as done before [33, 44]. The overall OSDI score delineated the OS from normal (0–12 points) and mild level of disorder (13–22 points) and moderate disorder (23–32 points) to severe stage of disease (33–100 points). The OSDI questionnaire was done during the medical appointments.

The effectiveness of A/ω3 was evaluated by studying the clinical and biochemical changes, as well as the subjective impressions of the participants through the 3 months of follow-up.

All participants were examined by the Occupational Medical Services staff at the general Treasury Administration Offices with the help of one ophthalmologist. Examinations included the Schirmer test to quantify tear secretion, blinking frequency (near), ocular surface inspection, and corneal characteristics under fluorescein staining. Schirmer test was performed by placing a small strip of filter paper in the lower

eyelid (inside), without previous anesthetic drop instillation, to observe the amount of wetting the strip during 5 min. The blinking frequency was determined by recording the spontaneous number of times of closing eyelids that occur in 1 min, with the participant seated in front of the computer station, under working conditions.

First ocular data considered for the DEs diagnosis and the effectiveness of the A-ω3 formulation were the Schirmer test and blinking frequency, and secondary outcome measures were the DEs symptoms and subjective sensations.

Special attention was paid to the workplace conditions in the office to better understand ocular surface changes in the employees. Data were obtained from the following homologized systems: heat stress monitor, indoor air quality (Microtherm IAQPROBE DAE 504002), light (luxometer Gossen Mavolux 5032 C/B n° serie 0C60759), and CO_2 concentration analyzer Ex 2000 Oldham/CO_2.

Next, we collected tears samples from all women participants to be analyzed through biochemical approaches. The gentle rubbing method was used to obtain reflex tears from the inferior meniscus of both eyes of our participants, by means of a micro Pasteur, as previously described [35, 36, 45] and shown in Figure 2. Collected tear samples from both eyes were immediately deposited in micro Eppendorfs to be frozen and stored at −80°C until assaying a specific set of inflammatory mediators. The human panel of cytokines/chemokines that was assayed in this study was composed of the following interleukins (IL): IL-1β, IL2, IL4, IL5, IL6, IL7, IL8, IL10, and IL12; tumor necrosis factor-alpha (TNF-α); vascular endothelial growth factor (VEGF); granulocyte-macrophage-colony stimulating factor (GM-CSF); and interferon-gamma (IF-γ). The analyses were performed by the Luminex R-200 multiplex system (Luminex, Austin, TX, USA), as reported before [33, 35, 36]. Polystyrene beads coupled covalently to specific antibodies (cytokines/chemokines) were prepared to react with an approximate amount of 20 μL of each tear sample (which contains an unknown amount of these molecules), or with a standard solution (having a known amount of molecules), at room temperature for 1 hour. For describing briefly the protocol, a series of washes (to remove unbound proteins) were done. Biotinylated detection antibody specific for a different epitope on the cytokine was added to the beads and incubated at room temperature for 30 min. Streptavidin-phycoerythrin (which binds to the biotinylated detection antibodies) was used to detect the reaction mixture. Next, the flow-based Bio-Plex (Bio-Rad Laboratories, Hercules, CA, USA) suspension array system was used to identify and quantify each antigen-antibody reaction. The assayed set of inflammatory molecules were identified by means of a method of bead color and fluorescence, using fluorescently labeled reporter molecules associated with each target protein. Unknown cytokine/chemokine levels were calculated automatically by the Bio-Plex Manager software (Bio-Rad Laboratories) by using a standard curve derived from a recombinant cytokine standard. Tear levels of the cytokine/chemokines were corrected for the initial total protein concentration and finally expressed as mean ± SD of three independent measurements.

FIGURE 2: Collecting method of reflex tears by a Pasteur micropipette.

TABLE 2: Analysis of the environmental conditions in the work place.

Environmental parameters	Data
Light intensity (lux)	500
Relative humidity (%)	32.673 ± 5.13
CO_2 (ppm)	2370.71 ± 646.89
Air speed (m/seg)	0.11 ± 0.031
CO (ppm)	0
Dry temperature (°C)	24.56 ± 0.60

Data were recorded in a designed Excel sheet (Microsoft Corporation, Redmond, WA, USA) and reflected as the mean ± SD. A parametric test (t-student) was used for comparing two independent sample groups by means of the SPSS software (IBM Corporation, Armonk, NY, USA). Results were statistically analyzed to detect differences between the two groups, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Demographics and Workplace Characteristics. Mean age of all women employees was 54 ± 8.5 years; among them, the AGE1 group (aged 40–52 years) displayed a median age of 46 ± 6 years, with 65% of these women being menopausal, whereas the AGE2 group (composed of the women aged 53–65 years) had a median age of 60 ± 4 years, with 100% of them being menopausal. Furthermore, mean age of the CUG was 53 ± 5 years versus 50 ± 10 years of the NCUG.

An important point to consider was the average duration of Cs uses during the office workday among the women employees, and it was 4.5 ± 2 hours. It has to be emphasized that the type of screen and the Cs were similar for all participants, and the clinical probes and tear collection were performed at the end of the daytime in all participants.

Moreover, all study participants were exposed to the same controlled environment during the working time. The environmental conditions were evaluated periodically by means of the workplace analyses (Table 2).

3.2. Evaluation of the Ocular Surface Status. A clinician global impression as well as a participant global self-assessment was

TABLE 3: Expression levels of the inflammatory molecules in tears from the women participants as expressed in picograms per microliter. These data are examined in greater detail in Figures 4 and 5.

	GM-CSF	IL2	IL-1β	IL5	IL10	IL6	TNF-α	IFN-γ
AGE1-CUG	5.5 ± 4	1.3 ± 0.7	12.9 ± 14.06	4.8 ± 5.9	2.2 ± 1.67	29.1 ± 2	215.1 ± 29.4	285.3 ± 32.5
AGE1-NCUG	6.5 ± 3	0.9 ± 1.25	6.7 ± 3	3.9 ± 6.3	3.4 ± 2.32	5.8 ± 1.2	233.6 ± 30	301.3 ± 257
P value	0.86	0.09	0.000	0.42	0.17	0.000	0.21	0.66
AGE2-CUG	7.6 ± 3	1.5 ± 0.2	43.1 ± 4.2	4.1 ± 7	3.02 ± 2.53	32.4 ± 5.22	223.3 ± 25.4	298.1 ± 321.5
AGE2-NCUG	7.5 ± 2.15	1.2 ± 2.3	18.7 ± 3.05	3.1 ± 5.4	2.5 ± 3	20.7 ± 7.6	217.1 ± 28	312.2 ± 77
P value	0.34	0.65	0.007	0.26	0.54	0.000	0.13	0.58

the endpoint to estimate the OS status that was completed by the OSDI questionnaire scores. The Cs user women from the AGE1 and AGE2 groups complained of one or more DEs signs/symptoms of the following: itchiness, soreness, irritation, foreign body sensation, photophobia, redness, eye strain, tired eyes, eye pain, blurred vision, vision loss, or headache associated with eye pain.

The overall OSDI score delineated the OS severity. It was diagnosed that 33% of the AGE1 and 64% of the AGE2 Cs users had mild or mild-to-moderate DEs, as confirmed by the anatomic and functional eye probes. Furthermore, most of these women participants (89%) utilized eye drops and none of them had severe dryness or Sjögren syndrome.

As shown in Figure 2, the Schirmer test scores (by wetting the paper strip during 5 min) were significantly lower in the AGE1-CUG and AGE2-CUG groups than in the NCUG of women employees ($P = 0.0002$ and $P = 0.0000$, resp.). These data reflect the altered tear film in the women using the Cs during the working time (Figure 2).

The blinking frequency (near) for the right and left eyelid values were combined and analyzed as a function of age and the results showed lower frequency in the AGE1-CUG and AGE2-CUG (9.5 ± 3.81/5.77 ± 2.27 blinking per 1 min, resp.) than in the NCUG (14.55 ± 6.50/9.61 ± 4.98/blinking per 1 min, resp.). Our results strongly suggest that there is a trend toward decreasing blink amplitude and peak velocity with age for spontaneous blinks. Furthermore, the blinking process is altered by the exposure to the visualization screen in women employees, compared to the nonusers ($P = 0.000$).

3.3. Multiplex Analysis of Inflammatory Molecules in Tears.
With the assayed amounts of tears utilized in the present work (mean 14 ± 8 mL) it was permitted to detect the majority of molecules related to inflammation (as in the human cytokine panel utilized herein) in 92% of the samples. Polystyrene beads coupled covalently to specifically directed antibodies (cytokines/chemokines) were allowed to react with each tear sample containing an unknown amount of them, or with a standard solution containing a known amount of these molecules, at room temperature for 1 hour, following the manufacturer's instructions. Detection data of the inflammation molecules from the tear samples of the women employees are summarized in Table 3 and expressed in picograms/μL. The following molecules showed very low or undetectable levels in the tear samples: IL4, IL8, and VEGF, and the results were excluded from Table 3.
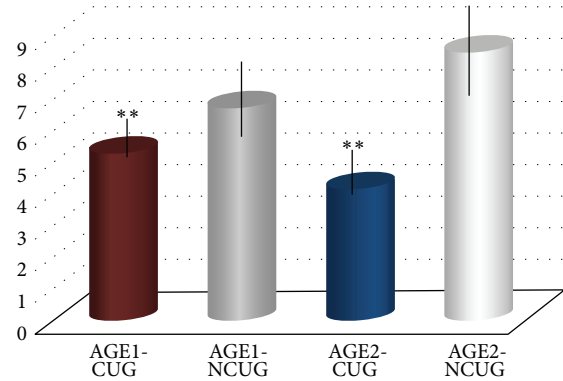

FIGURE 3: The Schirmer test scores in the age groups of women employees and in relation to being exposed or nonexposed to computer screens during the workday. Data are mean ± SD for all participants in each group.

Data comparison for the AGE1 and the AGE2 women employees revealed that the IL-1β and IL6 tear levels were significantly higher (a twofold increase) in the older women compared to the younger employees (Figure 3).

In relation to the Cs uses, when comparing the AGE1-CUG versus the AGE1-NCUG and the AGE2-CUG versus the AGE2-NCUG the results also showed statistically significant differences in the tear expression of the assayed kit of cytokines/chemokines, with the most relevant concentrations of proinflammatory mediators pertaining to the IL-1β and IL6, as reflected in Figure 4.

3.4. Influence of the Oral Supplementation with Antioxidants and Omega 3 Fatty Acids.
Average amount of the collected tear samples was 24.9 ± 6.8 μL from the controls versus 15.6 ± 4.8 μL in the DEs group. This latter augmented noticeably after supplementation (about 25%) in the AGE2 group and the CUG of women employees that were taking the A-ω3 supplement as compared to those not taking the A-ω3 pills.

A significant reduction in the expression levels of the inflammation biomarkers was detected in the AGE1-CUG and AGE2-CUG supplemented groups in contrast to the nonsupplemented women employees. A more precise analysis strongly indicated that the IL-1β and IL6 were the most significantly reduced proinflammatory biomarkers in the A/ω3 supplemented study subjects (Figure 5).

IL-1β expression in tears
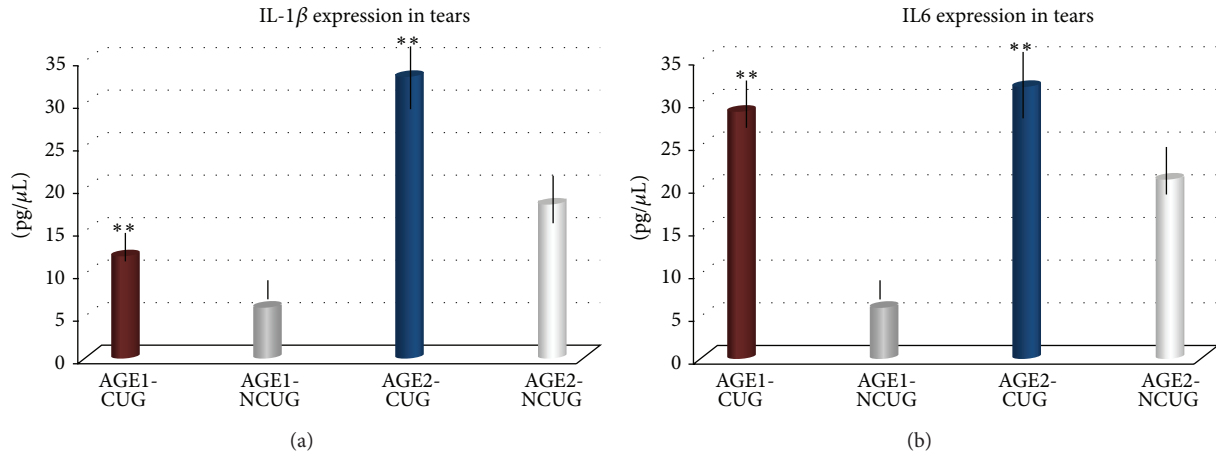
IL6 expression in tears

(a)

(b)

FIGURE 4: (a) Data comparison of the multiplex tear samples analyses for the two main age groups of women employees exposed and nonexposed to computer screens regarding the IL-1β tear expression levels. (b) Determination of IL6 expression in tears compared to the computer screen exposures in the age study groups of women participants. Bars, mean ± SD. Significance levels were taken at $^*P < 0.01$; $^{**}P < 0.001$.
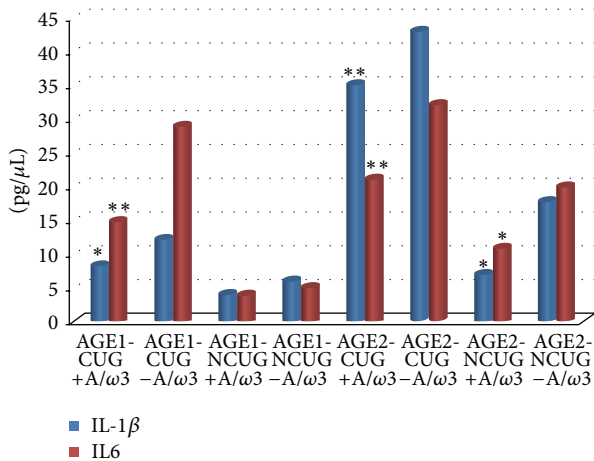

FIGURE 5: Expression levels of main inflammatory biomarkers in tears from the supplemented and the nonsupplemented women employees, according to the main age groups. Comparative analysis between groups (significance level) $^*P < 0.01$; $^{**}P < 0.001$.

Up to 70% of the AGE1-CUG and AGE2-CUG women taking the supplement pills according to the prescribed doses (+A/ω3) significantly improved their subjective/objective DEs-associated manifestations at the end of the study, as compared to the participants not taking the oral supplementation (−A/ω3).

No adverse effects were recorded in relation to the oral intake of this supplementation in the corresponding subgroups.

4. Discussion

4.1. Age-Related Ocular Surface Disorders.
Visual impairment in adults and older people is a major health problem worldwide. Age is a risk factor for OS disorders, especially in

women [5, 6, 10, 11, 38, 46]. The DEs are characterized by ocular surface damage, reduced tear film stability, and tear hyperosmolarity accompanied by signs and symptoms [1, 2, 4]. Inflammatory components are also considered within the DEs [28–32, 44], which is also an important point of the present work.

In this study we have evaluated the OS status in a sample of healthy adult women employees that were Cs users during the working time, following an age-related fashion. Demographic, ophthalmologic, and molecular data were obtained from all participants in the study and the integration of these data allowed us to better evaluate the incidence and severity of age-related DEs in our women employees. In fact, an abnormally low Schirmer test and reduced spontaneous blinking frequency were found in the older women compared to the younger employees, in agreement with previous reports [16, 20, 46, 47]. These data regarding the high DEs prevalence in relation to aging strongly agree with similar reports [5–7, 10, 11, 18, 46]. In a recent review it was confirmed that conditions predisposing older adults to DEs include systemic and topical medications, eyelid laxity, menopause, and chronic systemic inflammation [4, 7, 10, 11, 47]. In this context, early detection and adequate management of DEs in the older Cs user women may help in preventing ocular surface complications such as corneal ulcers and scarring leading to visual disability.

4.2. Computer Screens Exposures in the Women Employees.
It has been reported that in adult employees the Cs use and/or the body position may influence the visual performance and the eye comfort [15, 22]. Our representative population of Cs user women employees had a mean of 4.5 hours of exposure, and their symptoms were similar to those reported in surveys of video terminal users during the working time [19–22].

To fully evaluate the risk factors that may compromise the OS integrity in the employees, we took advantage of the controlled environmental conditions of the workplace

provided to our study employees. Individuals are vulnerable to adverse environments that may increase tear evaporation and decrease goblet cell density and acquired ocular surface pathology. Lifestyle factors contributing to DEs include working in a dry atmosphere; looking at visualization screens or reading without blinking frequently; treatments for allergy; use of diuretics, beta-blockers, antispasmodics, birth control pills, and other medications; diets that provide insufficient water or essential fatty acids; autoimmune disorders (arthritis, lupus erythematosus); and menopause [1, 3, 6, 9]. No systemic chronic disorders and no special local or systemic treatments and/or particular exposures to external or internal damaging agents were recorded. Therefore, our controlled parameters in the office allow us to exclude these factors for DEs, pointing to both the age and gender, as well as the Cs uses as major risk factors for OS pathologies in our middle-age and older population of administrative women office employees.

The incidence of DEs that was diagnosed during the present study in the Cs user employees was noticeable, with an important prevalence in the group of older women (33% of the AGE1 and 64% of the AGE2). It has to be emphasized that 100% of the older women were menopausal, with the hormonal disbalance (testosterone, progesterone, and estrogens), independently of the hormone replacement therapy, being a relevant factor in the initiation and progression of DEs. [12–15, 48]. Furthermore, decreased Schirmer test scores and blinking frequency were seen in the CUG as compared to the NCUG of women employees, as depicted in similar works [15, 16, 19, 22].

We also considered that Cs uses may extend beyond work activities and into leisure time. When asked about this, women of the CUG confirmed only low utilization of video games, internet, and social networks. Furthermore, given that the environmental conditions in the office were periodically recorded and accordant with a healthy workplace (see Table 2) and that no participants had systemic disease, aggressive treatment, or Sjögren syndrome, the fact of using Cs during the workday appears to be a major risk factor for the development and progression of DEs in our women employees. In spite of this, current information is insufficient to completely understand the basic cellular and molecular mechanisms underlying DEs.

4.3. Inflammatory Mediators in Tears of the Women Employees.

The role of inflammation in the pathogenic mechanisms of DEs has also been investigated in the present work. Previous reports demonstrated an altered tear composition in DEs [2, 15, 17, 23–27, 45], including in air-controlled conditions for the study participants [49], as in the present work. A common underlying cytokine/chemokine-mediated inflammatory disorder in all ocular surface pathologies has been suggested, independently of the etiology [28–36, 44]. Results from the quantification of tear components related to the immune system among the study participants showed significant differences between groups and subgroups. In fact, the set of cytokines/chemokines assayed herein showed a differential expression profile regarding age (as shown in Table 3 and Figure 3). The most relevant differences were detected in relation to IL-1β and IL6, these two cytokines being important proinflammatory mediators involved in DEs [31–33, 44]. Moreover, the IL-1β and IL6 also showed significantly higher levels in the CUG as compared to the NCUG of women employees, reflecting a relevant inflammatory background in tears from these computer user patients. Interestingly, the increased cytokines in tears of the AGE2-CUG versus the AGE1-CUG of women employees strongly correlated with clinical DEs parameters, with results being in agreement with previous reports [31, 33–36]. Outstanding statements regarding inflammation and DEs from the Cullen Symposium on Corneal and OS Inflammation (Baylor College of Medicine, Houston, Texas, USA; 2005) strengthen the main results of the present work. According to this, cytokines produced by activated T cells increase the immune response by mediating adhesion molecules expression from the conjunctival blood vessels [41]. With the data provided by the tear analysis performed during the present work we may also contribute to rising inflammation as a milestone for the development and progression of DEs in middle-age and older employees exposed to visualization screens in the office. The host defense against chitin-containing pathogens includes production of chitinases. In this context, Musumeci et al. [47] and Bucolo et al. [50] have studied the role of AMCase in relation to OSD suggesting that chitinases may be important mediators of the inflammatory processes, constituting a potential diagnostic and therapeutic target in these pathologies.

4.4. Effect of a Combination of Antioxidants and Omega 3 Fatty Acids in the Ocular Surface Disorders.

At this point the question as to whether a combined formulation of A/ω3 may influence the evolution of DEs signs and symptoms in the affected women employees arises. It has been described that antioxidant supplements may help in counteracting the oxidative stress generated in the anterior eye segment pathologies [24, 27, 37, 38]. In addition, specific ω3 metabolic by-products may play an essential role in modulating the inflammatory response in health and disease [40]. Main results of our study are the significantly lower expression levels of the inflammatory molecules found in the CUg +A/ω3, as compared to the CUG −A/ω3, in agreement with previous reports [51–53]. Likewise, recent research from our group [36, 37] described the fact that a combination of A/ω3 improved cytokine/chemokine expression levels in tears of the affected patients as well as the subjective and objective DEs manifestations. In the present work, global amelioration in the clinical signs and symptoms was registered among the CUG +A/ω3 compared to the −A/ω3, at the end of follow-up. Up to 70% of ocular signs (dryness, photophobia, eye heaviness, burning sensations, and blurred vision) displayed a noticeable improvement in the supplemented subgroups of women employees.

A limitation of this study is the absence of a placebo group to the A/ω3 oral supplementation participants. This was an open-label study with a potential bias, but it was reduced with the use of randomization and the utilization in parallel with the nonsupplemented subgroups.

5. Conclusions

Considering these findings, we may suggest that a specific appropriate combination of A-ω3, as in the present work, may benefit the OS integrity in women employees that were Cs users during the working time. Further research will indicate whether the supplement is also effective (or not) in redressing the OS damage. Moreover, cytokine/chemokine expression and availability in tear samples can be helpful biomarkers for diagnosing women at risk of DEs and visual impairment in relation to computer work.

Given the estimated number of the population at risk of DEs due to the Cs uses during the workday, the challenge that lies ahead is of real impact and requires screening campaigns among adult employees, mainly those women at age of 50+ and working with Cs, for detecting DEs cases that remain occult. Nutraceutics with an appropriate combined formulation of A-ω3 may help in managing DEs in women employees with daily computer work.

Abbreviations

A/ω3:   Antioxidants and omega 3 fatty acids
+A/ω3:   Oral supplemented group
−A/ω3:   Nonsupplemented group
AGE1:   Women aged 40–52 years
AGE2:   Women aged 53–65 years
Cs: Computers
CUG: Computer users group
DEs: Dry eyes
IL: Interleukins
NCUG: No computer users group
OS: Ocular surface
SD: Standard deviation
VEGF: Vascular endothelial growth factor
TF: Tear film.

Disclosure

All authors fully agree with the submission of this work to the OCD Ophthalmology Special Issue of Biomed Research International Journal and the corresponding copyright. The present work was not sent to any other journal.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Alfredo Ribelles and Carmen Galbis-Estrada share the first place within the authors of this work.

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Clinical Study

Femtosecond Laser Assisted Deep Anterior Lamellar Keratoplasty Outcomes and Healing Patterns Compared to Manual Technique

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The purpose of the study is to report the visual, refractive, and wound healing pattern outcomes of femtosecond assisted deep anterior lamellar keratoplasty (DALK) compared to the conventional manual technique. DALK was performed on 50 eyes of 47 advanced keratoconus patients. The patients were divided into two groups, 25 eyes each, depending on whether femtosecond assisted or manual DALK technique was performed for the side cut of the procedure only. Patients were followed up at 1 month, 6 months, and 1 year for visual acuity, clinical refraction, corneal cylinder, date of suture removal, and side cut corneal healing pattern according to new grading classification of the side cut scar (Grade 0 = transparent scar, 1 = faint healing opacity, 2 = evident healing opacity, 3 = significant opacity with some cosmetic imbalance, and 4 = highly significant opacity with very significant cosmetic imbalance). Outcomes are reported at one year. In conclusion, femtosecond assisted and manual DALK show comparable visual and refractive outcomes but femtosecond assisted DALK shows more evident corneal wound healing patterns at the side cut. This observation may indicate that an activated cornea wound healing might allow earlier suture removal when femtosecond technology is used to perform the side cut for DALK.

1. Introduction

Deep anterior lamellar keratoplasty (DALK) is a surgical procedure in which a diseased corneal stroma is excised until the Descemet membrane (DM) or as close as possible followed by transplantation of the donor corneal button free from DM and endothelium. This procedure can be considered the first line surgical choice for corneal stromal diseases with intact endothelium [1], better than penetrating keratoplasty (PK) as far as DALK is associated with a lower risk of graft rejection, secondary glaucoma, complicated cataract, and postoperative long term loss of endothelial cells [2].

A report from the American Academy of Ophthalmology concluded that DALK was equivalent to PK in terms of graft survival, best corrected visual acuity, and refractive errors, but DALK may be the superior procedure regarding the preservation of corneal endothelial cell density [3]. Femtosecond assisted DALK has been suggested as a more advanced and probably better procedure for the performance of DALK surgery, in particular the side cut [4].

Corneal grafting techniques are affected by many variables, biological, immunological, biomechanical, surgical, and technological variables, which make this procedure intrinsically variable. This variability of outcomes affects not only the visual and refractive outcomes but also the biological performance of the tissues affected by the graft and by the surgical trauma and, overall, the visual recovery of the patient [5].

The femtosecond laser (FSL) is able to make precise corneal incisions with customized graft edges and lamellar planes for both donor and recipient corneas [6]. The use of femtosecond laser due to its precision and control in sizing of the donor and recipient corneal buttons might help in the control of many of the previously mentioned variables making corneal grafting surgery a better and more controllable technique with better outcomes [5].

In addition, the different patterns that can be performed with the FSL allow an excellent apposition of the tissue that result in rapid wound healing, which may lead to earlier removal of the suture and faster patient recovery [7].

The aim of our study is to compare manual and femtosecond assisted DALK (Fs-DALK) in terms of refractive and visual outcomes and to ascertain whether corneal wound healing patterns appear in Fs-DALK different to those that are observed in the manual technique.

2. Materials and Methods

2.1. Study Design. Prospective and retrospective consecutive comparative clinical series of cases. The study was carried out in accordance with the Declaration of Helsinki [8] and was approved by the Ethical Committee (CEIC) of our institution in Alicante.

2.2. Inclusion Criteria. Patients included in this study underwent DALK due to advanced keratoconus. All patients were free of any other ocular comorbidity other than the corneal ectatic disorder leading to the indication of corneal graft. Patients were matched for age and sex to create equivalent groups for the purpose of the study. If complications such as perforation during the stromal dissection happened, the case was excluded from the investigation and replaced by another with similar profile.

We divided the patients into two groups according to the technique used to perform the side cut in the donor and the recipient cornea.

2.2.1. Group 1 Femtosecond Assisted DALK. 25 eyes of 22 patients underwent femtosecond laser mushroom configuration DALK between January 2010 and May 2013. All surgeries were performed by the same expert surgeon (JLA) at Vissum Instituto Oftalmologico, Alicante, Spain.

2.2.2. Group 2 Manual DALK. 10 eyes of 10 patients underwent manual trephine straight-edge configuration DALK between May 2012 and January 2013. Other 15 cases of manual DALK were performed by another expert surgeon (RB) during the same period of time at Institut Universitari Barraquer, Universitat Autónoma de Barcelona, Spain. These cases were analyzed retrospectively at one year of the follow-up using the same observational protocol as in the other cases. Both surgeons followed the same surgical and postoperative protocol.

2.3. Surgical Technique. Manual trephine straight-edge configuration DALK was performed using the Melles technique

FIGURE 1: Mushroom configuration.

implemented by the injection of air in the residual stroma left by the manual dissection to better accomplish the dissection of the deep stroma. The dissection was performed in all cases down to the Descemet layer or leaving minimal amounts of residual stroma tissue in case that big bubble was not accomplished. The donor cornea was in all cases the same diameter as the recipient button (8 mm of diameter). The donor was secured to the recipient with a double torque-antitorque 16 bites' continuous suture.

Femtosecond laser mushroom configuration DALK was performed by a 60 KHz Intralase Femtosecond Laser (IntraLase, Abbott Medical Optics, Santa Ana, California, USA). Only the side cut was performed. The corneal stroma was excised and completed down to the Descemet membrane or to deepest stromal layers assisted by the injection of air (big bubble technique).

For the side cut a full-thickness mushroom configuration cut was made on the donor cornea first and then a nonpenetrating mushroom configuration on the recipient, using the FS laser system. The energy used was 2 to 2.3 mJ depending on the case. In the recipient cornea, the depth of the anterior side cut was about 60% of the thinnest corneal pachymetry, and the depth of the posterior side cut was about 80% of the thinnest corneal pachymetry, leaving a ring lamellar cut of 1 mm (Figure 1).

In the donor cornea, the Descemet membrane (DM) and endothelium were debrided in all cases of both groups assisted by trypan blue dye (vision blue dye).

2.4. Postoperative Management

  (i) Topical antibiotic eye drops: cetraflux 3 mg/mL (ciprofloxacin) 4 times daily for 1 week till complete epithelial healing and removal of contact lens (once daily after 1 week if epithelium did not completely heal or contact lens were still not removed).

 (ii) Topical steroid eye drops: Pred Forte (prednisolone acetate) 8 times daily for 1 week and then tapering gradually through 1 month and finally once daily forever.

(iii) Contact lens for 1 week for comfort of the patient and till complete epithelial healing.

 (iv) Cycloplegic eye drops twice daily for 3 days.

  (v) Tear substitute if needed.

Postoperatively all patients were followed up by the surgeon and a cornea specialist at the cornea units of each institution at 1 month, 6 months, and 1 year for visual acuity (uncorrected and best corrected) and corneal cylinder studied by corneal

TABLE 1: Analysis of visual outcomes.

Time	Femtosecond DALK	Manual DALK	P value
UCDVA (mean)			
1 month	0.17	0.14	0.308
6 months	0.20	0.23	0.801
1 year	0.18	0.20	0.757
BCDVA (mean)			
1 month	0.30	0.39	0.118
6 months	0.45	0.52	0.262
1 year	0.55	0.54	0.965

UCDVA: uncorrected distant visual acuity; BCDVA: best corrected distant visual acuity.

TABLE 2: Analysis of corneal cylinder.

|	Femtosecond DALK	Manual DALK	P value
1 month	5.16 (1.03–13.58)	5.30 (1.09–10.01)	0.843
6 months	4.60 (1.13–8.47)	4.79 (1.26–20.20)	0.467
1 year	5.43 (1.00–10.27)	4.62 (0.49–13.70)	0.180

FIGURE 2: Healing in manual and femtosecond DALK. Healing is more evident in femtosecond assisted DALK.

topography map. Suture removal was not performed in any case before the end of the 12th month of follow-up. The side cut corneal healing pattern was evaluated according to a grading system established for the purpose of this investigation. The grading was performed as observed and registered photographically by slit lamp photography with illumination at 45° light angle of incidence concerning the slit lamp observation optics placed orthogonal to the corneal vertex as observed by the first Purkinje reflex. The grading of the scar was performed as follows: Grade 0 = transparent scar, Grade 1 = faint healing opacity, Grade 2 = evident healing opacity, Grade 3 = significant opacity with some cosmetic imbalance, and Grade 4 = highly significant opacity with very significant cosmetic imbalance. The same investigator (AA) performed all the slit lamp side cut wound healing gradings of this investigation.

2.5. Statistical Analysis. SPSS V.21 was used for the analysis. The postoperative outcomes between manual and femtosecond groups were compared using Mann-Whitney U test. For all the analysis, P value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline Characteristics. There were no significant differences in age or gender between the manual and femtosecond groups ($P = 0.211$). A big bubble was achieved following the stromal dissection in 20 of the cases of the Fs-DALK and in 21 of the manual cases.

3.2. Visual Outcomes. There were no significant differences in uncorrected distant visual acuity (UCDVA) and best corrected distant visual acuity (BCDVA) at 1 month, 6 months, and 1 year between the two groups (Table 1).

3.3. Corneal Topography Cylinder Analysis. There were no significant differences in corneal cylinder taken by corneal topography at 1 month, 6 months, and 1 year between the two groups (Table 2).

3.4. Healing Pattern at the Side Cut between the Donor and Recipient Cornea. Slit lamp pictures of all cases were
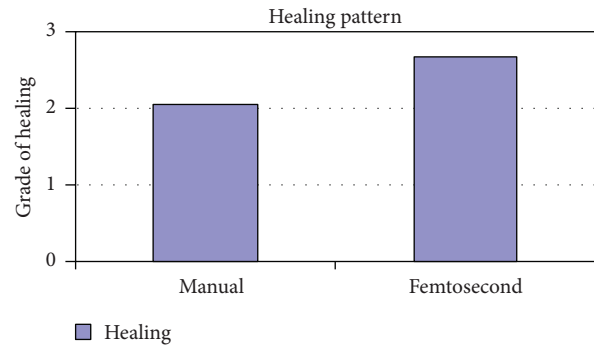
reviewed by an independent observer for both surgeons. There was a statistically significant difference in the side cut corneal healing pattern between the two groups (P value < 0.05), and healing is more evident in the femtosecond group (Figure 2).
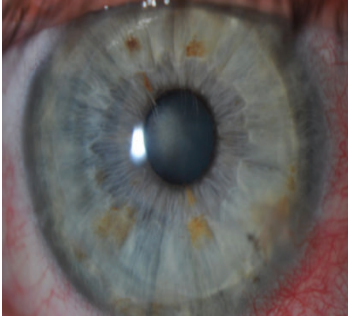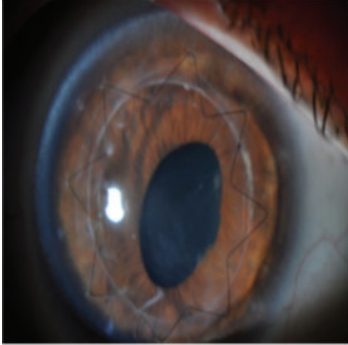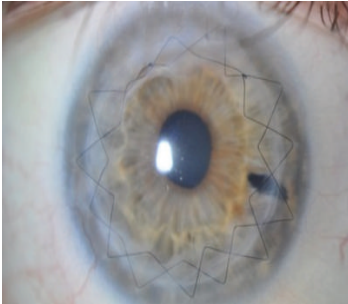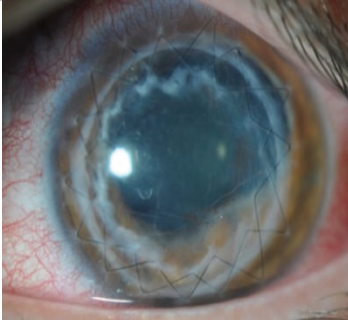
52% (13 eyes) of femtosecond assisted DALK cases showed wound healing patterns Grades 3 and 4 while only 12% (3 eyes) of manual DALK cases showed the same grades of wound healing (Table 3).

4. Discussion

The use of the femtosecond laser in DALK avoids manual trephination and allows more precise identification of tissue depth and insertion of the air needle by following the plane between the lamellar and posterior laser side cuts. Injection of air at this precisely predefined pre-Descemet plane may facilitate the big bubble formation with full baring of DM [9, 10]. Using the FSL to create shaped wound configurations in DALK may combine the mechanical and wound healing advantages found for stepped corneal wounds in PKP with the advantages of the lamellar surgery [11].

As variability in stromal thickness in eyes with advanced keratoconus, ectasia, or dense and deep stromal scars may limit the ability of the femtosecond laser to produce a uniform lamellar plane, we used the FS laser only to create the side cut both in donor and in recipient cornea, while leaving a minimal amount of residual corneal tissue. With this we tried to control the potential risk of creating a large buttonhole or uncontrolled Descemet membrane perforation with the femtosecond laser. In our study, femtosecond laser was programmed to leave a residual stroma according to the pachymetry of each case. Manual dissection of the posterior lamella assisted by air injection (big bubble technique) was chosen, as it allows the surgeon to create a lamellar plane

TABLE 3: Analysis of side cut corneal wound healing pattern.

			Femtosecond DALK (% of eyes)	Manual DALK (% of eyes)
Grade 0	Transparent scar		16% (4 eyes)	16% (4 eyes)
Grade 1	Faint healing opacity		8% (2 eyes)	8% (2 eyes)
Grade 2	Evident healing opacity		24% (6 eyes)	64% (16 eyes)
Grade 3	Significant opacity with some cosmetic imbalance		40% (10 eyes)	12% (3 eyes)
Grade 4	Highly significant opacity with very significant cosmetic imbalance		12% (3 eyes)	0%

parallel to the more regular posterior corneal surface as opposed to the front surface.

In addition to its advantage in facilitating the DALK procedure, using the FSL to create corneal-shaped wound configurations offers the advantages of better donor-recipient fit with increased surface area contact, which may accelerate wound healing [12, 13].

The mechanical stability of the mushroom configuration (larger anterior diameter cut) created using the FSL has been shown to be superior to traditional straight cuts [13]. In addition, it might have an advantage in keratoconus cases and extensive corneal scars because it provides a larger amount of donor-recipient tissue to interact for the purpose of corneal wound healing consistency [13].

In this study, we compared the outcomes after FSL-assisted mushroom configuration with manual trephine straight edge configuration DALK. Mean postoperative keratometric cylinder, uncorrected distant visual acuity (UCDVA), and best corrected distant visual acuity (BCDVA) were comparable between both groups. FSL and manual trephine DALK techniques provided patients with significantly improved vision postoperatively but the FSL group achieved this improvement faster (mean UCDVA is better at 1 month in the FSL group). The greatest improvement in mean BCDVA occurred at 1 year in both groups. Although it was better in the FSL group at 1 year, BCVA was not significantly different between either group at 1 month, 6 months, and 1 year ($P = 0.118$, $P = 0.262$, and $P = 0.965$, resp.). UCDVA was not significantly different between either group at 1 month, 6 months, and 1 year ($P = 0.308$, $P = 0.801$, and $P = 0.757$, resp.).

Corneal cylinder (topographic) was not significantly different between either group at 1 month, 6 months, and 1 year ($P = 0.843$, $P = 0.467$, and $P = 0.180$, resp.).

The main finding of this investigation was the observation of an evident and statistically significant difference in the side cut corneal healing pattern between the two groups ($P < 0.05$) as observed and graded by the slit lamp appearance by an independent observer, and healing is more evident in the femtosecond group.

The reasons for the femtosecond assisted DALK to show a more active wound healing leading to leucomatous wound could be either due to the larger area of contact between the donor and recipient tissues and/or due to femtosecond laser related biological activation of the corneal tissues, which should be related to the level of energy used for the creation of the side cut; 52% (13 eyes) of femtosecond assisted DALK cases showed wound healing patterns Grades 3 and 4 while only 12% (3 eyes) of manual DALK cases showed the same grades of wound healing.

Although it was previously suggested that using FSL may accelerate suture removal due to faster wound healing related to a better donor-recipient fit with the increased surface area contact [13, 14], in no study performed formerly has it been reported any consistent evidence that such enhanced corneal wound healing actually exists. The results of the present study demonstrate that different and more evident wound healing patterns do exist in Fs-DALK. According to the outcomes of this investigation, earlier suture removal might be possible in FS assisted cases once evidence of initial scarring is observed along the slit lamp biomicroscopic postoperative evaluation.

5. Conclusions

This study concluded that Fs-DALK is followed by an increase in the wound healing pattern as observed by clinical biomicroscopy. However, Fs assisted and manual techniques show comparable visual and refractive outcomes at one year of the surgery. The FSL group achieved a significantly improved visual and refractive outcome in terms of UCDVA only at 1 month, an outcome that in our opinion should be considered to be anecdotal. The differences in the wound healing patters in Fs-DALK should be considered to be relevant as the higher levels of the scale used in this investigation imply cosmetic imbalance to the patient, especially in darkly pigmented eyes.

To the best of our knowledge this is the first report in which an increased wound healing response in corneal grafting surgery following femtosecond assisted techniques is demonstrated. Such finding may have implications in the indication of Fs laser in the surgery of keratoconus and in the indication of suture removal following this procedure.

Conflict of Interests

The authors have no financial interests to disclose.

Acknowledgments

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Research Article

Diagnostic Accuracy of Nonmydriatic Fundus Photography for the Detection of Glaucoma in Diabetic Patients

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Purpose. To determine the diagnostic accuracy for glaucoma of a set of criteria with nonmydriatic monoscopic fundus photography (NMFP) in diabetics. *Methods.* Diabetics recruited from a screening program for diabetic retinopathy and diabetic glaucoma patients recruited from our glaucoma unit were included. Any patient with evidence of diabetic retinopathy was excluded. Diabetic patients had to have no visual field defects to be included as controls. Glaucoma patients had to have a glaucomatous field defect in at least one eye to be included. One NMFP was taken per eye for all subjects. These photographs were evaluated by two masked glaucoma specialists for the presence of the following: bilateral cup to disc (C/D) ratio ≥0.6, notching or thinning of the neuroretinal rim, disc hemorrhages, and asymmetry in the C/D ratio between both eyes ≥0.2. This evaluation led to a dichotomous classification: if any of the above criteria was present, the patient was classified as glaucoma. If none were present, the patient was classified as normal. *Results.* 72 control subjects and 72 glaucoma patients were included. Evaluation of NMFP had a sensitivity of 79.17% and a specificity of 80.56% for specialist 1 and a sensitivity of 72.22% and a specificity of 88.88% for specialist 2 for the detection of glaucoma. The overall accuracy was 79.83% and 80.55%, respectively. *Discussion.* NMFP evaluation by a glaucoma specialist may be useful for the detection of glaucoma in diabetics.

1. Introduction

Open-angle glaucoma is one of the leading causes of irreversible visual loss worldwide [1]. Globally, there are an estimated 60 million people with glaucomatous optic neuropathy and an estimated 8.4 million people who are blind as a result of glaucoma. These numbers are set to increase to 80 million and 11.2 million by 2020 [2]. Because initial glaucoma is asymptomatic, approximately 50% of patients with glaucoma are unaware that they suffer a disease that can lead to blindness if the condition goes untreated [3, 4]. Efforts have been made to develop screening programs for glaucoma; however, there is insufficient economic evidence on which to base recommendations regarding screening for glaucoma [5]. Nevertheless, targeted screening of subgroups at higher risk

of developing glaucoma may be viable. One such group may be diabetic patients. Although there is conflicting evidence, a meta-analysis published in 2004 reported that diabetic patients are at significantly increased risk of developing glaucoma [6].

Screening for diabetic retinopathy is now often performed through nonmydriatic fundus photography [7–9]. Although the primary objective is to detect patients with diabetic retinopathy which requires an evaluation by a retina specialist, the graders may also assess the optic nerve. Patient referral in each healthcare system varies, but patients with anomalous optic nerves will probably be referred for further investigation to glaucoma units. A recent publication has evaluated the glaucoma referrals from a local unit of the English National Screening Programme for Diabetic

Retinopathy, reporting a positive predictive value of 78.8% at detecting glaucoma of dilated fundus photography [10]. In contrast, in a large survey to determine the outcomes resulting from optometric referrals, only one in five subjects had glaucoma [11]. Thus, Ong et al. suggest that the opportunity of using images taken during diabetic retinopathy screening to detect glaucoma in one of the highest risk target populations should not be missed. However, there remain several obstacles: one of them is that the evaluation of the optic nerve is highly subjective. Another is that the diagnostic accuracy of fundus photography is still unknown.

In an attempt to reduce the subjectiveness of optic nerve assessment, we designed this study, in which a set of criteria for detecting glaucomatous damage was employed. Our aim was to evaluate the accuracy of optic nerve head evaluation using these criteria in nonmydriatic fundus photography (which is the method for diabetic retinopathy screening in our sanitary area) for the diagnosis of glaucoma in diabetic patients.

2. Methods

This study was designed as a case-control study; this design was chosen because of the low prevalence of glaucoma even in a high-risk population such as diabetics. Ethics committee approval was obtained; the study adhered to the tenets of the declaration of Helsinki. All patients were informed of the nature of the investigation and signed a written consent form. Diabetic nonglaucomatous subjects, which will be referred to from now on as control subjects, were randomly recruited from diabetic patients sent to our hospital's screening program for diabetic retinopathy. Glaucoma patients with diabetes were recruited from our hospital's glaucoma service.

All study subjects underwent an extensive ophthalmologic evaluation, including nonmydriatic fundus photography, best-corrected visual acuity (BCVA), intraocular pressure (IOP) measurement with a Goldmann applanation tonometer, central corneal thickness measurement with contact ultrasonography, anterior and posterior pole biomicroscopy (with cup-to-disc (C/D) ratio estimation after pupillary dilation), visual field testing, and optical coherence tomography (OCT) scanning of the optic nerve head. Visual fields were performed on a Humphrey perimeter with the Swedish Interactive Threshold Algorithm 24-2 strategy (Carl-Zeiss Meditec, Dublin, California, USA). Visual fields were considered reliable if there were fewer than 20% false-positive responses, false-negative responses, and fixation loses. Optical coherence tomography was performed and analysed with the Optic Disc Cube 200 × 200 protocol of Cirrus OCT (Carl-Zeiss Meditec, Dublin, California, USA). This protocol provides, in addition to other measurements, the average retinal nerve fiber layer (RNFL) thickness, disc area, and vertical C/D ratio.

Fundus photographs were taken by nurses trained by a technician and an ophthalmologist on how to operate a nonmydriatic fundus camera (TRC-NW200, Topcon Europe Medical, Netherlands). Ideally, one image of the posterior pole, including the optic nerve head and the macula, was to be taken for both eyes of all participants. Images were then forwarded through a safe telematic line to a tertiary care hospital, where they were arranged in order to be assessed in alphabetical order with no imposed time limits by two masked glaucoma specialist observers (Gema Rebolleda and Francisco J. Muñoz-Negrete). Another glaucoma specialist (Inés Contreras) oversaw all the examinations performed.

Subjects were classified as controls if no evidence was found of diabetic retinopathy or any other ocular disease apart from mild cataracts. Visual fields had to be reliable, with no glaucomatous defects. No cut-off point was set for C/D ratio in order for a subject to be included as a control. Since this study focused on the ability of fundus photography to detect glaucoma, including control subjects with previously normal C/D ratios was likely to bias the results in favour of subjective assessment of the optic disc. Glaucoma subjects had to have a reproducible glaucomatous visual field defect in at least one eye, defined as two or more contiguous points with a pattern deviation $P < 0.01$ sensitivity loss or more, or three or more contiguous points with $P < 0.05$ sensitivity loss or more, in the superior or inferior arcuate areas or an abnormal result in a glaucoma hemifield test. Glaucomatous field defect severity was classified according to the Hodapp classification [12]. In order to be included in the study, glaucomatous subjects must not have any evidence of any other ocular pathology, apart from mild cataracts. Exclusion criteria for both controls and glaucomatous subjects were the presence of a BCVA of less than 20/40 and of a spherical equivalent of more than 5 diopters.

Fundus photographs obtained by nonmydriatic fundus photography were presented for evaluation to two experienced observers who were masked to participant clinical details and the proportion of control and glaucoma participants. The observers were asked to evaluate each pair of images for the presence of the following criteria of glaucomatous optic nerve damage (adapted from O'Connor et al. [13]):

  (i) bilateral C/D ratio of 0.6 or higher (Figure 1),

 (ii) notching or thinning of the neuroretinal rim,

(iii) disc haemorrhages (defined as hemorrhages at the border of the optic, running parallel to the nerve fibers in the nerve fiber layer, shaped liked splinters),

(iv) asymmetry in the C/D ratio between the two eyes of 0.2 or higher.

This evaluation led to a dichotomous classification: if any of the above criteria was present, the patient was classified as glaucoma (Figure 1). If none were present, the patient was classified as normal (Figure 2). The observers were also asked to estimate the vertical C/D ratio for each eye. In order to estimate intraobserver variability, specialist 2 was asked to reevaluate the photographs of 50 subjects chosen randomly.

2.1. Statistics. Sample size calculation: previous reports of stereoscopic evaluation of the optic nerve head by glaucoma specialists have shown that its sensitivity for the diagnosis of glaucoma ranges between 70 and 80% for a specificity of 80% [14–16]. Our working hypothesis was that nonmydriatic fundus photography could reach similar values. Thus, for
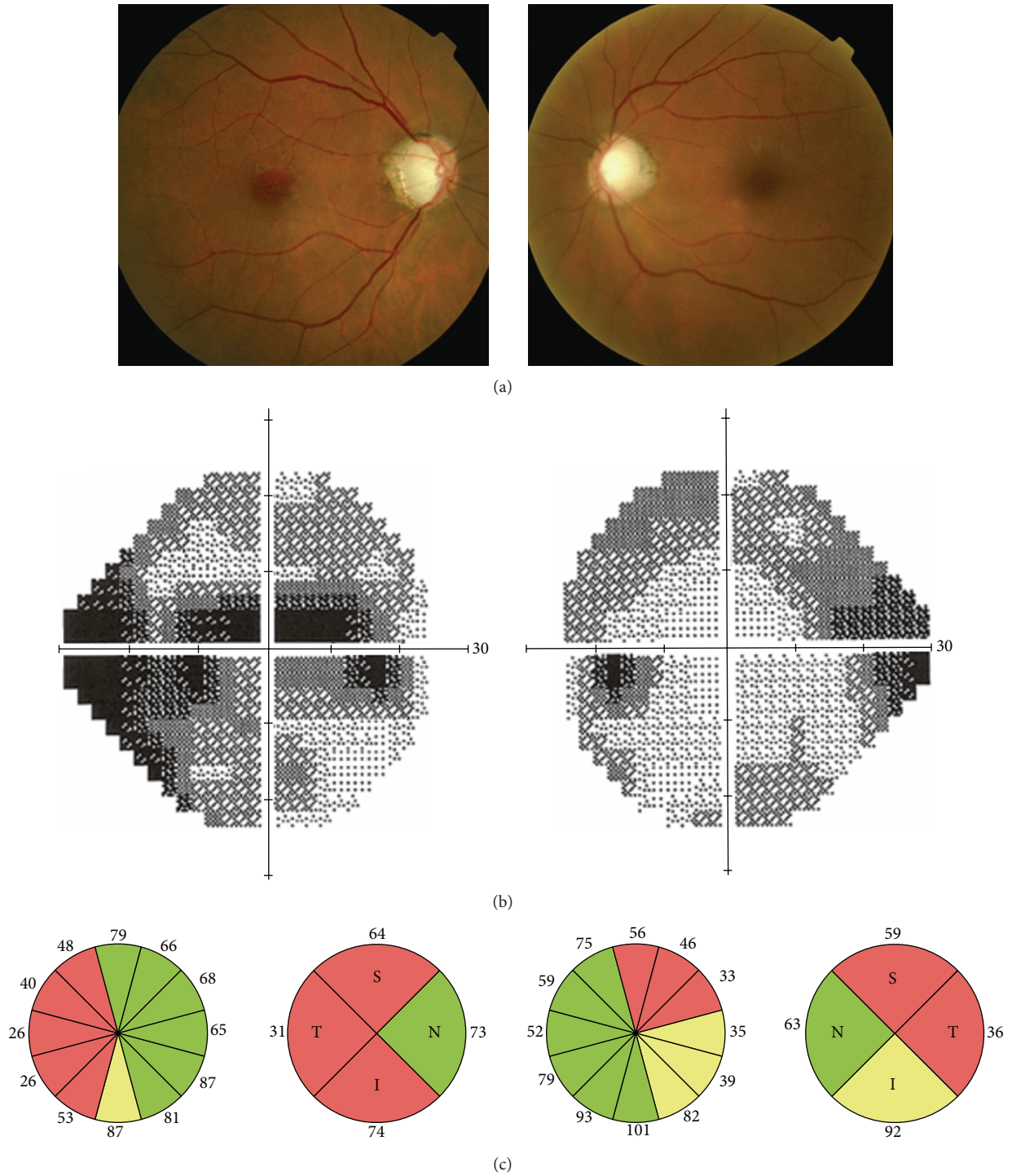
(a)

(b)

(c)

FIGURE 1: Nonmydriatic fundus photography, visual fields, and optical coherence tomography of a 77-year-old woman. Cup-to-disc ratio is 0.9 in both eyes, visual fields show severe diffuse defects, and optical coherence tomography reflects severe retinal nerve fiber layer loss in both eyes. Intraocular pressure was 23 mmHg in the right eye and 22 mmHg in the left eye. She was diagnosed with bilateral glaucoma.

an expected sensitivity of 70%, a precision of 10%, and a confidence level of 95% the number of glaucoma patients required would be 72 patients. Since the design of the study was a one to one case control, 72 healthy control subjects would be necessary for an expected specificity of 70%.

The sensitivity, specificity, and overall accuracy of the evaluation by the glaucoma specialists of nonmydriatic fundus photography for the diagnosis of glaucoma were calculated. Sensitivity was obtained dividing the number of glaucoma patients with signs of glaucomatous optic nerve damage
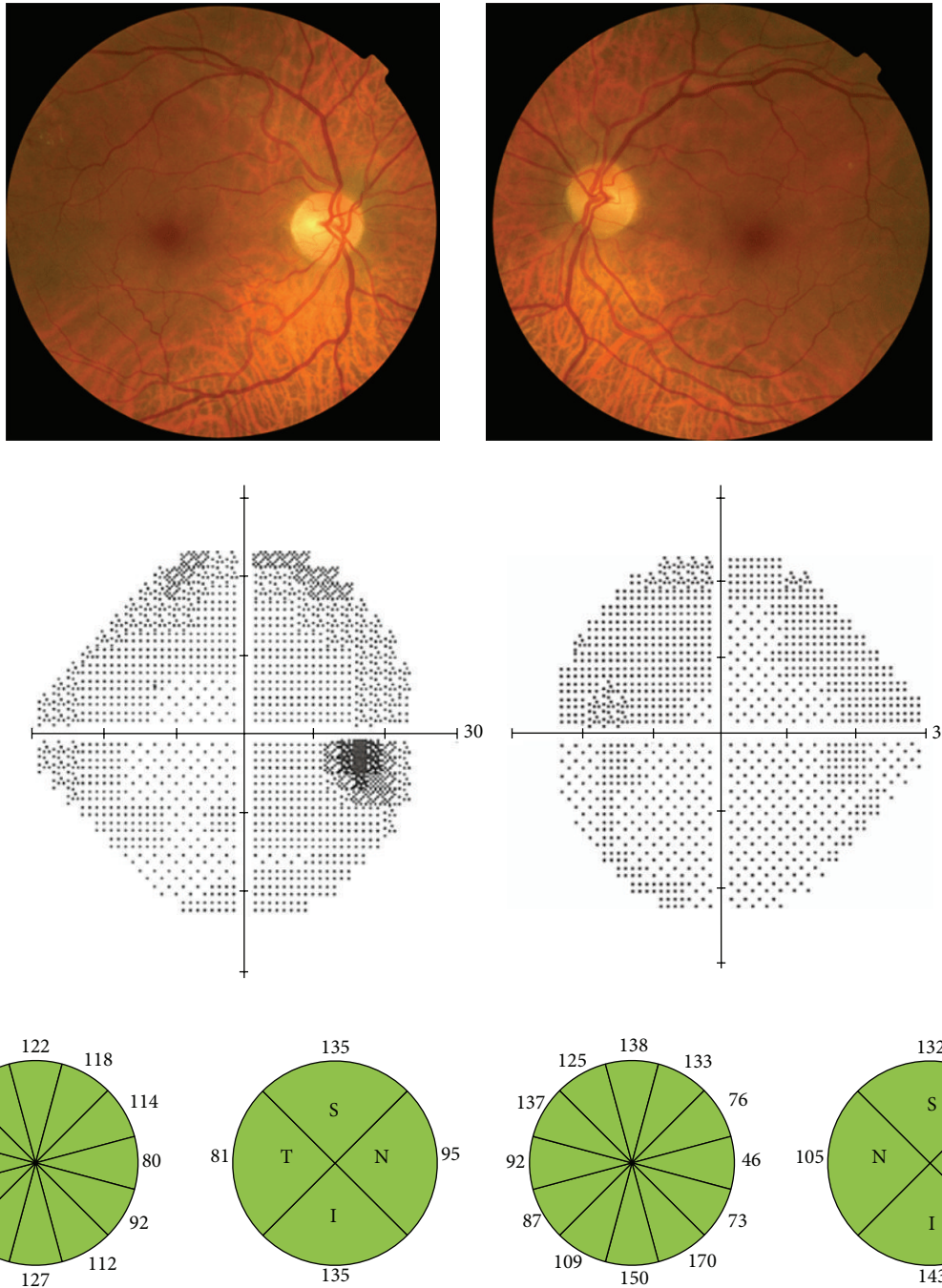
FIGURE 2: Nonmydriatic fundus photography, visual fields, and optical coherence tomography of a 68-year-old man. Cup-to-disc ratio is 0.1 in both eyes, visual fields are normal, and optical coherence tomography is within normal limits.

on nonmydriatic fundus photography by the total number of glaucoma patients. Specificity was obtained by dividing the number of controls who had no signs of optic nerve damage on nonmydriatic fundus photography by the total number of controls. Overall accuracy was defined as the sum of true positives and true negatives divided by the total number of subjects. Cohen's kappa test was calculated to estimate the agreement between the two observers as well as intraobserver variability. The SPSS for Windows software, version 12.0 (SPSS, Chicago, IL), was used to perform the statistical analysis.

3. Results

In order to fulfill the sample size requirements, 72 consecutive glaucoma cases and 72 consecutive control subjects were included. All participants were Caucasian. Eighty-six participants were men and 58 women. Among cases there were 40

Table 1: Distribution of signs of optic nerve damage in nonmydriatic fundus photography as evaluated by glaucoma specialist 1.

Signs of optic nerve damage		Controls (n = 72)	Glaucoma (n = 72)	Sensitivity and specificity
Vertical C/D ratio ≥0.6	No	65	24	Sensitivity 66.67% (55.78–77.56%)
	Yes	7	48	Specificity 90.28% (88.43–97.12%)
Vertical C/D ratio asymmetry ≥0.2	No	65	30	Sensitivity 58.33% (46.95–69.72%)
	Yes	7	42	Specificity 90.28% (83.43–97.12%)
Notches or thinning of the neuroretinal rim	No	72	32	Sensitivity 55.56% (44.08–67.03%)
	Yes	0	40	Specificity 100%
Optic disc hemorrhages	No	72	68	Sensitivity 5.56% (0.26–10.85%)
	Yes	0	4	Specificity 100%

C/D: cup to disc.

Table 2: Distribution of signs of optic nerve damage in nonmydriatic fundus photography as evaluated by glaucoma specialist 2.

Signs of optic nerve damage		Controls (n = 72)	Glaucoma (n = 72)	Sensitivity and specificity
Vertical C/D ratio ≥0.6	No	67	39	Sensitivity 45.83% (34.32–57.34%)
	Yes	7	33	Specificity 93.05% (87.18–98.92%)
Vertical C/D ratio asymmetry ≥0.2	No	66	37	Sensitivity 48.61% (37.06–60.15%)
	Yes	6	35	Specificity 91.66% (85.28–98.05%)
Notches or thinning of the neuroretinal rim	No	70	48	Sensitivity 33.33% (24.44–44.02%)
	Yes	2	24	Specificity 97.22% (93.42–101.01%)
Optic disc hemorrhages	No	72	68	Sensitivity 5.56% (0.26–10.85%)
	Yes	0	4	Specificity 100%

C/D: cup-to-disc.

men (55.6%) and among controls 46 (63.9%), although this difference was not statistically significant ($P = 0.396$, Chi-Square). Control subjects were slightly younger than glaucoma patients (66.1 ± 6.1 years versus 68.3 ± 8.0 years, resp.); the difference was not statistically significant ($P = 0.065$). Glaucoma was unilateral in 17 patients (23.6% of glaucoma subjects). Visual field damage in the most affected eye was mild in 22 (30.6%), moderate in 27 (37.5%), and severe in 23 patients (31.9%). Mean central corneal thickness was similar in cases and controls: $554\,\mu$m (SD 36.0) and $556\,\mu$m (SD 31.5). However, there was a statistically significant difference between eyes with primary open-angle glaucoma and eyes with normotensive glaucoma: $557\,\mu$m (SD 31.7) and $545\,\mu$m (SD 42.8), respectively, $P = 0.021$.

For specialist 1, 14 patients (19.44%) of the control group and 57 (79.17%) of the glaucoma group showed at least one of the criteria of optic nerve glaucomatous damage; the figures for specialist 2 were 8 patients (11.11%) in the control group and 52 (72.22%) in the glaucoma group. The frequency with which each of the criteria of optic nerve damage was found is recorded in Table 1, together with their sensitivity and specificity. The most sensitive criterion was the presence of a C/D ratio ≥0.6 for specialist 1 and the presence of cup-to-disc asymmetry for specialist 2 (Table 2). The presence of notches or thinning of the neuroretinal rim and that of optic disc hemorrhages both had a specificity close to 100%. However, these findings were less frequent, especially the presence of

hemorrhages, resulting in a very low sensitivity. Evaluation of NMFP with the proposed criteria had a sensitivity of 79.17% (95% confidence interval (95% CI) 69.79%–88.55%) and a specificity of 80.56% (95% CI 71.41%–89.70%) for specialist 1 and a sensitivity of 72.22% (95% CI 61.87%–82.56%) and a specificity of 88.88% (95% CI 81.62%–96.14%) for specialist 2 for the detection of glaucoma. The overall accuracy was 79.83% and 80.55%, respectively. The agreement between both glaucoma specialists was high, with a kappa value of 0.763, $P < 0.01$. Intraobserver variability was also high, with a kappa value of 0.830, $P < 0.01$.

A careful analysis of the misdiagnosis of nonmydriatic fundus photography was performed. Fifteen cases were undetected by specialist 1 (false negatives). In six patients this was due to an underestimation of the C/D ratio by the glaucoma specialist that evaluated the photographs. Four of these pairs of photographs had a very low quality. In the other two pairs, the cup size was difficult to estimate because of diffuse pallor. In the remaining nine patients, the C/D ratio estimated by the glaucoma specialist was similar to that estimated by biomicroscopic evaluation: these were patients in which there was no detectable increase in C/D ratio despite the presence of glaucomatous field defects. Visual field damage in the most affected eye in these "missed" cases was mild in 7, moderate in 3, and severe in 5 patients. Specialist 2 "missed" 20 patients. In 10 cases there was no detectable increase in C/D ratio, and in the other 10 cases the specialist underestimated C/R ratio.

Visual field damage in the most affected eye in these "missed" cases was mild in 8, moderate in 6, and severe in 6 patients.

On the other hand, 14 controls were classified as glaucoma by specialist 1 (false positives). Seven subjects had a C/D ratio ≥0.6 and the other 7 had an asymmetry in C/D ratio. Biomicroscopic evaluation by a glaucoma specialist agreed with the C/D evaluation of specialist 1 in 13 subjects; only in one control was the asymmetry in the C/D ratio overestimated. Specialist 2 classified 8 controls as cases: 5 subjects had a C/D ratio ≥0.6 and 2 had an asymmetry in C/D ratio. Again, only in one control was the asymmetry in the C/D ratio overestimated. Mean optic disc size as estimated by OCT in control subjects with a C/D ratio ≥0.6 was 2.96 mm^2 (SD 0.49; range 2.10–3.64), compared to a mean of 1.94 mm^2 (SD 0.39) for all other study eyes; that is, these eyes had macrodiscs. The six controls with a real asymmetry in C/D ratio seemed to have a physiologic asymmetry.

4. Discussion

Nonmydriatic fundus photography has been proven to be an adequate method for screening for diabetic retinopathy. It makes screening available to more patients at a lower cost than conventional in-office evaluation by an ophthalmologist, and it is more convenient for patients and helps to reduce the burden on ophthalmology services [17]. Glaucoma is a disease that is initially asymptomatic and it is estimated that approximately 50% of patients are unaware that they suffer from this disease [3, 4]. Efforts have been made to develop screening programs for the detection of glaucoma. However, given the relatively low prevalence in the general population and the cost of the explorations performed, attempts at screening have remained isolated since they do not appear to be cost-effective [18]. But the progressive expansion of nonmydriatic fundus photography for diabetic retinopathy screening is providing the graders with high-quality photographs in which the optic nerve head can be readily assessed for glaucomatous damage, at no additional cost.

The idea of employing nonmydriatic fundus photography for glaucoma screening is not new. As early as 1990, a study was performed in which 183 first-degree relatives of glaucoma patients were photographed by a technician with a nonmydriatic fundus camera. The images were examined by an ophthalmologist for glaucomatous damage; 31 subjects (17%) were referred to further examinations, leading to the diagnosis of glaucoma in 6 cases (3%) [19]. Detry-Morel et al. evaluated the usefulness of nonmydriatic fundus photography, combined with frequency doubling perimetry and IOP measurement for detecting glaucoma in a general population. A total of 1620 subjects were included in the study; glaucomatous optic discs were detected in 3.5% of the subjects [20]. Steele et al. evaluated the optic nerve for signs of glaucomatous damage in nonmydriatic photographs taken for diabetic retinopathy screening; 1.42% were considered to have optic disc changes compatible with glaucoma [21]. Recently, the results of a study carried out to investigate the positive predictive value of the glaucoma referral process from a local unit of the English National Screening Programme for Diabetic Retinopathy (DRSP) have been reported. Of

11,565 diabetic patients screened, 216 were suspected to have glaucoma (1.87%). After independent grading by a glaucoma specialist, a total of 170 were graded glaucoma positive and referred to a clinic for further evaluation. After one year, 113 were found to be true cases and 22 were false cases. The authors concluded that optic discs imaging could be useful as part of a glaucoma screening strategy to identify the new disease within a diabetic population [10]. However, the first step towards evaluating the possible use of a diagnostic tool as a screening method is defining its accuracy for the diagnosis of the disease. The purpose of our study was to evaluate the accuracy of a set of criteria in evaluating the optic nerve head in nonmydriatic fundus photography for the diagnosis of glaucoma in diabetic patients (adapted from O'Connor et al. [13]). Although wedge defects of the RNFL are typically considered as glaucomatous damage signs, the RNFL defects are best detected with red-free and black-and-white fundus photograph [22]. For this reason only glaucomatous optic nerve signs of damage were considered in the present study.

A case-control study was chosen because, even in diabetics, the reported rate of glaucoma is low (5.5% [23]). When deciding on the criteria for glaucomatous optic nerve damage, the cut-off value for bilateral C/D ratio was chosen as 0.6 because when C/D ratio equals or exceeds 0.6, the probability of abnormality increases dramatically [24]. The side difference in C/D ratio was set at ≥0.2 because 88% of normal subjects have a C/D vertical ratio side difference equal or less than 0.1 [25]. The appearance of the optic nerve head was not used as a restriction criterion for the entry of subjects into either the normal or glaucoma groups. This tried to avoid sample bias that might influence the outcome.

In the present study, we have found that the assessment by two glaucoma specialists of nonmydriatic fundus photographs taken by trained nurses and forwarded telematically to a tertiary care hospital performs well for the diagnosis of glaucoma, with a sensitivity of 72.22%–79.17% and a specificity of 80.56%–88.88%. Overall accuracy was close to 80%. This compares favourably with previous reports of subjective assessment of stereophotographs [14–16, 26] and even with newer imaging devices [27–29]. In fact, in a high proportion of the images misclassified, the C/D ratio had been estimated correctly. These images corresponded to healthy eyes with macrodiscs or to glaucomatous eyes with no increased cupping.

Greaney et al. compared the ability of qualitative assessment by glaucoma specialists of optic nerve head stereophotographs, confocal scanning laser ophthalmoscopy (CSLO), scanning laser polarimetry (SLP), and OCT to distinguish normal eyes from those with early to moderate glaucomatous visual field defects. The sensitivity of stereophotograph grading ranged between 76 and 86% and the specificity between 85 and 92%. No single quantitative imaging technique, CSLO, SLP, or OCT, was better than qualitative assessment by a glaucoma specialist [28]. However, it must be taken into account that the accuracy of subjective assessment depends on the experience of the observer. Thus, Reus et al. performed a study in which 243 of 875 invited ophthalmologists in 11 European countries evaluated the stereoscopic slides of 40 healthy eyes and 48 glaucomatous eyes with varying severity

and classified them as normal or glaucomatous. The overall accuracy was of 80.5%, with a sensitivity of 74.7% and a specificity of 87.4%. Imaging devices outperformed general ophthalmologists for the diagnosis of glaucoma; however, if glaucoma specialist assessments were considered, they were found to be slightly better than imaging devices [30]. In another study, it was found that glaucoma specialists classified the optic discs better than general ophthalmologists, who in turn outperformed hospital-based optometrists. The worst classifiers were junior residents [29]. Monoscopic digital images taken by a nonmydriatic fundus camera and forwarded telematically to a reading center have the advantage of being easier and quicker to acquire than stereoscopic images. We have shown that evaluation by a glaucoma specialist reaches a high accuracy for the diagnosis of glaucoma.

In summary, this is, as far as we could ascertain, the first study to evaluate the accuracy of monoscopic images taken with nonmydriatic fundus photography for the diagnosis of glaucoma in diabetic patients. The accuracy we have found is comparable to other imaging methods for glaucoma and may be high enough for it to be included in a screening program, in combination with other diagnostic techniques, although the best method for screening and whether screening is feasible requires further studies. An important setback of our study is that it is a case-control study with a very high proportion of patients compared with controls, when in a screening setting for glaucoma the prevalence of the disease would be much lower.

Disclosure

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Research Article

Analysis of Retinal Peripapillary Segmentation in Early Alzheimer's Disease Patients

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Decreased thickness of the retinal nerve fiber layer (RNFL) may reflect retinal neuronal-ganglion cell death. A decrease in the RNFL has been demonstrated in Alzheimer's disease (AD) in addition to aging by optical coherence tomography (OCT). Twenty-three mild-AD patients and 28 age-matched control subjects with mean Mini-Mental State Examination 23.3 and 28.2, respectively, with no ocular disease or systemic disorders affecting vision, were considered for study. OCT peripapillary and macular segmentation thickness were examined in the right eye of each patient. Compared to controls, eyes of patients with mild-AD patients showed no statistical difference in peripapillary RNFL thickness ($P > 0.05$); however, sectors 2, 3, 4, 8, 9, and 11 of the papilla showed thinning, while in sectors 1, 5, 6, 7, and 10 there was thickening. Total macular volume and RNFL thickness of the fovea in all four inner quadrants and in the outer temporal quadrants proved to be significantly decreased ($P < 0.01$). Despite the fact that peripapillary RNFL thickness did not statistically differ in comparison to control eyes, the increase in peripapillary thickness in our mild-AD patients could correspond to an early neurodegeneration stage and may entail the existence of an inflammatory process that could lead to progressive peripapillary fiber damage.

1. Introduction

Alzheimer's disease (AD), the most common cause of dementia, afflicts 67 of every 1000 persons over age 65. Its prevalence and incidence increase exponentially with age [1, 2]. In 2006, the worldwide prevalence of Alzheimer's was 26.6 million, and by 2050, the prevalence will quadruple, meaning that by that time 1 in 85 persons worldwide will be living with the disease [2].

AD is characterized by a decline in cognitive function, loss of learning and memory, and the formation of neuritic plaques and neurofibrillary tangles, primarily in the cerebral cortex [3, 4].

The retina is a projection of the brain, and a number of similarities between AD pathology and several distinct retinal degenerations have been described [5, 6]. The retinal nerve fiber layer (RNFL) is composed of retinal-ganglion cell axons that form the optic nerve. Decreased thickness of the RNFL can reflect retinal neuronal-ganglion cell death and axonal loss in the optic nerve [7, 8].

The RNFL reportedly thins with aging [9, 10]. Some studies have also shown a decrease of the RNFL in AD in addition to aging [7, 8, 11–16]. Hinton et al. [17] were the first to show histopathological evidence of retinal-ganglion cell loss and optic-nerve degeneration in patients with AD. These findings were then confirmed in several follow-up studies [18–21]. Indeed, the large magnocellular cell axon degeneration in AD has been documented [19, 22]. Other histopathology studies [23–28], however, have failed to confirm these findings and suggest that methodological differences were responsible for

the contradictory results, due to a different postmortem delay in axon count or difficulties in obtaining well-preserved myelinated axons.

Currently it is thought that retinal ganglion cell (RGC) loss in AD might result from amyloid deposits in the eye and/or retina. Amyloid-beta plaques as well as oligomers have been reported in postmortem retinal tissue from patients with AD and in a mouse model of AD, as well as in human retinal tissue *in vivo* [29]. Therefore, amyloid accumulation in the eye or retina of patients with AD may result in the degeneration of RGC in parallel to amyloid-beta-related neurodegeneration in the brain [29].

Diagnosis and progression of AD, especially early cases, are complicated because of imprecise neuropsychological testing, sophisticated but expensive neuroimaging techniques, and invasive sampling of cerebrospinal fluid [30, 31]. Improved methods for screening and early detection are essential to identify cognitively normal individuals who have a high risk of developing AD, so that treatment can be developed to delay the progression of the disease [32]. Currently, there is no definitive antemortem diagnosis for AD, and new biomarkers for diagnosis are therefore needed. Over the last few decades, very accurate tools for analyzing the eye fundus have been developed (i.e., OCT, laser polarimetry), opening new ways of examining the retina *in vivo*.

OCT is a reliable noninvasive technique, routinely used in ophthalmology to visualize and quantify the layers of the retina. OCT enables quantitative cross-sectional imaging of the RNFL and macular volume. A recent study published by our group [33] has shown that in mild-AD patients the first affected area of the retina is the macular area. As the neurodegeneration progresses, a significant decline in peripapillary RNFL thickness will become apparent.

The goal of the present study was to examine in detail peripapillary and macular segmentation in order to determine which is the earliest thinned area in patients with mild AD which may be used, in the future, as a predictive tool.

2. Material and Methods

2.1. Subjects. To select patients, we reviewed the Database of the Memory Unit of the Hospital Clinico San Carlos in Madrid (Spain), consisting of a total of 2635 patients. First, we excluded the patients with a Global Deterioration Scale (GDS) over 4 and then those with a mood or psychiatric disorder. Next, we took into account 87 patients with mild AD. These patients, according to the National Institute of Neurological and Communicative Disorders and Stroke-AD and Related Disorders Association and the Diagnostic and Statistical Manual of Mental Disorders IV, had mild cognitive impairment according to the Clinical Dementia Rating scale. Then ophthalmic medical records of these patients were reviewed, excluding patients who were previously diagnosed with an ophthalmological pathology (glaucoma or suspected glaucoma, media opacity, and retinal diseases). After this analysis, 29 patients with AD satisfied all the requirements to participate in the study (GDS over 4 and free of ocular disease and systemic disorders affecting vision

in their medical record). Of the 29 mild-AD patients and 37 age-matched control subjects selected (normal MMSE scores), 6 mild-AD patients and 9 age-matched control subjects were subsequently excluded due to posterior pole pathology including macular degeneration, drusen, suspicion of glaucoma, glaucoma, epiretinal membrane, or cataract that prevented ocular examination. Because of this selection, 23 patients with mild AD and 28 age-matched control subjects were considered for the study. Informed consent was obtained from both groups. The research followed the tenets of the Declaration of Helsinki, and the protocol was approved by the local ethics committee.

2.2. Methods. For the ophthalmological part of the study, the right eye of each patient was analyzed. All participants met the following inclusion criteria: being free of ocular disease, AREDS Clinical Lens Standards <2, retinal drusen, and systemic disorders affecting vision; having a best corrected VA of 20/40; having a ±5 spherocylindrical refractive error; and having intraocular pressure of less than 20 mmHg. For screening, all AD patients and control subjects underwent a complete ophthalmologic examination, including assessment of VA, refraction, anterior segment biomicroscopy, applanation tonometry (Perkins MKII tonometer, Haag Streit-Reliance Medical, Switzerland), dilated fundus examination, and OCT. The RNFL thickness and macular thickness were measured by OCT Model 3D OCT-1000 (Topcon, Japan) after pupil dilatation. The RNFL thickness was scanned 3 consecutive times per patient in each area studied. The mean values were considered for statistical analysis. All tests were performed by the same optometrist (ESG) under the same conditions. These tests were selected considering that in this developmental stage of the disease the results were not influenced by the patient's cognitive impairment.

The peripapillary RNFL thickness parameters evaluated in this study were average thickness (360° measurement), thickness for each 12-o'clock hour position with the 3-o'clock position as nasal, 6-o'clock position as inferior, 9-o'clock position as temporal, and 12-o'clock position as superior. Macular RNFL thickness data were displayed in three concentric rings centered in the foveola that were distributed as follows: a central macular ring, 1 mm away from the fovea; an inner macular ring, 3 mm away from the fovea; and an outer macular ring, 6 mm away from the fovea. As a result, the total area studied made up a 6 mm macular map. In addition, the inner and outer rings were each divided into four quadrants (superior, inferior, nasal, and temporal) (Figure 1). The total volume of the macula as provided by the OCT was also calculated. The good scan criteria were determined as the signal-to-noise ratio >30 and accepted A-scans >95% in fast RNFL scanning. All measurements are given in microns, according to the calibration provided by the manufacturers and the total volume in mm^3.

2.3. Statistical Analysis. The data are reported as mean values ± SD. The differences between mild AD and control eyes were analyzed using the Mann-Whitney test. Data for the statistical analysis were introduced and processed in a SPSS
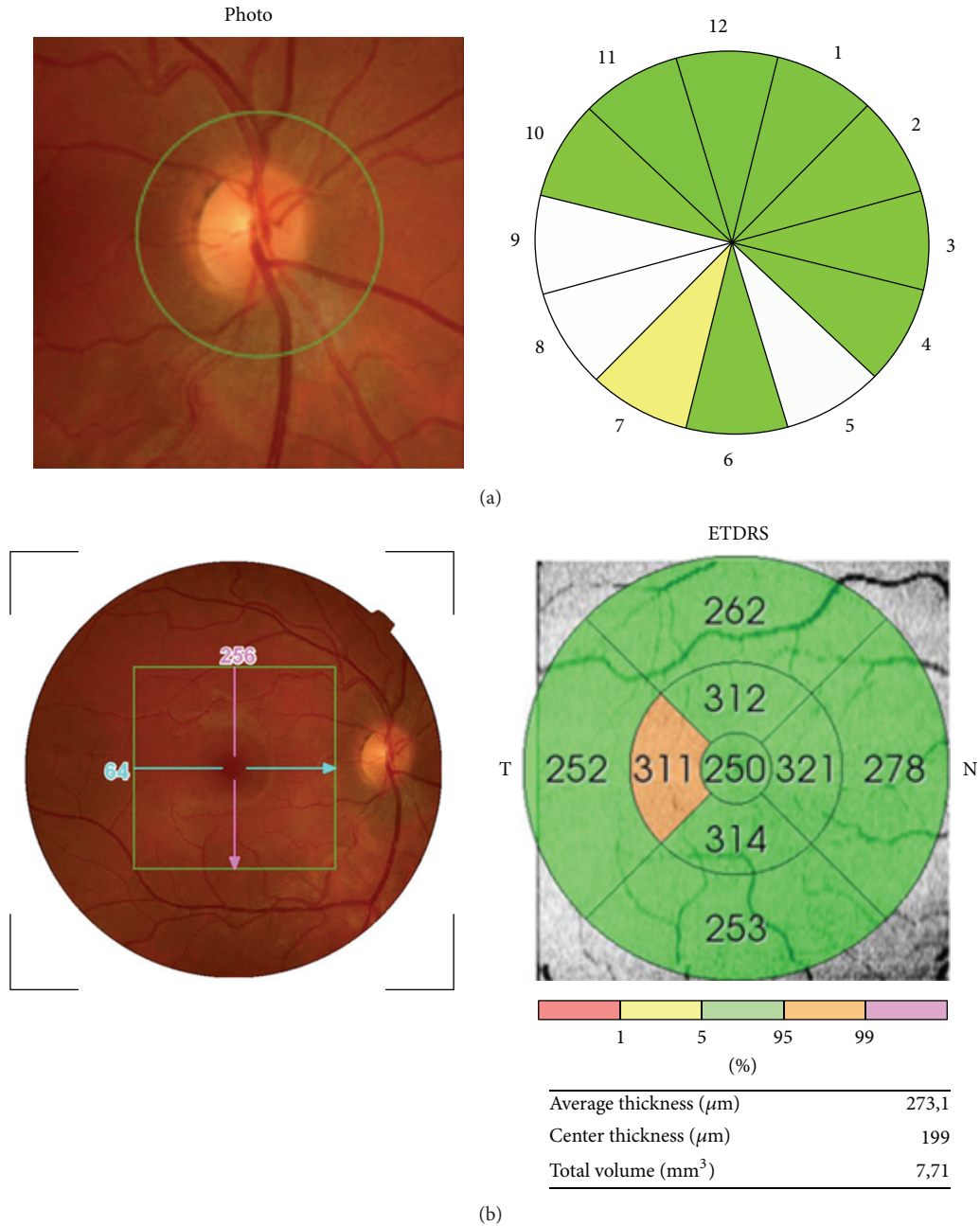
(a)

(b)

FIGURE 1: OCT report of retinal nerve fiber layer (RNFL) thickness analysis. (a) Peripapillary OCT. The thickness for each 12-o'clock hour position with the 3-o'clock position as nasal, 6-o'clock position as inferior, 9-o'clock position as temporal, and 12-o'clock position as superior was evaluated. (b) Macular OCT. Diagram showing the concentric rings and quadrants considered for analysis of the macular RNFL thickness and measurements automatically provided by the analyzer.

19.0 (SPSS Inc©, Inc, Chicago, IL, USA). A P value of <0.05 was considered statistically significant.

3. Results

Demographic and clinical data for the mild-AD patients and control group are shown in Table 1. No statistically significant differences in age, gender, or educational level were found between the study groups. The MMSE scores in mild-AD patients were significantly decreased in comparison with age-matched control subjects (Table 1). All mild-AD patients had MMSE values higher than 17.

3.1. Optical Coherence Tomography

Peripapillary RNFL Segmentation Thickness. Peripapillary RNFL thickness values (Figure 2(a)) showed no statistical
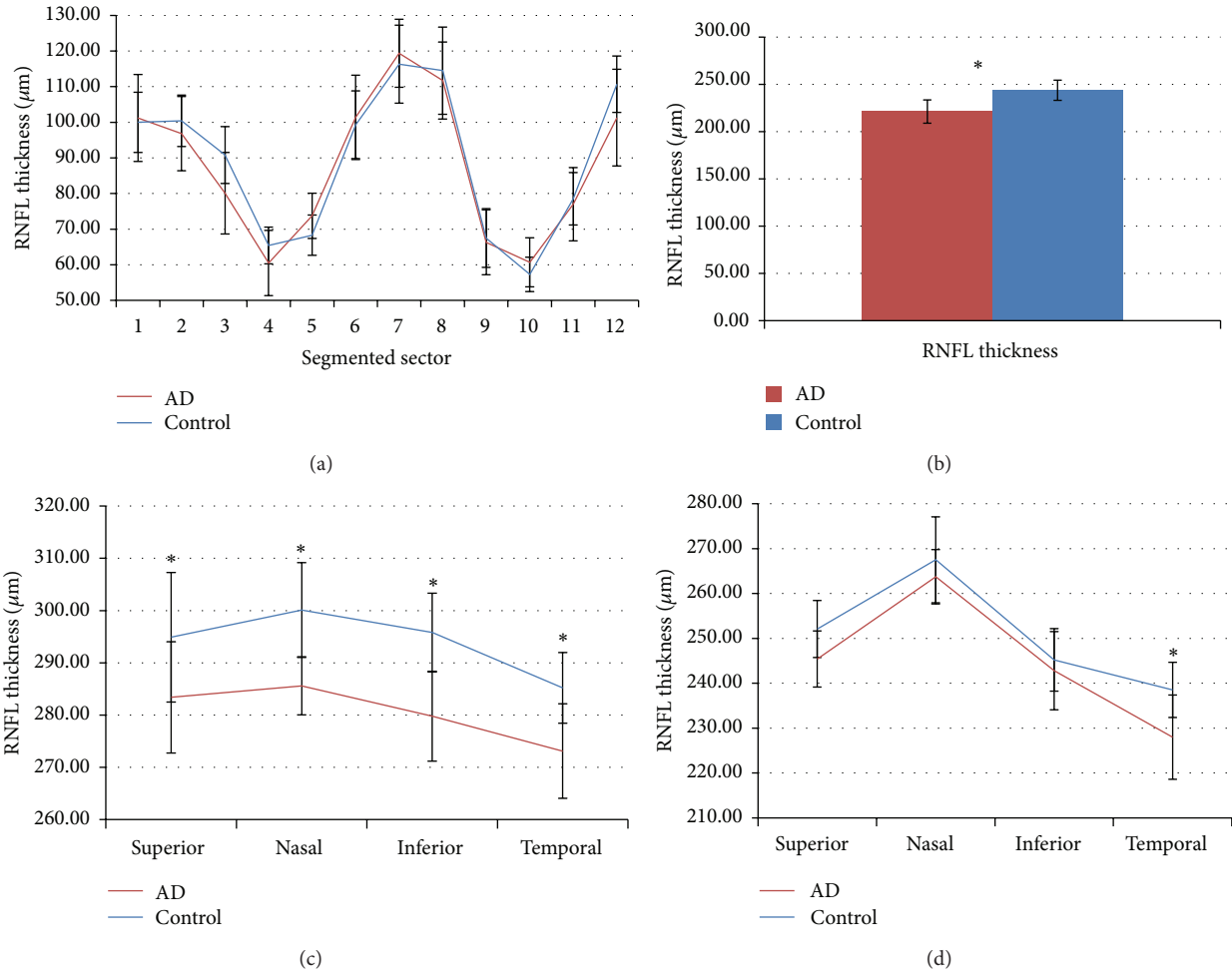
(a)

(b)

(c)

(d)

Figure 2: Mean data of RNFL thickness against eye quadrants assessed with optical coherence tomography (OCT). (a) Peripapillary segmentation retinal nerve fiber layer, (b) Central macular ring (1 mm away from the fovea). (c) Inner macular ring (3 mm away from the fovea). (d) Outer macular ring (6 mm away from the fovea). *P value < 0.01.

Table 1: Demographic and clinical data of the study groups.

	AD ($n = 23$)	Control ($n = 28$)	P value
Age§	79.3 ± 4.6	72.3 ± 5.1	0.274
Gender			
Male	9	9	0.615
Female	14	19	
Race	Caucasian	Caucasian	
MMSE§	23.3 ± 3.1 Range (17–29)	28.2 ± 1.9 Range (25–31)	0.001*

§Mean value ± SD; *P < 0.01 [AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; SD, standard deviation].

difference between mild-AD patients and control subjects (Table 2).

Although the differences were not significant in any of the sectors, it was shown that peripapillary sectors 2, 3, 4, 8, 9, 11, and 12 were thinner in the mild-AD patients than in controls; in peripapillary sectors 1, 5, 6, 7, and 10 the retina in mild-AD patients was thicker with respect to the control (Figure 2(a); Table 2).

Macular RNFL Thickness and Total Volume. As we reported in a previous study [33] the analysis of the RNFL revealed that, in patients with mild AD, the values for the central ring (fovea) (Figure 2(b)) and the four inner quadrants (3 mm from the fovea) (Figure 2(c)) were significantly decreased in comparison with control subject (P < 0.05 in both instances; Mann-Whitney U test) (Table 2). The RNFL thickness of the outer macular quadrants (6 mm from the fovea) (Figure 2(d)) in patients with mild AD was diminished in comparison with control subjects; however, only the values of the outer temporal quadrant were significantly lower (P < 0.05; Mann-Whitney U test) (Table 2).

The total macular volume was significantly reduced in mild-AD patients in comparison with control subjects (P < 0.05; Mann-Whitney U test) (Table 2).

4. Discussion

Alzheimer's dementia syndromes, like all neurodegenerative diseases, lack objective disease- and stage-specific biomarkers [34]. As a part of the CNS, the retina or neural portion

TABLE 2: RNFL thickness and total macular volume.

Retinal area of study		AD group[§]	Control group[§]	% RNFL decrease	P value
Peripapillary thickness (μm)	Sector 1	101.2 ± 24.4	100.0 ± 16.9	1.24	0.790
	Sector 2	96.8 ± 20.8	100.4 ± 14.4	−3.62	0.618
	Sector 3	80.1 ± 22.9	90.8 ± 16.0	−11.85	0.084
	Sector 4	60.5 ± 18.3	65.4 ± 10.3	−7.60	0.464
	Sector 5	73.7 ± 12.7	68.3 ± 11.3	7.83	0.173
	Sector 6	101.4 ± 23.7	99.3 ± 19.0	2.02	0.790
	Sector 7	119.4 ± 19.1	116.3 ± 21.9	2.62	0.756
	Sector 8	111.7 ± 21.6	114.5 ± 24.5	−2.48	0.564
	Sector 9	66.3 ± 18.2	67.5 ± 16.5	−1.80	0.877
	Sector 10	60.7 ± 13.8	57.3 ± 9.6	5.99	0.464
	Sector 11	77.0 ± 20.6	78.5 ± 14.7	−1.96	0.94
	Sector 12	101.3 ± 27.1	110.7 ± 15.8	−8.45	0.335
Foveal thickness (μm)	Fovea	221.2 ± 21.6	243.7 ± 24.8	−9.24	0.015*
Inner macular quadrant (μm)	Superior area	283.4 ± 11.1	294.9 ± 18.1	−3.91	0.002*
	Inferior area	279.8 ± 18.1	295.8 ± 13.5	−5.40	0.002*
	Nasal area	285.6 ± 17.2	300.1 ± 15.1	−4.83	0.007*
	Temporal area	273.1 ± 12.7	285.2 ± 14.6	−4.22	0.002*
Outer macular quadrant (μm)	Superior area	245.4 ± 12.5	252.1 ± 13.7	−2.65	0.084
	Inferior area	242.8 ± 17.4	245.2 ± 13.9	−0.99	0.531
	Nasal area	263.7 ± 12.1	267.5 ± 19.1	−1.41	0.110
	Temporal area	228.0 ± 18.8	238.5 ± 12.3	−4.43	0.009*
Total macular volume (mm^3)		7.1 ± 0.3	7.3 ± 0.3	9.34	0.024*

[§]Mean value ± SD; *$P < 0.05$ [AD, Alzheimer's disease; RNFL: retinal nerve fiber layer; SD, standard deviation].

of the eye shares many features with the brain, including embryological origin as well as anatomical and physiological characteristics. Its peripheral location provides an accessible and noninvasive way of examining brain pathology [35]. OCT is a reliable noninvasive technique that enables quantitative cross-sectional imaging of the RNFL [36].

Thinning of the RNFL has been found in several neurological diseases, such as Parkinson's disease [16, 37–39], dementia with Lewy Bodies [16], amnestic mild cognitive impairment [8, 15], neuromyelitis optica [40], migraine [41], and AD [8, 11–17, 20, 27, 36, 37, 42–45]. The loss of RNFL thickness in AD is linked to a depletion of retinal-ganglion cells and optic-nerve axons [13, 14, 32, 46, 47]. It has been postulated that the defects in RNFL may be the earliest sign of AD, even prior to damage to the hippocampal region that impairs memory [36]. In addition, published data suggest an association between the thinning of RNFL and severity of AD [8, 11].

In the present work, we compare the peripapillary RNFL segmentation thickness, macular thickness, and the total macular volume in mild-AD patients and age-matched control subjects. One of the relevant issues of the study was that the sample analyzed here was homogeneous in that (i) all patients had recently been diagnosed as having mild AD (GDS 4, Reisberg scale [48]) with mean MMSE score values of 23.7 ± 3.3; (ii) all the individuals were Caucasians; and (iii) there were no significant differences in age or educational level among the groups. The results of our study showed a

difference between the peripapillary RNFL segmentation and the macula thickness in our mild-AD patients in that only the macular thickness was significantly decreased in comparison with the control group.

Widespread axonal degeneration in the optic nerve was found in a postmortem study of patients with AD [17]. Morphometric analysis of the whole-mount retina has shown that Alzheimer's patients had a predominant loss of the largest class of retinal-ganglion cells (M-cells), which could be a primary process or a consequence of retrograde neurodegeneration occurring in the cortical regions [19]. *In vivo* studies using different methodologies have confirmed optic-nerve-fiber damage in AD when compared with controls. Optic disc pallor, pathologic disc cupping, and thinning of the neuroretinal rim and the RNFL have been reported in studies based on the subjective evaluation of fundus photographs [11, 42] and the optic-nerve analyzer [42].

There is controversy on the reduction of the peripapillary RNFL thickness measured by OCT in AD. A reduction in the thickness of all peripapillary RNFL quadrants as measured by OCT has been reported [12, 43], and it has been suggested that this morphologic abnormality is related to retinal dysfunction as revealed by abnormal patterns in electroretinogram responses [43]. However, some OCT studies on peripapillary thickness in AD [8, 13, 15, 16, 36, 44, 45, 49] found that the RNFL thinning was restricted to the superior quadrant [13, 44, 50–52] or to the superior and inferior quadrants [15, 37, 45] in comparison with control subjects. Some studies have

correlated cognitive decline with decreased RNFL thickness [33, 50, 53]. It has been suggested that the inferior quadrant of the RNFL may be a more specific and sensitive area than other RNFL quadrants in predicting the deterioration of cognitive status to reflect retinal abnormality in the early stages of AD [15, 50]. The reason for the variability of the results among studies could be related to MMSE scores. Thus, Parisi et al. [43] and Iseri et al. [12], whose patients had more advanced AD (ranges of MMSE scores 11 to 19 and 8 to 28, resp.), showed a reduction in RNFL thickness in all peripapillary quadrants. Kesler et al. [15], whose patients had a mean MMSE score of 23.6, showed a decrease in the superior and inferior peripapillary quadrants. By contrast, both Berisha et al. [13], whose Alzheimer's patients had higher MMSE scores (17 to 30), and Paquet et al. [8], whose patients had a mean MMSE score of 22.6, found a thickness reduction only in the superior peripapillary quadrant, postulating this finding as being the earliest peripapillary retinal damage in AD patients. In our patients, with MMSE values similar to those reported by Berisha and Paquet, the reduction of mean peripapillary RNFL thickness did not reach statistical significance in comparison to control, but peripapillary RNFL thickness diminished or increased, depending on the segment studied. It should be noted that the thinning sectors of papilla corresponded to 2, 3, 4, 8, 9, and 11, while sectors 1, 5, 6, 7, and 10 showed a thickening. These values differ from those found in the same sectors of the controls but in any case reach statistical significance.

Most authors, although working in more advanced stages of disease (MMSE > 23.7), agree that the peripapillary RNFL thinning is significant in the superior and inferior sectors [13, 37, 44]. However, sectors 1, 5, 6, 7, and 10 in our patients showed thickening. This dissimilarity could be explained because of difference in the stage of the disease, which in our case corresponded to a much earlier stage. This tendency towards greater thickness, although not statistically significant, could be related to the findings of Ascaso et al. in the macula of patients with mild cognitive impairment (MCI) and AD. Patients with MCI had greater RNFL thickness compared to AD and controls, suggesting that this difference could be caused by inflammation after gliosis neuronal death [54]. Similarly, the increase in peripapillary thickness in our mild-AD patients in the sectors 1, 5, 6, 7, and 10, corresponding to the superior and inferior sectors, may indicate a phase of inflammation and gliosis of neural tissue prior to the degenerative process.

Reactive astrogliosis in the brain is a well-known feature of AD, but its role in AD is not well understood. Reactive astrogliosis tends to be focal in AD. Reactive astrocytes are intimately associated with amyloid plaques or diffuse amyloid deposits. Astrocytes surround them with dense layers of processes as if forming miniature scars around them, perhaps to wall them off and act as neuroprotective barriers [55]. It is plausible that, in the early stages of the disease, microglial activation could help remove amyloid plaques, while in later phases proinflammatory cytokines induced by microglia could contribute to neurodegenerative process [56, 57].

In the same way, retinal neurodegenerative diseases are also associated with chronic microglial activation and neuroinflammation. In the degenerating retina, endogenous signals activate microglial cells, leading to their local proliferation, migration, enhanced phagocytosis, and secretion of cytokines, chemokines, and neurotoxins. These immunological responses and the loss of limiting control mechanisms may contribute significantly to retinal tissue damage and proapoptotic events in retinal neurodegeneration [57–59]. A limitation to be considered in our study, as well as those reported in the literature on RNFL thickness evaluation by OCT, is the number of patients included. Studies on early-stage Alzheimer's patients are difficult to perform, one reason being that these patients usually come for diagnosis at advanced stages of the disease. Taking this into consideration and the homogeneity of the patients included in the present work, we consider that our data provide preliminary evidence to warrant a more extensive study.

5. Conclusions

In the present study, the analysis of the OCT values of both peripapillary and macular RNFL thickness in patients with mild AD (MMSE = 23.7) showed that only in the macula was there a significant thickness reduction compared to aged-matched controls. Our data, taken together with those reported in the literature, move us to propose the hypothesis that the first affected area of the retina in mild AD is the macular area, where, due to the arrangement of the multilayer bodies of the ganglion cells, the decrease is easier to detect.

Subsequently, as the neurodegeneration progresses, a significant decline in peripapillary RNFL thickness will become apparent. The study of the peripapillary segmentation reveals, in a more accurate way, the changes that occur in RNFL thickness in relation to the macular-thickness changes. In this sense, our patients with mild AD differed with respect to controls, although without reaching statistical significance; perhaps due to the early stage of the disease. In addition, the increase in peripapillary thickness in our mild-AD patients may indicate the existence of an inflammatory process that would lead to neurodegeneration of the peripapillary fibers. More extensive studies should be conducted to test these findings.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Review Article

The Association between Primary Open-Angle Glaucoma and Blood Pressure: Two Aspects of Hypertension and Hypotension

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Glaucoma is the second leading cause of blindness worldwide. Although the mechanism of the development of primary open-angle glaucoma (POAG) is not fully understood, elevated intraocular pressure (IOP) is considered the most important risk factor. Several vascular factors have also been identified as risk factors and can lead to hypoperfusion of the optic nerve head and thus may play an important role in the pathogenesis and progression of POAG. The results of the present study suggest that both high and low blood pressure (BP) are associated with an increased risk of POAG based on a comprehensive literature review. Elevated BP is associated with elevated IOP, leading to increased risk of glaucoma, but excessive BP lowering in glaucoma patients may cause a drop in ocular perfusion pressure (OPP) and subsequent ischemic injury. The relationship between IOP, OPP, and BP suggests that the relationship between BP and glaucoma progression is U-shaped.

1. Introduction

Glaucoma is commonly defined as optic neuropathy characterized by progressive loss of retinal ganglion cells (RGCs) which is associated with characteristic structural damage to the optic nerve and visual field loss. Risk factors related to glaucoma include intraocular pressure (IOP), age, family history, clinical appearance of the optic nerve, race, and potential vascular disease [1–4].

Although the mechanism of RGC death is not fully understood, elevated IOP is considered the most important risk factor [5, 6]. Several large randomized clinical trials showed a relationship between IOP and glaucoma development and progression [5–9]. Besides the mechanical effect of raised IOP on the optic nerve head (ONH), several vascular factors have also been identified as risk factors [10]. Such factors can lead to hypoperfusion of the ONH and may thus play an important role in the pathogenesis and progression of primary open-angle glaucoma (POAG) [11–15].

Among vascular factors, systemic hypertension may contribute to increases in IOP via overproduction or impaired outflow of aqueous humor [16]. However, the relationship between glaucoma and blood pressure (BP) remains under debate. While some studies report that systemic hypertension is a risk factor for glaucoma [4, 17, 18], other studies indicate that low systemic BP is a risk factor for the development and progression of glaucoma. A direct and clear relationship between glaucomatous damage and BP level has not been established [19]. Moreover, the association between BP and IOP is inconsistent.

Some but not all population studies found statistically significant positive associations between systolic blood pressure (SBP) and diastolic blood pressure (DBP) with IOP [3, 4, 20–28].

In the present study, we reviewed the relationship between POAG and BP, focusing on two aspects: hypertension and hypotension.

2. Method of Literature Search

The Medline database was used for the literature search in this review. Although every effort was made to use the most recent references possible, articles were used irrespective of the year

of publication if deemed appropriate. The key words searched included the following: intraocular pressure, ocular perfusion pressure, glaucoma, blood pressure, circadian fluctuation, and risk factors. After retrieving relevant articles using these key words, a search was conducted through the studies cited in these articles, and additional papers were identified. Abstracts of papers in languages other than English were also surveyed. Medical Subject Headings (MeSH) searches were also performed. Case reports and abstracts from meeting presentations were excluded.

3. Blood Supply of ONH

The ophthalmic artery, which is the first branch of the internal carotid artery, gives off 2–4 posterior ciliary arteries. Posterior ciliary arteries later divide into 10–20 short posterior ciliary arteries that pierce the sclera and enter the globe around the optic nerve. It was reported that the superficial layers of the ONH are supplied by the central retinal artery while the deeper prelaminar regions are supplied by the posterior ciliary arteries, which branch off the circle of Zinn-Haller [16].

ONH circulation is thought to be anatomically and physiologically similar to the circulation in the retina, which is characterized by tight junctions, abundant pericytes, and nonfenestrated endothelium [29]. The capillaries of ONH do not leak fluorescein and may represent a nerve-blood barrier, supporting the concept of the retina-nerve vasculature as a continuous system with the central nerve system [29, 30]. Histologic examination of glaucomatous optic nerves showed a reduction in the number of capillaries, consistent with the degree of neural loss.

Blood flow in the anterior optic nerve depends on many factors, which include the ocular perfusion pressure (OPP) and the resistance to flow as determined by the vascular caliber in the arterioles and capillaries [31]. The ability to keep local tissue blood flow constant and counteract changes in the local metabolic environment is called autoregulation [32]. Moderate increments in IOP and systemic BP have little effect on anterior optic nerve-blood flow, and autoregulatory mechanisms maintain flow in hyperoxic and hypercapnic conditions. In contrast to the extraocular and choroidal vessels, retinal vessels have no neural innervation. Therefore, local vascular mechanisms are mainly responsible for matching perfusion to the changes in metabolic demand [33, 34]. The process of autoregulation in a vascular bed maintains constant or nearly constant blood flow through a wide range of perfusion pressures. However, if autoregulation is impaired, elevated IOP may reduce optic nerve perfusion. The circulatory networks of the optic nerve and retina have deficient autoregulation in POAG.

Substances produced by the vascular endothelium play a major role in the control of ocular blood flow, and these include the vasodilators nitric oxide and prostacyclin and vasoconstrictors such as angiotensin and the endothelins [35].

Regulation of blood flow through the choroid is under the control of the autonomic nervous system. Data regarding choroidal autoregulation are contradictory. The autonomic
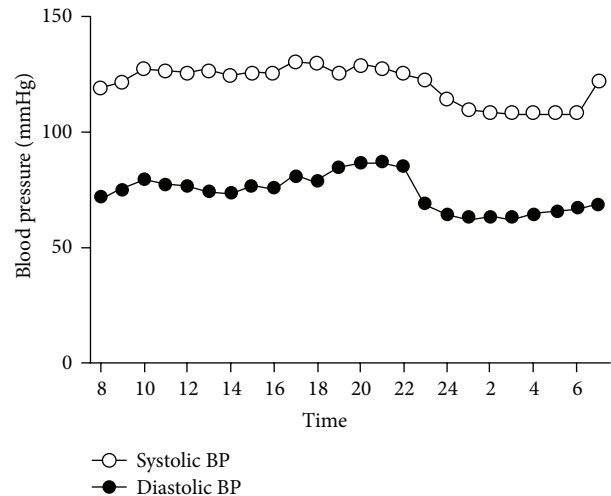

Figure 1: Circadian curve of mean systolic and diastolic blood pressure (BP) in 34 combined dipper and nondipper patients. Modified from Quaranta et al. [36] and Staessen et al. [39].

tonus may protect the eye from transient elevations in systemic BP under normal circumstances; however, the autonomic nervous regulation may break down in the presence of systemic hypertension.

4. BP and IOP

BP is one of numerous metabolic systems in humans that exhibit a circadian rhythm [36–39] (Figure 1). Millar-Craig et al. showed that BP is lowest at around 3 AM and increases gradually during the early morning hours before waking, reaching a peak at midmorning [36, 37]. These fluctuations have been attributed to the nocturnal decrease in sympathetic activity and circulating catecholamine levels. In humans, resting levels of plasma epinephrine and norepinephrine (markers of sympathetic nervous activity) exhibit endogenous circadian rhythmicity with a broad peak during the middle of the biological day, and the BP rise that begins before waking is independent of behaviors [40].

Circadian variations in IOP also exist, and many studies were conducted to characterize these rhythmic patterns. The traditional view is that IOP is generally higher in the morning, but recent research in both healthy and glaucomatous eyes questioned this pattern [36, 40, 41] (Figure 2).

Lui et al. performed IOP measurements every 2 hours over 24 hours in young healthy volunteers [40]. The average IOP was significantly higher in the dark period than in the light-wake period. In comparison with the sitting IOP values in the first group, the supine IOP in the second group was significantly higher during the light-wake period. The authors concluded that a nocturnal IOP elevation can appear independent of body position change, but change in posture from upright to recumbent may contribute to the relative nocturnal IOP elevation. Another study showed that there were no significant changes in supine IOP at any time point, although the IOP peaked at midnight (16.5 mmHg) and troughed at noon and 4 PM (14.2 mmHg), nor any significant changes
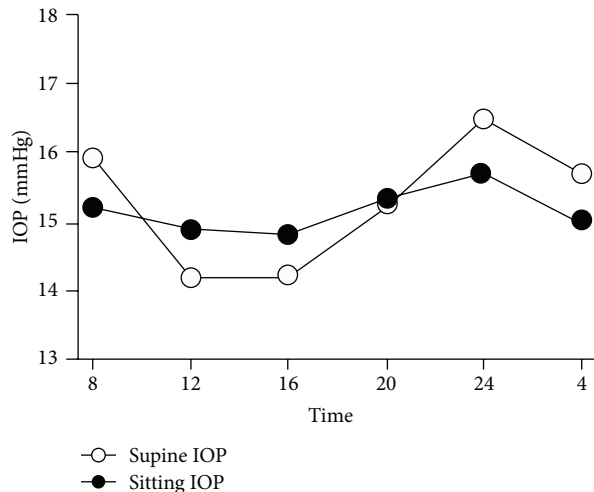
Figure 2: Circadian curve of mean supine and sitting intraocular pressure (IOP). Modified from Fogagnolo et al. [41].

in sitting IOP over time (mean values between 14.8 mmHg and 15.7 mmHg). The same was true when the daytime and nighttime measurements were compared [41]. In addition, a recent study confirmed that 24-hour IOP fluctuations were not highly reproducible and that IOP patterns were not sustained from day to day in healthy young volunteers [42]. Unlike circadian rhythm of BP, controversy exists on the circadian IOP cycle.

The majority of population-based studies reported a positive association or correlation between SBP, DBP, and IOP [20, 21, 26–28, 43–45]. A recent meta-analysis showed a pooled average IOP increase of 0.26 mmHg (95% CI, 0.23–0.28; I^2, 42.5%) and 0.17 mm Hg (95% CI, 0.11–0.23; I^2, 91.2%) associated with a 10 mmHg and 5 mmHg increase in DBP, respectively, with similar results seen in cross-sectional and longitudinal studies [46]. These trends may be because systemic hypertension increases IOP via overproduction or impaired outflow of aqueous humor [16].

Several studies investigated the vascular risk factors in the pathogenesis of glaucoma, with BP and OPP being the most studied. The vascular hypothesis is based on the assumption that abnormal perfusion and the subsequent ischemia of the ONH play a major role in the loss of RGCs.

OPP can be defined as the systolic, diastolic, or mean OPP. The mean OPP (MOPP) can be calculated as 2/3 of the mean arterial BP-IOP, where mean arterial pressure = DBP + 1/3(SBP − DBP). The factor of 2/3 accounts for the drop in BP between the brachial and ophthalmic artery when the subject is seated [47]. Systolic OPP is defined as the difference between the systemic SBP and IOP, whereas diastolic OPP (DOPP) equals the systemic DBP-IOP [48]. DOPP is especially useful for displaying the lowest OPP values and is regarded as an independent risk factor for open-angle glaucoma (OAG).

When calculated by this equation, a certain change in IOP or BP results in the same value of MOPP. However, an experimental study showed that IOP is more important than

BP in determining retinal function and that, for a given OPP, a higher IOP elevation induces greater retinal dysfunction [49]. This is possibly because BP modification influences vascular supply only, whereas an IOP elevation affects the vascular supply via a reduction in OPP and produces mechanical stress on retinal neurons which is OPP independent.

5. POAG and Hypertension

As previously mentioned, data on the association between hypertension and IOP is consistent across studies. However, the relationship between POAG and BP is complex and poorly understood. Several large-scale epidemiologic studies investigated this relationship, with most studies describing conflicting reports. Several studies reported a low risk of glaucoma in individuals with elevated BP [50–53], whereas others reported significant associations between high systemic BP and POAG using cross-sectional data [17, 44, 45, 54]. However, the Barbados Eye and the Proyecto VER studies failed to demonstrate a significant relationship between BP and POAG [55, 56].

Although the influence of systemic hypertension on glaucoma is complex, several mechanisms are suggested. The Baltimore Eye Survey showed an age-related association between BP and glaucoma [2]. In younger patients, hypertension showed a protective effect that might improve OPP. However, in older patients, this positive effect is lost and an increased risk of glaucoma is seen, most likely as a result of blood vessel alterations induced by arterial hypertension with disturbed oxygen and nutrition supply [57]. In systemic hypertension, chronically elevated BP may result in arteriosclerosis, changes in the size of the precapillary arterioles, and capillary dropout leading to increased resistance to blood flow and, thus, reduced perfusion [58]. Also, disruption of the autoregulatory mechanisms of blood flow in the ONH vascular beds at high levels of BP may further contribute to reduced perfusion, which may counteract any protective effect afforded by higher perfusion pressure [13]. These findings lead to the assumption of a U-shaped relationship between BP and the progression of glaucoma [54].

Another important consideration is the relationship between BP, IOP, and POAG. Elevated IOP is considered the most important risk factor for the development and progression of POAG. Therefore, the relationship between BP and IOP should be considered when evaluating the association between POAG and hypertension. Moreover, OPP is regarded as another important risk factor for disease development and progression. As previously mentioned, as OPP includes IOP, it is possible that some of the findings attributed to OPP are in fact exclusively secondary to IOP. Therefore, it is always important to verify whether previous studies adjusted for IOP. Several large epidemiology studies that adjusted for IOP are shown in Table 1. Interestingly, Memarzadeh et al. showed no association between OAG and conventionally defined systemic hypertension; however, the relationship was found across a range of BPs rather than by arbitrary divisions and definitions. Elevated systolic and mean arterial BPs were significantly associated with a high prevalence of OAG, independent of the impact of IOP.

TABLE 1: Characteristics of the studies investigating the association between primary open-angle glaucoma (POAG) and blood pressure (BP) with adjustment for intraocular pressure (IOP).

Reference	Country	Sample size	Study design	Exposure	Outcome
Blue mountains eye study [44]	Australia	3654	Population-based survey	HTN	OAG
The Beijing eye study [20]	China	3222	Population-based survey	HTN	POAG
The Singapore Malay eye study [64]	Singapore	3280	Cross-sectional population-based study	SBP, DBP, HTN	POAG
Los Angeles Latino eye study [54]	USA	6130	Cross-sectional population-based study	SBP, DBP	OAG
Barbados eye study [52]	India	3222	Cohort study of prospective population-based study	SBP, DBP, HTN	OAG
The Rotterdam study [65]	Netherlands	5317	Cross-sectional prospective population-based study	SBP, DBP	OAG

HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; OAG, open-angle glaucoma; POAG, primary open-angle glaucoma.

6. POAG and Hypotension

Pache and Flammer reported hypotension, and in particular a nocturnal drop in BP, as an important risk factor for OAG [59]. Randomized clinical trials also suggested that low BP is associated with risk and progression of glaucoma. In the Early Manifest Glaucoma Trial, lower SBP in patients with lower baseline IOP was associated with faster progression to OAG [50]. However, this J-shape association between systolic and diastolic BP and IOP may be confounded by antihypertensive treatment status, as treated or overtreated hypertensive patients can have a normal or low BP but elevated POAG risk [46]. In the Thessaloniki Eye Study, low DOPP was associated with an increased risk for POAG in subjects undergoing antihypertensive treatment [51]. In the Baltimore Eye Study, a DOPP of less than 35 mmHg was associated with a significant increase in the prevalence of glaucoma [2]. In the Egna-Neumarkt Study, the prevalence of glaucoma decreased progressively with increased DOPP, whereas no correlation was detected with either systolic or mean OPP [17].

In terms of the association between BP and glaucoma, nocturnal hypotension may exacerbate the progression of visual field loss in patients with glaucoma [60, 61]. When a nocturnal BP dip coincides with an IOP spike, a substantial OPP reduction is thought to produce an intermittent insult that increases the risk of disease progression [62]. DOPP is especially useful for displaying the lowest OPP values and is regarded as an independent risk factor for OAG. A recent study suggested that nocturnal BP could be a modifiable risk factor for glaucoma severity and progression [63]. Nocturnal hypotension is caused primarily by sleep, presumably owing to sympathetic withdrawal. However, physiologic nocturnal hypotension is regarded as a protective mechanism during sleep; therefore, artificial regulation of nighttime BP should be considered with caution.

7. Conclusion

Several studies demonstrated that both high and low BP are associated with increased risk of POAG. An increase in BP is associated with an elevated IOP, leading to increased risk of glaucoma. In addition, the microangiopathy of hypertension can result in end organ damage including the retina and optic nerve. Hypertension must be treated because it is one of the most important risk factors for cardiovascular morbidity and mortality. But excessive BP lowering in glaucoma patients may cause a drop in OPP and subsequent ischemic injury. In particular, DOPP is useful for displaying the lowest OPP values and is regarded as an independent risk factor for OAG. Although low OPP is an established risk factor in POAG, as OPP includes IOP, it is possible that some of the findings attributed to OPP are in fact exclusively secondary to IOP. Current treatment of POAG aims to reduce IOP; however, there is no evidence to support the value of increasing BP as therapy for POAG. Such recommendations are not currently warranted, since we lack crucial information about the microvascular beds in which perfusion is important in glaucoma, and the appropriate methods to evaluate their blood flow [16]. More research on treatments designed to increase OPP by increasing BP is needed.

The relationship between IOP, OPP, and BP may be related to a U-shaped relationship between BP and the progression of glaucoma. Therefore, both high and low BP should be monitored with caution especially in patients with progressive glaucoma despite controlled IOP.

Disclaimer

The authors alone are responsible for the content and writing of the paper.

Conflict of Interests

The authors report no conflict of interests.

Authors' Contribution

Hye Jin Chung and Hyung Bin Hwang are co-first authors and equally contributed to this work.

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Review Article

Ocular Manifestations of Ebola Virus Disease: An Ophthalmologist's Guide to Prevent Infection and Panic

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Ebola virus disease (EVD—formerly known as Ebola hemorrhagic fever) is a severe hemorrhagic fever caused by lipid-enveloped, nonsegmented, negative-stranded RNA viruses belonging to the genus *Ebolavirus*. Case fatality rates may reach up to 76% of infected individuals, making this infection a deadly health problem in the sub-Saharan population. At the moment, there are still no indications on ophthalmological clinical signs and security suggestions for healthcare professionals (doctors and nurses or cooperative persons). This paper provides a short but complete guide to reduce infection risks.

1. Introduction

Ebola virus disease (EVD—formerly known as Ebola hemorrhagic fever) is a severe hemorrhagic fever caused by lipid-enveloped, nonsegmented, negative-stranded RNA viruses belonging to the genus *Ebolavirus*. Ebola virus and Marburg virus constitute the family Filoviridae in the order of Mononegavirales. These viruses have characteristic twisted filamentous particles that give the virus family its name. Ebola virus particles have a uniform diameter of 80 nm but can greatly vary in length, 1 μm or even longer [1].

The first reported cases of EVD occurred in 1976 in southern South Sudan (former Sudan) and in northern Democratic Republic of the Congo (former Zaire). The name Ebola corresponds to the name of a small river located in the endemic area (northwestern Zaire) [2, 3]. Five different species of Ebola viruses are recognised, *Bundibugyo*, *Zaire*, *Sudan*, *Reston*, and *Taï Forest*. The three first species caused epidemics in Africa with high case fatality rates, while no human death due to the two other species has ever been reported [4].

EVD is a classic zoonosis with persistence of the virus in reservoir species which live in endemic areas. Viral transmission chain was unveiled after a long absence of epidemic EVD. Animal trapping missions were carried out in areas where several cases had occurred. Three species of fruit bats were found asymptomatically and naturally infected with Ebola virus, thus suggesting that these animals are the natural reservoir [5]. Such idea was corroborated by experimental studies which reported successful Ebola virus infection transmission in fruit bats [6] and by the identification of Marburg virus in fruit bats [7]. Indeed, virus is present in saliva of chronically and asymptomatically infected fruit bats, which expose other species through direct transmission via bites. Indirect transmission also is postulated, as infected bats drop to the ground partially eaten fruit, which may be eaten by nonhuman primates. This process would hypothetically promote their infection. Whatever the route of infection is, it is necessary to amplify the number of infected individuals of the secondary host species to promote the emergence of human disease. A single infected human does not make an epidemic which is, however, promoted by the persistence of prerequisites for Ebola virus infection transmission [5].

Ebola virus infects human monocytes and induces the production of virus-cell particles that result in the loss of endothelial barrier function. In addition, infected monocytes

release proinflammatory cytokines and chemokines, which further decrease endothelial barrier function [8]. Animal and in vitro studies show that the Ebola virus has a number of physical and biological mechanisms to evade host innate and acquired humoral and cellular immune responses. These mechanisms promote rapid virus dissemination and replication [9].

Accurate description of the clinical manifestations of EVD was made, for the first time, during the outbreak that occurred in 1995 in the Democratic Republic of the Congo, because it secondarily affected a large number of healthcare workers. The evolution of the disease was followed from the beginning and differences between survivors and fatal cases were directly observed. Although the incubation period is thought to range between two and twenty-one days, the mean observed incubation period among secondary cases was six days, ranging between five and eight days after the contact. Common EVD manifestations are fever, asthenia, diarrhea, abdominal pain, myalgia, arthralgia, melena, sore throat, conjunctival injection, and rush. Fatal cases also show anuria, shock, tachypnea, hiccups, dysesthesia, and bleeding (e.g., gum bleeding, epistaxis, bleeding on injection sites, hematuria, melena, hematemesis, and hemoptysis). Survivors show severe asthenia, tinnitus, hearing loss, coughing, vision loss, conjunctivitis, and uveitis [10].

2. Epidemiology

Case fatality rate, assessed during outbreaks that occurred between 1976 and 2012, is 65% (95% confidence interval, 55–75%). Case fatality rates of the single species are 76% (95% confidence interval, 63–87%), 55% (95% confidence interval, 50–59%), and 37% (95% confidence interval, 14–63%) for the *Zaire*, *Sudan*, and *Bundibugyo* species, respectively [11].

The current outbreak is the largest known. It started in February 2014 in Guinea and spread into Liberia in March and Sierra Leone in May, followed by other countries. Its exponential expansion in the first period raised great public health concern. The peculiar characteristic of the Ebola virus strain responsible for the last outbreak is that, given its genetic closeness with the two strains responsible for the most recent outbreaks, it probably derived from both these specimens. However, it underwent human adaptive mutations, which ultimately increased the person-to-person transmission [12]. Indeed, on March 4, 2015, the World Health Organization reported 24,000 cases and 10,000 deaths. These figures show without any doubt that this is the most widespread and long-term outbreak since the discovery of the Ebola virus [13].

EVD may be characterized by high fever, arthralgia, and mild coagulopathy or be asymptomatic at all. Indeed, during various outbreaks, prevalence of individuals with high levels of anti-Ebola virus IgG was relatively high, ranging between 1 and 6% in villages and 20 and 30% in the forest (Table 1) [2, 3, 14–18]. Only a fraction of these individuals reported fever in the days of the outbreak, while the majority were asymptomatic. Early during the infectious process, asymptomatic individuals show strong inflammatory response, which causes virus clearance. Conversely, the delayed inflammatory response, observed among symptomatic subjects, is at

Table 1: Prevalence of individuals with high serum levels of IgG anti-Ebola virus (i.e., immune against EVD), who were not close contacts of EVD patients, during Ebola virus outbreaks.

First author, year	Country	Setting	Prevalence
WHO, 1978a [2]	Sudan	Overall	6%
WHO, 1978b [3]	Zaire	Overall	1%
Baron, 1983 [18]	Sudan	Village	18%
Busico, 1999 [14]	Democratic Republic of the Congo	Village	2.2%
Gonzalez, 2000 [15]	Central African Republic	Village	3.1–3.7%
		Forest	1.9–12.1%
Becquart, 2010 [16]	Gabon	Village	2–7–12.4%
		Forest	18.4–21.2%
Nkoghe, 2011 [17]	Gabon	Overall	15.3%
		Deep forest	5.0–32.4%

the basis of the most severe hemorrhagic symptoms of EVD [19].

3. Human-to-Human Ebola Virus Infection Transmission

Human-to-human transmission of EVD is reported in large outbreaks which occur in remote locations, where proper medical, public health, transportation, and communication infrastructure are limited. Ebola virus infection and, sometimes, amplification in hospital settings are frequently described. Widespread transmission events typically involve hospital settings where protective equipment is limited or unavailable, thus suggesting that transmission in healthcare settings can be largely prevented by basic infection control precautions and proper disposal of contaminated items. Indeed, the infectious nature of person-to-person transmission is not efficient. It is limited to direct contact, through broken skin or mucous membranes, with blood, secretions, organs, or other bodily fluids of infected people, and with contaminated surfaces and materials (e.g., bedding and clothing). During outbreaks, there can be several secondary cases (i.e., infection transmission from the index case) and few tertiary cases (i.e., infection transmissions from the secondary cases) [19].

According to epidemiologic studies among households, most secondary cases have direct physical contact with blood, organs, or bodily fluids of diseased persons or cadavers [18, 20]. However, since some individuals do not report direct contact with EVD patients other routes of transmission are plausible [21–23]. An exhaustive example of transmission tree was performed with all cases and contacts reported from Nigeria during the 2014 outbreak. The index case was an infected individual coming from Liberia and EVD was diagnosed after three days. Since then, specific preventive measures were applied that resulted in the end of the outbreak within few weeks. A total of 898 contacts were subsequently identified and linked to the index case, split

into 351 primary/secondary contacts and 547 tertiary/higher-order contacts. The outbreak resulted in 19 cases; more than half of them were healthcare workers. The index patient generated twelve secondary cases, while five tertiary and two quaternary cases occurred. Therefore, only 2% of all contacts developed Ebola virus infection or EVD [24].

A transmission route that raises public concern is through the air. One study on three healthy rhesus macaques housed in cages located three meters away from cages that housed infected animals found that two of them became infected and developed EVD [25]. Conversely, another similar study performed on two cynomolgus macaques housed in cages placed thirty centimeters away from the cages of infected animals reported that healthy macaques did not develop the infection [26]. These discrepant studies suggest that Ebola virus airborne transmission is unlikely to occur and is probably due to bloody coughing or sneezing with spatter production (i.e., droplets larger than $50–100\,\mu m$ in size that fall in the environment within 1/100 seconds) that fall within few meters from the source, rather than through nonbloody aerosol and droplet nuclei (i.e., droplets smaller than $50\,\mu m$ in size that suddenly desiccate and become droplet nuclei, $1–5\,\mu m$ in size), which may be suspended in air for hours before their sedimentation.

The main problem in assessing human-to-human Ebola virus transmission is that the minimum infectious dose of the microorganisms is unknown. This makes it difficult to assess how much blood or bodily fluids are necessary to develop the infection.

4. Ebola Virus Infection Transmission to Healthcare Workers

Healthcare workers are the category at the highest risk of secondary Ebola virus infection. For example, during the 1995 outbreak in the Democratic Republic of the Congo, one-fourth of all cases and the majority of secondary cases were healthcare workers who had provided care to EVD patients without appropriate contact precautions. Only one healthcare worker, who reported inadvertently rubbing her eyes with contaminated gloves, developed EVD despite the implementation of precautions based on Personal Protective Equipment (PPE) [27]. If adequate control measures are properly applied, the risk of infection among healthcare workers is minimal. Indeed, during the 2014 outbreak, most infections developed among healthcare workers occurred before PPE-based measures were applied in hospitals or in emergency departments, where EVD patients might be confused with patients with malaria, typhoid fever, or other tropical diseases characterized by high fever [28]. The situation in Ebola treatment units in countries where the virus is endemic is terrible, with patients who fall out of bed or in delirium and try to crawl out. The environment is extensively contaminated by blood, vomit, and diarrhea; instruments and fomites also are heavily contaminated. The risk for healthcare workers of being infected without PPE is close to 100%; such a risk is, however, minimal with proper PPE [29].

Ebola virus transmission to healthcare workers is due to blood or secretions and organs contaminated by blood.

Indeed, Ebola virus serum level in EVD patients is high soon after the onset of symptoms. Such level is as high as 10^8 viral particles per serum milliliter in fatal cases and 10^6 in nonfatal cases [30]. Detectable but low virus levels are occasionally reported in tears, saliva, semen, breast milk, and urine of EVD subjects during the acute phase, while the virus is virtually undetectable in convalescent patients. These data suggest that nonbloody bodily fluids are unlikely to transmit the Ebola virus infection [31, 32]. Ebola virus is not detectable in the environment of Ebola treatment units and in medical instruments used on EVD patients where there are no visible blood stains and surface disinfection is routinely performed. Thus, once again, blood is responsible for transmission, while fomites and other environmental surfaces, which are not contaminated by infected blood, are not [31].

These data suggest that Ebola virus transmission through bodily fluids without blood as well as airborne transmission is unlikely. However, since Ebola virus transmission is not completely understood, exceptional precautions, such as pressurized suits with oxygen tanks, are strongly suggested for interventions that generate huge amounts of aerosols (invasive explorations or intubations), for specific situations (e.g., massive hemorrhage), or in laboratories where the Ebola virus is cultivated. Goggles and masks might not even be necessary to speak with conscious patients, as long as a distance of one to two meters or a glass/plastic barrier is placed between the two subjects [33]. The use of extremely sophisticated PPE in African countries where outbreaks occur may be even counterproductive. Indeed, the image of healthcare workers with spectacular protective clothing encourages panic in some communities because they suggest that the only defense against Ebola virus is sophisticated PPE, which is inaccessible to the general population [33]. In addition, extreme PPE raises concerns in the general population regarding malevolent activities, such as intentional killing and stealing blood or body parts, being performed and local healthcare workers may not cooperate in wearing PPE. For this reason, local healthcare workers may not cooperate in wearing any PPE, thus exposing themselves to Ebola virus infection transmission [30].

5. What Are the Chances of Someone Infected with Ebola Virus Seeking Ophthalmologic Care? What Are the Effective Infection Control Measures?

It is generally believed that it is unlikely that EVD patients may seek specialized healthcare, as ophthalmologic care is. However, the number of people coming from West Africa to Western countries, such as Europe, USA, and Australia, is not trivial. For example, approximately 850 individuals come from Ebola virus endemic areas to London each month. If it is true that subjects with severe EVD, who are infectious, are likely to seek emergency services and hospitalization rather than other healthcare services [34], asymptomatic individuals and those with mild EVD are largely prevailing (Table 1) and although poorly infectious, they may seek ophthalmologic care, because of their symptoms.

TABLE 2: Summary of the main recommendations for the treatment of suspected Ebola virus infected patients in ophthalmologic care settings (from CDC and WHO websites).

Personal Protective Equipment (PPE)	Ebola virus infection may be transmitted through broken skin and mucosae	Gown, gloves (possibly double gloves), surgical mask, eye visor/goggles, or face shield to protect conjunctival, nasal, and oral mucosae at the same time. Choose PPE of exact size. Gloves or other PPE that becomes contaminated by blood or bodily fluids must be cleaned or changed before touching other instruments or surfaces. Gloved/ungloved hand hygiene. Use alcohol-based hand rub or soap and running water	Strength of the evidence High
Sharp instruments	Sharp instruments are extremely dangerous because they become contaminated by blood or bodily fluids and may break skin/mucosae even if protected by PPE	Use of needles and other sharp instruments must be limited. These instruments must be handled with extreme care and disposed after use in dedicated seal containers	Strength of the evidence High
Droplets	Airborne transmission is not demonstrated. Preventive measures are recommended under the Precautionary Principle	If aerosol generating procedures or events, such as coughing or sputum induction, occur, the use of powered air-purifying respirator or respirator (FFP2 or EN certified equivalent or US NIOSH-certified N95) is recommended	Strength of the evidence Low
Nonsharp instruments	Indirect transmission through nonsharp contaminated instruments is not demonstrated. Preventive measures are recommended under the Precautionary Principle	Use of disposable medical equipment is recommended or, alternatively, nondisposable medical equipment must be cleaned and disinfected after use according to manufacturer's instructions	Strength of the evidence Low
Environmental surfaces	Environmental surfaces do not pose a risk of infection. However, Ebola virus is nonenveloped and is able to survive in the environment for long time. Preventive measures regarding surfaces visibly contaminated with blood and bodily fluids are recommended under the Precautionary Principle	Use of standard hospital detergents and disinfectants (e.g., 0.5% chlorine solution or a solution containing 5000 ppm available free chlorine), preceded by cleaning to prevent inactivation of disinfectants by organic matter, is recommended	Strength of the evidence Low

Several international organizations, such as the Centers for Disease Control and Prevention (CDC) (available at http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html; accessed version updated November 4, 2014) and World Health Organization (WHO) (available at http://www.who.int/csr/disease/ebola/protective-measures-staff/en/; accessed version updated October 3, 2014), have released and periodically update guidelines for infection control directed to healthcare workers who treat EVD confirmed/suspected patients. These measures are based on the available evidence and on the Precautionary Principle [35]. The most important procedures to prevent Ebola virus infection transmission are displayed in Table 2. PPE use and careful management of sharp instruments are highly recommended, since the strength of the evidence of their effectiveness is strong. The control of droplets, nonsharp instruments, and environmental surfaces also is recommended but at a lower degree of strength based on the Precautionary Principle, since airborne transmission and contact transmission without broken skin and mucosae are hypothesized but undemonstrated.

Globally, these measures are practical and are not particularly different from those generally put into practice by the majority of ophthalmologists. They are enough to prevent Ebola virus infection transmission to healthcare workers, staff, and following patients. In addition, they may help prevent panic and anxiety, a prerequisite for safe and good practice.

6. Conjunctivitis: A Key Ophthalmologic EVD Symptom

During outbreaks, the risk of dealing with false alarms largely overwhelms the chance that infectious individuals

may unexpectedly present at an ophthalmologist office, due to panic generated by the severity of the disease and by misinformation and alarming campaigns through the media [34]. Thus, the key question is whether ophthalmologists are prepared to recognize Ebola virus infected patients.

In endemic areas, primary EVD cases at the initial stages of the disease are undistinguishable from patients affected by malaria (high fever), shigellosis and typhoid fever (diarrhea), and other protozoal, bacterial, and viral zoonoses. However, as long as hemorrhagic fever becomes manifest, the differential diagnosis between Ebola (or Marburg) virus and Lassa fever is to be made and the characteristics of conjunctivitis, one of the main hemorrhagic symptoms, become crucial. Indeed, in Lassa fever conjunctivitis is severe with periorbital swelling and pain, while in EVD conjunctivae are often injected but asymptomatic [2, 10, 36].

The situation is largely different in Western countries. The risk of false alarms is much higher and, therefore, the first issue to consider when evaluating a person for exposure to Ebola virus is to investigate epidemiologic risk factors through the anamnesis. The checklist provided by CDC, available at http://www.cdc.gov/vhf/ebola/exposure/risk-factors-when-evaluating-person-for-exposure.html, is exhaustive enough. The risk is classified into high, some, low, and no. For example, there is no epidemiologic risk if a patient has been in a country where Ebola virus is endemic more than twenty-one days before presenting at the healthcare worker, because twenty-one days is the longer possible incubation period.

On physical examination, conjunctivitis is a key EVD sign. It is typically bilateral, asymptomatic, and nonicteric. In secondary EVD, conjunctivitis is the earlier sign together with influenza-like symptoms (e.g., asthenia and fever) and may appear even 6-7 days before patients seek EVD-related care [37]. Thus, it is possible that these subjects may seek ophthalmologic care before seeking EVD-related care. Persistent and nonhemorrhagic conjunctivitis in EVD patients is a good prognostic factor, while hemorrhagic conjunctivitis is predictive of death within a few days [3, 10, 19, 36–39].

Summarizing, bilateral, asymptomatic, and nonicteric conjunctivitis is one of the earliest and most frequent signs of EVD and has an important prognostic value.

7. Uveitis: A Late Ophthalmologic Symptom

A relevant proportion of convalescent patients, as many as 20% according to one survey [40], who may be asymptomatic for up to 1-2 months, may develop uveitis characterized by ocular pain, photophobia, hyperlacrimation, and loss of visual acuity. Uveitis also is reported in patients who recover from Marburg disease after being asymptomatic for at least two months [41]. Pathogenesis of uveitis may be a delayed hypersensitivity reaction to RNA viral antigens. Uveitis can be treated with topical steroids [40].

It is possible that convalescent patients with late-onset uveitis may seek ophthalmologic care. These patients are considered safe and not infectious, although Ebola virus is detectable in tears of acute-phase EVD patients [31].

Nevertheless, PPE, as in Table 2, must always be adopted in patients with ascertained epidemiological risk.

8. Conclusion

EVD is typically a zoonosis and outbreaks with human-to-human transmission periodically occur. The severity of this disease with its high fatality rate and its awful hemorrhagic symptoms has been largely emphasized by mass media, thus generating panic in the general population and, most of all, healthcare providers who are the category at highest risk of secondary cases. Yet, false alarms are largely prevailing on true EVD patients. In addition, none of the healthcare workers from Western countries secondarily infected by the Ebola virus developed severe EVD and no tertiary cases occurred (see the CDC website at http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/united-states-imported-case.html). The risk for EVD among ophthalmologists from Western countries is, therefore, minimal. However, it is not impossible that mild, asymptomatic, and convalescent EVD patients may seek ophthalmologic care. Proper anamnesis and physical examination are enough to distinguish between false alarms and potential Ebola virus carriers and, in the latter case, preventive measures are effective in minimizing the risk of transmission.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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