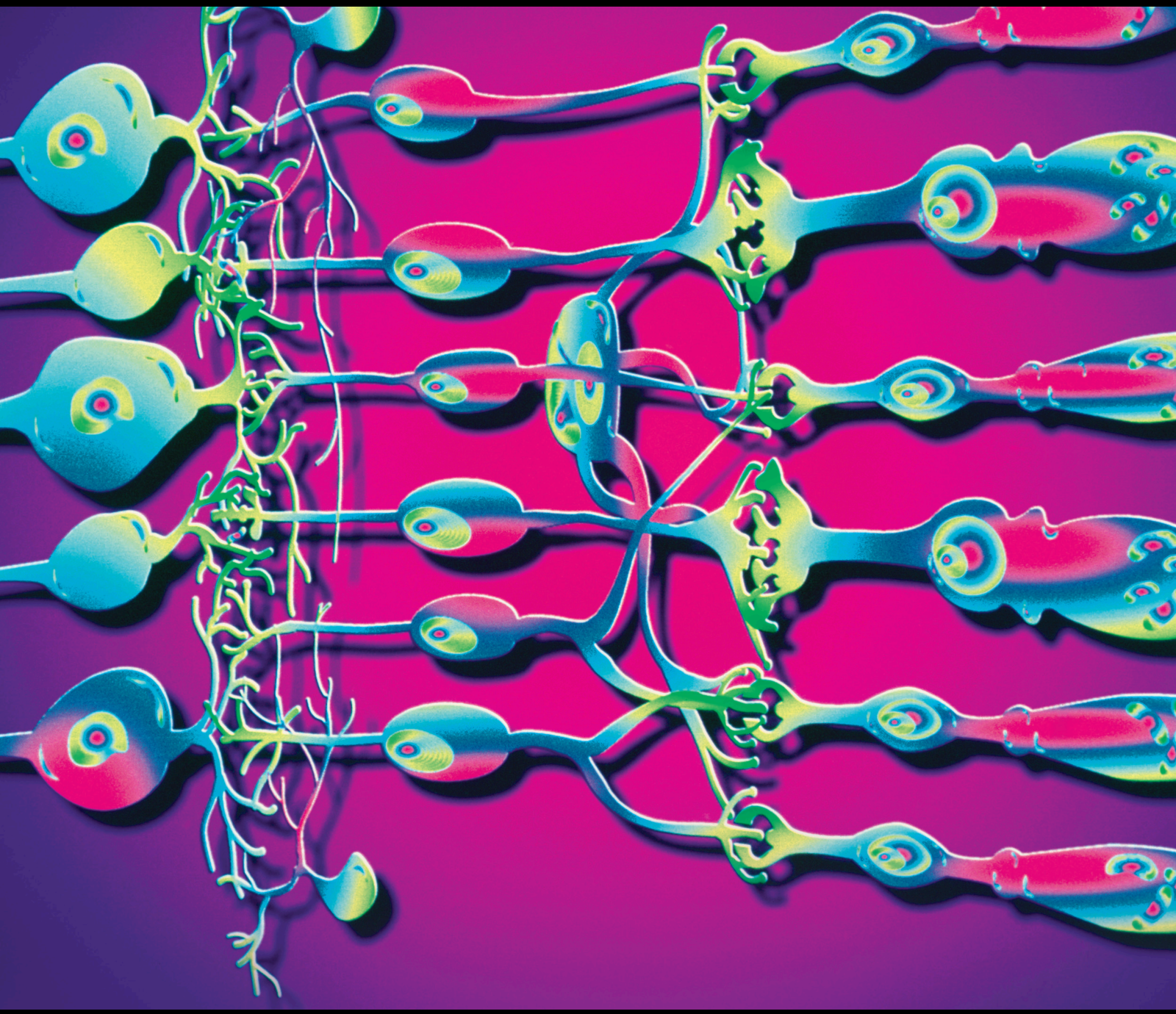
Journal of Ophthalmology

Novel Approaches to Optimize Treatment Strategies in Glaucoma

Lead Guest Editor: Miriam Kolko
Guest Editors: Barbara Cvenkel and Steffen Heegaard



Novel Approaches to Optimize Treatment Strategies in Glaucoma

Journal of Ophthalmology

Novel Approaches to Optimize Treatment Strategies in Glaucoma

Lead Guest Editor: Miriam Kolko
Guest Editors: Barbara Cvenkel and Steffen Heegaard

Contents

Hindawi
Journal of Ophthalmology
Volume 2021, Article ID 9876478, 2 pages
https://doi.org/10.1155/2021/9876478

Editorial

Novel Approaches to Optimize Treatment Strategies in Glaucoma

Miriam Kolko,[1,2] **Steffen Heegaard**,[2,3] **and Barbara Cvenkel**[4,5]

[1]*Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark*
[2]*Department of Ophthalmology, Copenhagen University Hospital, Rigshospitalet-Glostrup, Denmark*
[3]*Department of Pathology, Copenhagen University Hospital, Rigshospitalet-Glostrup, Denmark*
[4]*Department of Ophthalmology, University Medical Centre Ljubljana, Ljubljana, Slovenia*
[5]*Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

Correspondence should be addressed to Miriam Kolko; miriamk@sund.ku.dk

Received 28 July 2021; Accepted 28 July 2021; Published 16 August 2021

Preserving lifelong vision in glaucoma patients with minimal impact on quality of life in terms of inconvenience and side effects is the goal of all treatment guidelines. The current understanding of glaucoma is limited, and only strategies that reduce intraocular pressure (IOP) have been shown to be effective in just delaying the progression of glaucoma. However, IOP-lowering treatments come with the risk of side effects that affect adherence and patients' quality of life. Often, treatments are initiated too late due to the asymptomatic nature of the disease, and progression to severe visual impairment or blindness is inevitable.

The current Special Issue addresses some aspects of basic and clinical research on neuroprotection, neurodegeneration, and medical treatment of glaucoma with an overview of the current status, including novel drugs and drug delivery systems to improve adherence as well as new approaches to potential treatment modalities in the future.

Glaucoma, like many chronic diseases, has a low adherence rate, which may lead to a progression of the disease and therefore to higher costs. Meier-Gibbons and Toteberg-Harms present an overview of the published literature on the costs of glaucoma care, dividing them into indirect, associated with the consequences of an advanced disease, and direct costs, associated with diagnostic tests and treatment. The published studies focus primarily on the direct cost of glaucoma care and show great differences in the price of antiglaucomatous drugs across countries and trends of prescribing generic drugs in Europe. Despite the fact that patients appear to be better informed about their disease and there has been an improvement in medical and surgical treatment, adherence has not improved in recent decades. More studies are needed to investigate the relationship between cost and adherence and focus on strategies for improving patients' adherence.

The use of generic medications for glaucoma has grown considerably in recent years. This is due to patent expiration dates for the innovators and the relative ease and short time to bring generic eye drops to the market, which does not require studies comparing branded and generic eye drops. With approval by the relevant regulatory authority, generics are required to have the same ingredient, route of administration, dosage, and be manufactured to the same quality standards as the reference medicinal product but may have different ingredients and packaging. Tatham reviews the potential pros and cons of generic drugs in terms of efficacy, cost, tolerability, and differences in formulation, adherence, and ease of use and perceptions of generic medicines among healthcare professionals and the general public. The switch to generic drugs reduces health-related costs, but there are some limitations of generic drugs that need to be addressed, such as the influence of inactive ingredients on bioavailability and thus efficacy and bottle design to improve consistency in drug delivery.

Long-term glaucoma treatment with multiple benzalkonium chloride- (BAK-) preserved eye drops may lead to increased aqueous humour flare. Pakuliene et al. used laser flare photometry, a noninvasive quantitative measurement of anterior chamber protein levels, to investigate intraocular inflammation in glaucoma patients on topical treatment for more than 2 years scheduled for cataract surgery without any

other ocular abnormalities. The control group included cataract subjects without glaucoma. Investigators found higher aqueous humour flare in the glaucoma patients than in the control group. Several factors were associated with the aqueous humour flare increase, including pseudoexfoliation syndrome, number of eye drops, and presence of BAK.

An update of currently available IOP-lowering medications and potential new treatment targets for IOP-lowering and neuroprotective therapy are discussed by Cvenkel and Kolko along with the future trends in glaucoma therapy such as sustained drug delivery systems and drug formulations. For future personalized medicine based on genetic and other characteristics of the individual patients, it will be appropriate to identify patients with high risk of progression and treat them more vigorously.

In a review of the literature on vitamin D and glaucoma, Abouzeid and Samer concluded that vitamin D metabolites may play a role in glaucoma, either through a lowering effect on IOP or a neuroprotection pathway, but the exact molecular mechanism is not known. However, the limited number of clinical studies and significant bias in the study design preclude conclusions regarding the involvement of vitamin D in primary open-angle glaucoma (POAG).

Several mechanisms have been implicated in retinal ganglion cell death, and therapeutic strategies may be needed to target this to delay progressive retinal ganglion cell death in glaucoma. Mitochondrial dysfunction has been linked to optic neuropathy and brain neurodegenerative diseases. The review of Duarte focuses on how damaged mitochondria may impact the ability of neurons and glial cells to maintain homeostasis and induce sterile inflammation and neurodegeneration via mitochondrial damage-associated molecular patterns.

Tsai focuses on IOP-independent strategies for neuroprotection and/or neurodegeneration, including research into neurotrophic factors, gene therapy, immune system modulation, and novel neuroregeneration pathways. However, these innovative strategies should be critically balanced and weighed against the risk of disrupting the complex central nervous system environment.

Nuzzi et al. address the current new treatment strategies, including medical therapy and delivery modes, laser treatment, and minimally invasive glaucoma surgery. Furthermore, the most recent approaches to treat glaucoma in different stages are presented, including IOP-lowering effect of trans-palbebral electrostimulation of trabecular meshwork and novel techniques of cilioablation, as well as the advantages and risks of stem cell therapy, neurotrophic factors and potential therapy with stem cell-derived exosomes.

An important research goal is identifying systemic risk factors that can help in predicting POAG. Pfahler et al. using nailfold capillaroscopy documented increased levels of nailfold haemorrhage, dilated capillaries, and avascular zones in POAG patients. These findings suggest that systemic microvascular dysfunction is frequent in POAG. In addition, the presence of any haemorrhage was found to be a highly significant risk factor for glaucoma.

An enhanced physiological stress response to reduced oxygen supply has been documented in patients with normal tension glaucoma (NTG) compared to age-matched healthy controls, supporting the role of vascular dysfunction. Dalgaard et al. measured serum adrenaline and endothelin-1 levels and changes in distal finger temperature. In patients with NTG, a relative increase in adrenaline was found during hypoxia and a relative decrease during recovery, but not in controls. Hypoxia also induced elevated temperature in distal finger in patients with NTG only.

The role and function of synucleins, a family of small proteins involved in neurodegeneration of the central nervous system, have been studied in an animal model of glaucoma. Liu et al. found that IOP elevation caused a significant loss of retinal ganglion cells in older animals, but no significant change in young rats. Beta-synuclein was significantly downregulated in young animals without any significant change in old ones. These results show a link between aging and beta-synuclein regulation. Hydrogen sulfide, a potent reductant, was effective in downregulating beta-synuclein and was neuroprotective against acute IOP elevation.

Finally, Russel et al. devised a low-cost method assessing glaucoma using digital image analysis of the angle and optic nerve. Colour fundus photographs, standard optic disc OCT, and digital slit lamp gonioscopy images were used for digital image conversion and analysis. Requiring only retina digital cameras, the volumetric, geometric, and segmentational data acquired through digital image analysis correspond well with the data obtained by OCT imaging, but importantly reduce the costs.

In summary, this special issue offers an overview of novel treatment strategies and future targets in the treatment of glaucoma. It also provides new findings that may be a clue for new research in this important field.

Conflicts of Interest

The editors declare no conflicts of interest.

Acknowledgments

The editors would like to thank all the authors and reviewers whose work made it possible to publish this special issue.

Miriam Kolko
Steffen Heegaard
Barbara Cvenkel

Hindawi
Journal of Ophthalmology
Volume 2021, Article ID 4581909, 18 pages
https://doi.org/10.1155/2021/4581909

Review Article

Neuroinflammatory Mechanisms of Mitochondrial Dysfunction and Neurodegeneration in Glaucoma

Joao N. Duarte [1,2,3,4]

[1]*Neuroinflammation Unit, Biotech Research & Innovation Center, University of Copenhagen, Copenhagen, Denmark*
[2]*Department of Ophthalmology, Rigshospitalet, Copenhagen, Denmark*
[3]*Department of Clinical Immunology, Section 7631, Rigshospitalet, Copenhagen, Denmark*
[4]*Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark*

Correspondence should be addressed to Joao N. Duarte; joao.duarte@bric.ku.dk

Received 12 January 2020; Revised 29 June 2020; Accepted 23 March 2021; Published 16 April 2021

Academic Editor: Biju B. Thomas

The exact mechanism of retinal ganglion cell loss in the pathogenesis of glaucoma is yet to be understood. Mitochondrial damage-associated molecular patterns (DAMPs) resulting from mitochondrial dysfunction have been linked to Leber's hereditary optic neuropathy and autosomal dominant optic atrophy, as well as to brain neurodegenerative diseases. Recent evidence shows that, in conditions where mitochondria are damaged, a sustained inflammatory response and downstream pathological inflammation may ensue. Mitochondrial damage has been linked to the accumulation of age-related mitochondrial DNA mutations and mitochondrial dysfunction, possibly through aberrant reactive oxygen species production and defective mitophagy. The present review focuses on how mitochondrial dysfunction may overwhelm the ability of neurons and glial cells to adequately maintain homeostasis and how mitochondria-derived DAMPs trigger the immune system and induce neurodegeneration.

1. Introduction

Glaucoma is a complex and multifactorial neurodegenerative disease characterized by the irreversible loss of retinal ganglion cells' (RGCs) soma and degeneration of the optic nerve axons [1]. Glaucoma is the most common optic neuropathy and the leading cause of irreversible blindness worldwide [2, 3]. It is generally accepted that elevated intraocular pressure (IOP) is a major risk factor [2].

The exact mechanisms by which elevated IOP triggers axonal degeneration and RGC death are yet to be known. IOP-induced mechanical compression to the optic nerve head (ONH), at the level of the lamina cribrosa, might directly lead to ischemia-hypoxia damage, blockage of axonal transport, and deprivation of growth factors [4–7]. However, indirectly, damage to RGCs may also result from the action of factors released by activated glial cells located in the lamina cribrosa [8–11]. Regardless of the mechanisms that initiate damage, there is evidence that, in glaucoma, these events converge into axonal degeneration, RGC death, and clinical overlap between the different glaucoma subphenotypes.

Notwithstanding the major role of IOP in the progression of glaucoma, in patients with normal-tension glaucoma (NTG), the elevation of IOP is not necessary for the development of glaucomatous damage [12]. Moreover, the reduction of IOP does not always prevent neurodegeneration, and many patients progress with the disease despite having IOP within normal range [13, 14]. This suggests that mechanisms other than IOP biomechanical and/or ischemic injury may be responsible for the neurodegenerative process. Although the precise mechanisms that lead to RGC insult and loss have not been identified, accumulating evidence supports a primary role of inflammation and the immune system [15–17]. Despite these indications, ocular hypertension remains the only target for current glaucoma therapies.

The central nervous system (CNS), which also includes the retina and optic nerve, is an immune-privileged site where immune functions are tightly regulated and are

mediated by a limited number of cell types [18]. The onset of inflammation in glaucoma is hypothesized to be triggered by an altered crosstalk between RGCs and glial cells that involves the release of proinflammatory mediators, such as reactive oxygen species (ROS), nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β) [9, 19–23]. Despite the recognized role of inflammation in glaucoma, to date, the key inflammatory signals and events that lead to polarization of microglia and astrocytes in the disease progression are not known. Transcriptomic studies showed that many inflammatory genes are upregulated in the retina and ONH in the early stage of the disease [17, 24, 25]. Some of the pathogenic events in glaucoma have been attributed to modifications of neurotrophin and glutamate signaling, excitotoxicity, oxidative stress, mitochondrial dysfunction, protein misfolding, hypoxia, ischemia, autoimmunity, and autophagy dysfunction [26–28].

The present review focuses on the role of the immune system in glaucomatous neurodegeneration and its contribution to disease progression. In particular, it discusses how mitochondrial dysfunction and concomitant neuronal and glial pathological processes can induce and perpetuate sterile inflammation in glaucoma, and which crossroads may be implicated in that. In the first part of the review, potential causes of neuronal mitochondrial dysfunction are addressed. In the second and third parts, the discussion will focus on currently known mechanisms of neuroglial dysfunction and the immune response secondary to mitochondrial damage.

To obtain a comprehensive collection of publications dealing with the neurodegenerative inflammatory response in glaucoma, multiple unrestricted PubMed searches specifying the occurrence of the term "glaucoma" in combination with "inflammation," "neurodegeneration," "immune response," "mitochondrial dysfunction," "oxidative stress," "mitophagy," and "mutation" were performed. The search was limited to primary open-angle glaucoma (POAG). Human studies that dealt with primary closed-angle glaucoma or secondary glaucoma were not considered for this review.

2. Mitochondrial Dysfunction in Retinal Ganglion Cells

The retina is one of the most metabolically active tissues in the body and requires a precise regulation of energy production to meet its consumption needs [29, 30]. Energy in the form of adenosine triphosphate (ATP) is required to synthesize neurotransmitters, organize synaptic vesicles, restore ion gradients, buffer calcium, and transport cargo bidirectionally along axons [30, 31]. Due to the absence of saltatory conduction, the unmyelinated portion of RGC axons within the retina requires more energy for the generation of action potentials [32]. In response to this high metabolic demand, a large proportion of mitochondria populate the unmyelinated portion of RGC axons [32–35].

Strict coordination between mitochondrial biogenesis, dynamics, transport, and degradation is essential to preserve the integrity of mitochondria within RGCs [30, 35]. These events are tightly regulated to ensure that mitochondria can adapt to fluctuations in energy requirements [36, 37]. Even though these metabolic processes are not unique to RGCs, these cells have lower tolerance for mitochondrial damage, and an inadequate supply of healthy mitochondria or the accumulation of defective mitochondria may be the origin of an energy crisis in RGCs [28, 30]. Among other potential causes, mitochondrial dysfunction has been linked to oxidative stress, mutations in mitochondrial DNA (mtDNA), and deficient mitophagy [38, 39].

2.1. Oxidative Stress. Oxidative stress is broadly defined as an imbalance that favors the production of ROS over antioxidant defenses. A consequence of electron transport through mitochondrial oxidative phosphorylation (OXPHOS) complexes is the generation of ROS, such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2). Although ROS are key second messengers in various redox-sensitive signaling pathways, they can damage cellular proteins, lipids, and nucleic acids via oxidation [40]. As mitochondria are a significant source of ROS in many eukaryotic cells, especially in the context of age-related deterioration of mitochondrial electron chain transfer, mitochondrial ROS have been suggested to be an important immunostimulatory stimulus in glaucoma [40, 41].

Oxidative stress occurs as a result of malfunctions in one or more of the mitochondria's four main functions: generation of energy in the form of ATP; regulation of ROS production; regulation of cytosolic calcium levels; and modulation of apoptosis via mitochondrial permeability [42, 43]. In normal conditions, the generation of ROS in low levels is blocked by antioxidants, such as glutathione peroxidase, superoxide dismutase, and catalase. The most active sites of mitochondrial ROS production (i.e., sites of electron leakage) are complex I and complex III of the OXPHOS chain [44]. Although these sites do not constitute a major source of ROS within the cell, the damage inflicted by mitochondrial ROS can be very detrimental due to a particular vulnerability of the mitochondria to oxidative stress, notably to mtDNA. ROS, when in excess, can also induce lipid peroxidation and apoptosis by increasing mitochondrial membrane permeability and by inhibiting the mitochondrial respiratory chain [45–48].

The accumulation of neurotoxic levels of glutamate is another effect of oxidative stress. Glutamine synthase, which converts retinal glutamate into a nontoxic form, and glutamate transporter proteins were modified by ROS in experimental models of ocular hypertension [49–51]. Many of these cellular pathways and components that are damaged by oxidative stress become themselves the cause of further ROS production, perpetuating a cycle of cellular injury.

The cellular accumulation of ROS, regardless of the triggering stress condition, can mediate the induction of autophagy [52]. Autophagy is a nonselective degradation pathway that primarily promotes cellular protection through clearance of damaged organelles and protein aggregates. The autophagic process is fundamental to neuronal homeostasis and can be distinguished into more organelle-specific

pathways, such as mitophagy for selective removal of mitochondria (see Section 2.3). In RGCs, ROS serve as signaling molecules in the induction of autophagosomes by regulating the activity of the cysteine protease ATG4, which seems to be a key element in the activation of autophagy [53]. Yoshimura et al. found that mRNA levels of ATG4B are high in brain tissues and retina, among other sites [54]. Dysfunction of autophagy has also been linked to brain chronic neurodegenerative diseases, suggesting common mechanisms of autophagy-related neuronal loss [52].

A long list of factors has been linked to ROS formation in glaucoma. To some extent, these factors seem to be related to intrinsic abnormal function of mitochondria (primary) or to external events that expose the RGCs and neuroglial mitochondria to stress (secondary), such as compromised blood flow, hypoxia, nutrient deficiency, calcium dysregulation, or mechanical injury (Figure 1) [49, 55–59].

Oxidative stress in POAG has been linked to specific OXPHOS defects, in particular to defects in complex I. Using a lymphoblast-based model to measure systemic mitochondrial function, Lee et al. and Van Bergen et al. showed, in two different glaucoma patient cohorts, decrease rates of ATP synthesis related to complex I defects [60, 61]. Although those defects had a low impact on the in vitro growth of lymphoblasts, they possibly rendered RGCs more susceptible to oxidative stress and metabolic crisis due to the high energetic requirements of RGCs and the presence of multiple cellular stressors in glaucoma. These results are in line with similar findings for complex I defects in hereditary optic neuropathies and brain neurodegenerative diseases [62, 63].

Oxygen and nutrient deficits not only cause RGCs to become bioenergetically compromised but also dramatically increase ROS production. This increase is due to slower electron transportation in the OXPHOS, which augments the reduction state of electron carriers and favors superoxide production at low oxygen concentrations [64]. Studies have also shown the upregulation of hypoxia-inducible factor 1 (HIF1) in glaucoma as a cellular response to reduced oxygen levels in the retina and ONH [65]. HIF1-alpha is a transcriptional activator that induces the expression of several proteins whose main function is to increase oxygen availability in hypoxic tissues [43]. These findings suggest the importance of hypoxia signaling mechanisms in the pathogenesis of glaucoma.

Oxidative stress has also been linked to abnormal cellular calcium influx [66]. Even though calcium serves a major role in normal intracellular metabolism and signaling (including the generation of ROS), an uncontrolled influx may occur through a variety of insults, such as loss of cell membrane integrity and inhibition of calcium ATPase activity [67]. Once the cell has been exposed to an excess of calcium, a synergic effect between calcium and ROS may occur, as ROS can also inhibit calcium ATPase activity in neurons [68]. An excessive influx of calcium and neuronal damage has also been linked to homocysteine accumulation. The mechanism of this homocysteine-induced RGC toxicity seems to take place through direct overstimulation of N-methyl-D-aspartate (NMDA) receptors and a subsequent increase in intracellular calcium and formation of ROS [69].

In addition to retinal and optic nerve damage caused by oxidative stress, the trabecular meshwork in the anterior segment of the eye is also vulnerable to ROS [70, 71]. This event, which has been connected in some cases to genetic mutations in myocilin, may lead to increased resistance of the aqueous humor outflow and cause further retinal exposure to high IOP [71–73]. Mechanical injury to the ONH caused by increased IOP induces neuronal oxidative stress and lipid peroxidation, which increments the risk of neurodegeneration [49, 74, 75]. In a study conducted in aged mice by Kong et al., even short-term elevations of IOP were shown to significantly increase oxidative stress [76].

Increasing evidence also points out the impact of oxidative stress and mitochondrial dysfunction in Müller cells, which may contribute to the pathogenesis neurodegenerative conditions in the retina. As Müller cells have numerous supporting functions to maintain RGC homeostasis, with many of them being metabolic-related, the impact of restricted energy availability in Müller cells has been implicated in glaucomatous RGC loss [77, 78]. A published review by Toft-Kehler et al. on mitochondrial function in Müller cells can provide an in-depth discussion on the topic [28].

As oxidative stress also represents a failure of endogenous antioxidant defenses to meet an increase of ROS, many studies have been demonstrating an accumulation oxidative stress markers in the serum, aqueous humor, and retina of glaucoma patients, as well as suggesting a general compromise of antioxidant defense in these patients [41, 48, 49, 75, 79, 80]. In a case-controlled study conducted by Yuki et al., the incidence of NTG was significantly correlated with high serum total antioxidant levels and low urinary 8-hydroxy-2′-deoxyguanosine/creatinine (a marker of DNA damage from oxidative stress), suggesting systemic oxidative stress [81]. Similarly, a long prospective study in a cohort of 3500 patients with glaucoma showed an association between low intake of antioxidant nutrients and a higher risk of POAG [82]. Identical results have been also documented in experimental models of glaucoma [83].

Several works have assessed the impact of antioxidant treatment in the reduction of oxidative stress in RGCs. Brimonidine, an alpha-adrenergic receptor agonist currently used to lower IOP, has been found to have a neuroprotective effect beyond IOP lowering [84–86]. Brimonidine seems to prevent abnormal elevations of cytosolic calcium and RGC apoptosis through mechanisms not yet fully elucidated [85]. Tempol, a nitroxyl antioxidant, resulted in increased survival of RGCs exposed to TNF-α and hypoxia in the presence of a caspase inhibitor. Crocin, a carotenoid, has antioxidant properties that are capable of suppressing ROS production, increasing mitochondrial membrane potential, and enhancing RGC viability upon H_2O_2 treatment [45, 87]. Coenzyme Q10 and N-acetyl cysteine are antioxidant compounds that directly target mitochondria and have shown beneficial effects in experimental models of glaucoma. Coenzyme Q10, as an essential cofactor in mitochondrial OXPHOS, afforded retinal protection in an ischemia-reperfusion rat model [88]. Similarly, the administration of N-acetyl cysteine, which enhances mitochondrial OXPHOS, decreased oxidative stress in a rat model of ocular hypertension [84].

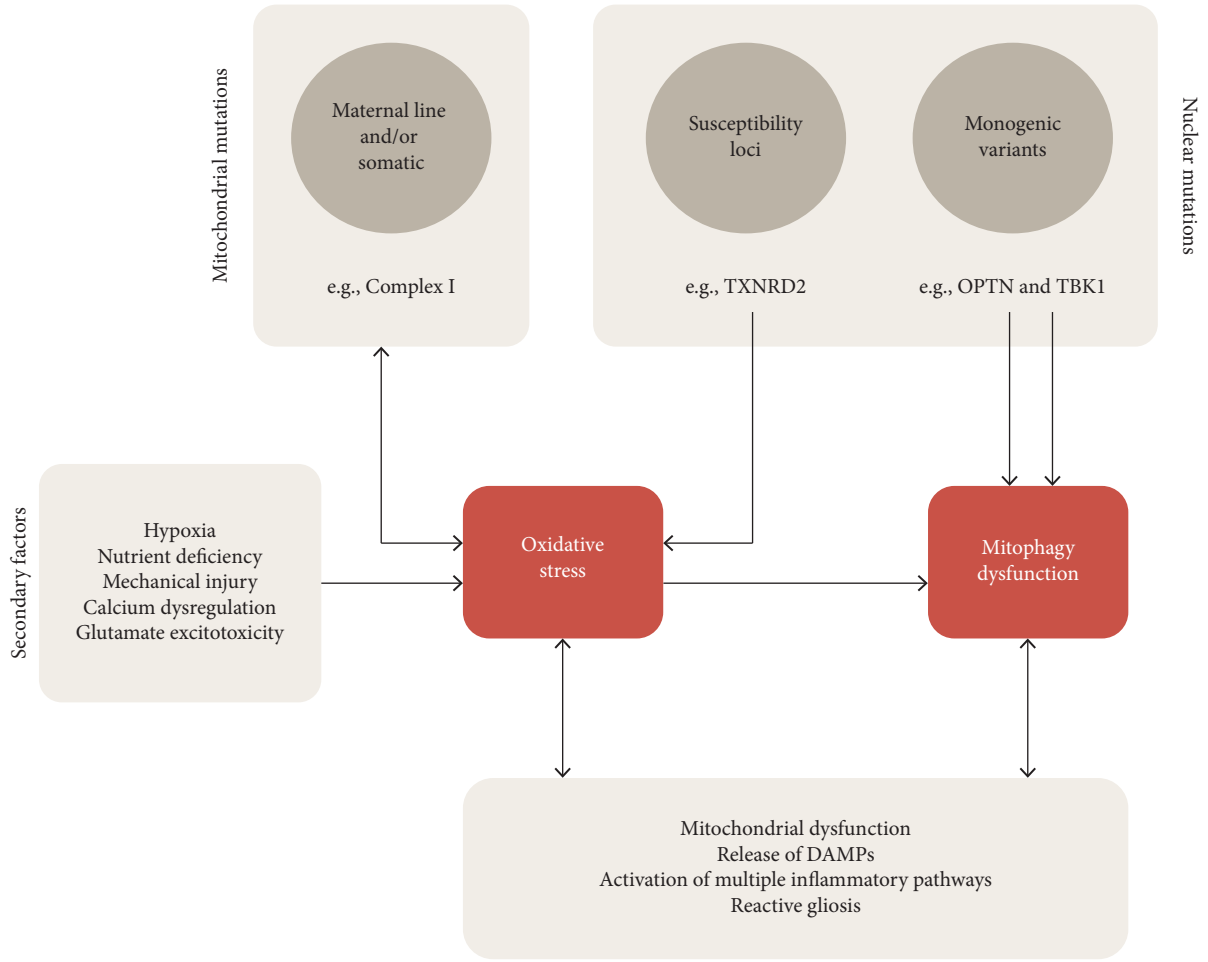


Figure 1: Mechanisms of mitochondrial dysfunction in retinal ganglion cells (RGCs). Mutations in mitochondrial and nuclear genomes can cause mitochondrial dysfunction via oxidative stress or defective mitophagy. Oxidative stress can also be generated through secondary causes external to the mitochondria, such as hypoxia. Mitochondrial-derived components can act as damage-associated molecular patterns (DAMPs) and trigger multiple inflammatory pathways that cause reactive gliosis and RGC loss.

The incorporation of antioxidants into mitochondrial membranes has also been shown to prevent lipid peroxidation, a process known to be initiated by ROS that can lead to cell membrane damage and RGC loss. In a rabbit model of glaucoma, 10-(6′-plastoquinonyl) decyltriphenylphosphonium (SkQ1), a mitochondrial-targeted plastoquinone-containing antioxidant, was able to reverse signs of glaucomatous injury [47]. Likewise, in a mouse model of glaucoma, choric acid and 3,5-dicaffeoylquinic acid of ethanol extract of *Crepidiastrum denticulatum* seemed to reduce lipid peroxidation and protect against RGC death [89]. Yokota et al. also showed that molecular hydrogen, through peroxynitrite scavenging capacity, protected lipid peroxidation and prevented retinal cell apoptosis [69].

2.2. Mitochondrial DNA Mutations. Glaucoma has not been clearly linked to nuclear genome mutations that result in mitochondrial dysfunction [90]. Large genome-wide association studies (GWAS) identified, with limited success, susceptibility loci associated with mitochondrial dysfunction [60, 61, 91]. A large meta-analyzed GWAS conducted by Bailey et al. identified, for example, a single nucleotide polymorphism in thioredoxin reductase 2 (TXNRD2), a mitochondrial protein required for redox homeostasis [92]. The effects of TXNRD2 in vivo were reported by Caprioli et al. who observed reduced RGC death after optic nerve axotomy in experimental models of glaucoma overexpressing TXNRD2 when treated with pharmacologically induced oxidative stress [93].

Mutations with Mendelian inheritance have only been associated with rare cases of POAG with early disease onset. These monogenic variants have been identified in myocilin (MYOC), optineurin (OPTN), and TANK binding kinase 1 (TBK1) [94–99]. Mutations in MYOC, as mentioned above, primarily cause oxidative stress abnormalities in the trabecular meshwork leading to increased IOP. Mutations in OPTN and TBK1 have been linked to dysregulation of mitophagy and apoptosis, and activation of inflammation with RGC loss (Figure 1). A more detailed discussion about the role of OPTN and TBK1 mutations in POAG is provided in the next section.

Several attempts to sequence and identify mutations in mitochondrial DNA have been hindered by mtDNA heteroplasmy, i.e., the multiple variants of mtDNA that can be

present within a cell or a tissue. However, massive parallel sequencing tools have allowed better recognition of large deletions in the mitochondrial genome in a few studies using small cohorts of patients with glaucoma [100–102]. Even though these studies extrapolated mitochondrial variants from peripheral blood leukocytes, they provide evidence of an association between systemic mtDNA defects and glaucoma. Moreover, from peripheral blood leukocytes, Sundaresan et al. identified mtDNA mutations in complex I in approximately one-third of POAG patients [102]. These results are in line with previous studies that addressed the possibility of defects in complex I resulting in impaired respiration rates and ATP production in lymphocytes of patients with POAG [60, 61]. To what proportion mtDNA variants are inherited through maternal line or acquired throughout life as somatic mutations is currently not known.

Somatic mutations are prone to accumulate more frequently in the mitochondrial genome due to the lack of protective histones and an efficient DNA repair system associated with the nuclear genome [103]. Mitochondrial abnormalities are associated with a number of optic neuropathies, and accumulating evidence indicates that age-related mitochondrial defects play a central role in the pathogenesis of glaucoma [44, 104, 105]. Mitochondrial genetic variants have also been linked to other neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease, which are thought to have some overlapping pathologic features with glaucomatous neurodegeneration [106–109].

Exposure of mtDNA to ROS can induce its degradation, and mtDNA degradation products are found in human cerebrospinal fluid and plasma [110]. Transfection of mouse primary astrocytes with degraded mitochondrial polynucleotides was shown to cause a proinflammatory response, which was characterized by the upregulation of TNF-α, IL-1β, IL-6, and monocyte chemotactic protein 1 [110]. This observation suggests a mechanism for mtDNA degradation and downstream activation of proinflammatory phenotypes in glial cells (Figure 1). It was also previously reported that mtDNA deteriorates in response to hydrogen peroxide in HA-1 hamster ovarian cells, an effect that was not observed with nuclear DNA or cytoplasmic RNA [111]. Degradation of the mitochondrial genome was apparent in both mtDNA and mitochondrial RNA species.

Even though an immunostimulatory release of mtDNA may occur through the formation of mitochondrial-derived vesicles or necrotic cell death, a more controlled and less immunogenic mechanism of mtDNA processing has also been proposed [40, 112]. PTEN-induced kinase 1 (PINK1) and Parkin, two mitochondrial proteins linked to mitophagy and Parkinson's disease, have been shown to actively inhibit mitochondrial-derived vesicles and mitochondrial antigen presentation in favor of mitophagy [112]. This suggests that impairments in PINK1-Parkin signaling may contribute to inflammation in neurodegenerative conditions.

2.3. Deficient Mitophagy. Mitophagy is the process in which damaged mitochondria are degraded by the autophagy system. This process is vital for removing debris and aggregating proteins from the cells, thereby protecting cells of potential cell-damaging proteins. For postmitotic cells like neurons, mitophagy is an essential survival mechanism for neuroprotection and elimination of toxic compounds. Damaged mitochondria are singled out and degraded, through the activities of PINK1 and Parkin [113].

In physiological conditions, PINK1, imported from the cytosol via mitochondrial translocases, is usually found within the inner mitochondrial membrane, where it is exported, proteolytically cleaved, and degraded. PINK1 has a mitochondrial targeting sequence that directs the protein into the correct mitochondrial subcompartment [114]. During mitochondrial damage, the mitochondrial membrane potential is altered and PINK1 is prevented from entering through the outer mitochondrial membrane (OMM) [114]. As PINK1 molecules accumulate on the OMM, they start a cascade of events to recruit and activate cytosolic Parkin. Parkin initiates the process of degradation of the mitochondrion, which involves ubiquitination of the mitochondria, autophagosome engulfment, and lysosomal fusion. Both Parkin and PINK1, therefore, contribute to the build-up of phosphorylated ubiquitin and abundant accumulation of PINK1 on the OMM, which acts as an indicator to the cell where the mitochondrion is damaged and will need to be removed [115].

Mitophagy can be activated by ROS in response to various conditions, such as oxidative stress, starvation, and mechanical injury [52, 116]. Wang et al. showed that ROS have a direct effect in PINK1-Parkin signaling and these effects can be reversed with antioxidant treatment using superoxide dismutase-2 [116]. Independently from the direct effects of ROS in mitophagy, autosomal recessive forms of glaucoma, familial Parkinson's disease, and amyotrophic lateral sclerosis (ALS) have been associated with dysfunctions of PINK1-Parkin pathway.

Specific mutations in OPTN and TBK1 have been implicated in the pathogenesis of a subgroup of NTG with early onset (Figure 1) [99]. OPTN is a ubiquitously expressed protein involved in neuroinflammation, Golgi maintenance, vesicular trafficking, and autophagy. In mitophagy, OPTN acts as a receptor protein, downstream of PINK1-Parkin, that is translocated to damaged mitochondria via binding to ubiquitinated mitochondrial proteins and through interaction with microtubule-associated protein 1 light chain 3 (LC3) to couple the mitochondria with an autophagosome for degradation [99, 117]. Several hypotheses about the role of OPTN in glaucoma have indicated a neuroprotective function and shown that mutations may lead to RGC loss through downregulation or dysfunction of this protein. Due to a relatively high expression of OPTN in the retina, mutations in this protein may increase RGC vulnerability. Chernyshova et al. showed that several glaucoma OPTN-mutant proteins (E50K, A377T, H486R, H26D, E103D, T202R, and A336G) seemed to restore mitophagy in HeLa cells where Parkin-dependent mitophagy was previously inhibited [117]. Such results were not seen in OPTN-mutant proteins associated with ALS, which suggests that glaucoma associated with OPTN mutations may occur through a

mechanism independent from mitophagy dysfunction. A different study, conducted by Shim et al., found that acute overexpression of OPTN E50K mutant in rat RGCs caused increased mitophagy, ROS production, and activation of apoptosis via Bax pathway [118]. A possible explanation for an increased mitophagy was elucidated by reports that observed that the OPTN E50K and M98K mutants displayed striking affinity to TBK1 [119–121]. As TBK1 stimulates autophagy by phosphorylating and activating OPTN, abnormal TBK1 activation of OPTN may explain the increase in mitophagy [98]. Minegishi et al. also reported that the OPTN E50K mutant forms aggregates of insoluble protein in neuronal cells derived from induced pluripotent stem cells, which seem to lead to cell death [121].

Copy number variation mutations of the TKB1 gene have also been found in a subgroup of NTG [98]. A duplication of TBK1 has been found to increment TBK1 transcription, which is linked to a gain of function role of TBK1. Transgenic mice that had the TBK1 gene duplicated or triplicated showed progressive loss of RGCs, proportional to the number of gene copies, with no increase in IOP [122]. Apart from OPTN phosphorylation, TBK-1 is also able to activate mitophagy in an OPTN-independent manner through p62 phosphorylation, suggesting that OPTN and p62 regulate mitophagy by different mechanisms [123].

Apart from a direct role of TBK1 and OPTN in mitophagy, both proteins are also involved in innate immune inflammatory signaling, such as through the activation of nuclear factor-kappa B (NF-κB) [124]. NF-κB is a family of transcription factors that are key regulators of cytokine production and respond to a wide variety of inflammatory stimuli.

The role of OPTN in NF-κB regulation has been somewhat controversial due to divergent results that came from different cell types and stimuli [125, 126]. OPTN was shown to interact with cylindromatosis (CYLD), an enzyme that deubiquitinates receptor-interacting protein (RIP) to inhibit TNF-α-induced NF-κB activation [127]. Studies with the glaucoma-associated OPTN H486R mutant demonstrated loss of interaction of OPTN and CYLD, which resulted in increased NF-κB signaling induced by inflammatory cytokines [128, 129]. Tanishima et al. also found another binding partner of OPTN, which is involved in NF-κB signaling [128]. The interaction of OPTN with IL-1β receptor-associated kinase 1 (IRAK1) also prevents NF-κB activation, induced not by TNF-α but also by IL-1β and TLR signaling. Taken together, these studies suggest multiple links between OPTN and NF-κB pathway, although not yet clearly characterized for glaucoma. It is important also to point out that OPTN expression is regulated by NF-κB itself by binding to an OPTN promoter and making a negative feedback loop to NF-κB activation [130]. The impact of glaucoma-associated OPTN mutants in this NF-κB negative feedback loop is currently not known.

TBK1 plays a key role in innate immunity by regulating the expression of inflammatory factors, such as NF-κB, IRF3, and IRF7, which has been linked to type-I interferon production [131, 132]. Despite TBK1 immune regulatory capacity, direct inflammatory effects derived from the glaucoma-associated TBK1 duplication have not been documented to date.

3. Dysfunction of Neuroglial Cells

Similar to many other neurodegenerative diseases, glial activation is recognized as a hallmark of neuroinflammation in glaucoma. When proinflammatory stimuli arise during injury, astrocytes, Müller cells, and microglia become activated to produce cytokines and chemokines. Although Müller cells, astrocytes, and microglia each have a different developmental origin, they share many functions within the retina, and there is an intricate interrelationship among these cells in the induction of an inflammatory phenotype [133, 134]. However, along with prolonged inflammatory activation of glial cells, there is also a failure in the regulation of immunity, which may ultimately tilt an initial beneficial inflammatory response towards a dysfunctional immune response and neuronal injury.

3.1. Astroglia. Astrocytes and Müller cells facilitate the interface between neurons, endothelia, and other glial cells to mediate and modulate metabolic functions, synaptic activity, and homeostasis of the blood-retina barrier [135–138]. These neurosupportive cells also contribute to the defense and homeostasis of neurons by recognizing and responding to local insults. However, under pathological conditions, astrocytes and Müller cells can undergo a pronounced transformation termed gliosis [19, 139–141].

Previous studies revealed that reactive astroglial cells can have both protective and detrimental influences on neuronal survival in glaucoma and other neurodegenerative conditions [19]. Astroglia was shown to become highly reactive in the retina and ONH in glaucomatous human donor eyes, and experimental models of glaucoma [141–143]. Sun et al. demonstrated that a brief period of ocular hypertension may be sufficient to initiate astroglial reactivity in experimental glaucoma [144]. This activation, seen in early stages of the disease, is characterized by morphological alterations and molecular responses that may be detectable even before the damage of RGCs and axons [145]. Although gliosis has the primary aim of circumscribing injured tissues to protect uninjured neurons, astroglial activation may lead to tissue remodeling that result in further biomechanical stress on optic nerve axons and in inadequate metabolic support to RGCs [143–145]. A proinflammatory signature acquired by astrocytes upon stress signals may, therefore, lead to tissue destruction and RGC death [146, 147]. Along with the capacity to trigger innate immunity signaling pathways, reactive astroglia may also compromise the blood-retina barrier, which further increases the access of systemic immune cells into the retina and the optic nerve [137, 145].

3.2. Microglia. Microglial cells are myeloid-derived cells that reside in the CNS, providing neurotrophic support and promoting tissue renewal through their phagocytic functions [148]. In inflammatory conditions, microglial cells may

also contribute to neuron injury [27, 149, 150]. Despite having a more limited antigen-presentation capacity than that of peripheric professional antigen-presenting cells, microglial cells also act as the first and main form of active immune response in the CNS [138, 148].

Similar to other glial cells, microglia show increased reactivity in the retina and ONH in experimental models and human donor eyes with glaucoma [133, 149, 150]. Microglia react to neural injury with morphological changes, proliferation, migration, and production of inflammatory cytokines that further propagate neuroinflammation. This reaction also includes the release of ROS, NO, and TNF-α, leading to neurotoxic effects and aggravated neuronal loss [22, 23, 151]. It has been reported that microglial activation is one of the first events in glaucomatous neural damage occurring prior to RGC loss [152]. In a mouse model of inherited glaucoma (DBA/2J mice), the extent of neurodegeneration correlated with early microglial alterations in vivo [152]. Treatments with minocycline, which inhibit microglial activation, reduced RGC death in the same mouse model [153]. In addition to resident microglia, data from DBA/2J glaucoma model also indicate that glaucomatous proinflammatory state may be amplified by monocytes and other circulating immune cells that invade the ONH and contribute to neurodegeneration [17].

Nitric oxide (NO) is also known to be secreted by microglia in inflammatory conditions [154]. Upregulation of inducible nitric oxide synthase (iNOS) and increased NO levels were found in the ONH of glaucomatous patients and in the retina and ONH of experimental models of glaucoma [155–158]. Inhibition of iNOS by aminoguanidine provided protection to RGCs in a glaucoma model, supporting the possibility of a role of NO in the pathophysiology of glaucoma [159].

4. Activation of the Immune System

Neuronal metabolic dysfunction and reactive gliosis are known to activate glial Toll-like receptor (TLR) signaling and induce complement activation (Figure 2) [20, 160]. In addition to the costimulatory role of ROS in antigen presentation of glial cells, oxidative stress may modify the antigenic features of retina and optic nerve proteins with accumulation of advanced glycation end-products, activation of NF-κB transcriptional program, and stimulation of glial cytokine production in glaucomatous tissues [50, 161, 162].

In addition to the activation of innate immunity in the retina and ONH, glial cells are capable of stimulating systemic immune responses by displaying and releasing signals that favor the recruitment of circulating T lymphocytes. Several factors seem to contribute to the increment of immunogenicity and the activation of inflammatory cues for autoimmunity, namely, an increased exposure of antigens due to neuronal injury, increased expression of immunostimulatory stress proteins, and increased antigenicity due to protein modifications [25, 50, 162–166]. In a similar fashion to peripheral antigen-presenting cells, reactive glia in human glaucoma and animal models display high surface levels of major histocompatibility complex II molecules and stress-associated costimulatory molecules that may enhance antigen presentation to T lymphocytes [161, 167–169].

4.1. Release of Damage-Associated Molecular Patterns (DAMPs). The sterile inflammatory response following glaucomatous damage activates innate immunity mechanisms that are also triggered in infection-induced inflammation. Following infection, microorganisms are initially sensed by pattern-recognition receptors (PRRs) of the innate immune system, which bind conserved molecular patterns that are shared by different classes of microorganisms. These pathogen-associated molecular patterns (PAMPs) include microbial structural components, nucleic acids, and proteins [170]. The list of PRRs that are known to be able to sense PAMPs is extensive and is comprised most notably of four families: TLRs, nucleotide oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) [170, 171]. PRR ligation triggers multiple signaling pathways that may culminate in the activation of NF-κB, mitogen-activated protein kinases (MAPKs), and interferon regulatory factors (IRFs), which control the expression of proinflammatory cytokines, chemokines, and costimulatory molecules [170–172]. The resulting proinflammatory state is necessary for the generation of a robust antimicrobial response and for the activation of the adaptive immune system.

In addition to recognizing PAMPs, PRRs can be triggered by cellular damage and stress in the absence of microbial infection. Sterile tissue injury and cellular necrosis elicit robust responses characterized by proinflammatory cytokine production and leukocyte recruitment, which are triggered by TLR-, NLR-, and RLR-dependent sensing of "alarmins" or damage-associated molecular patterns (DAMPs) (Figure 2) [170, 173]. DAMPs are endogenous molecules that are isolated within intracellular compartments (e.g., DNA and N-formylated peptides) or are subject to robust metabolism and/or editing in healthy cells (e.g., DNA and double-stranded RNA) [170]. These molecules often exhibit similarities with PAMPs and can be recognized by PRRs during pathological injury [174]. Therefore, this "hidden-self" recognition serves to alert the immune system of cellular or tissue dysfunction.

Due to ancestral bacterial origin, eukaryotic mitochondria maintain prokaryotic features, including a double-membrane structure, the unique cell membrane lipids (e.g., cardiolipin), a circular genome containing CpG DNA, the absence of histones, the ability to replicate independently of the nucleus, the ability to form N-formyl peptides, which are distinct byproducts of mitochondrial translation that reflect their prokaryotic origin, and the use of separate sets of rRNAs and tRNAs encoded by the mitochondrial genome [40, 175]. Thus, cellular damage leading to the release of prokaryotic-like mitochondrial constituents through different modes of cell death can engage PRRs and act as a potent trigger of innate immune responses during stress and injury [40, 175].

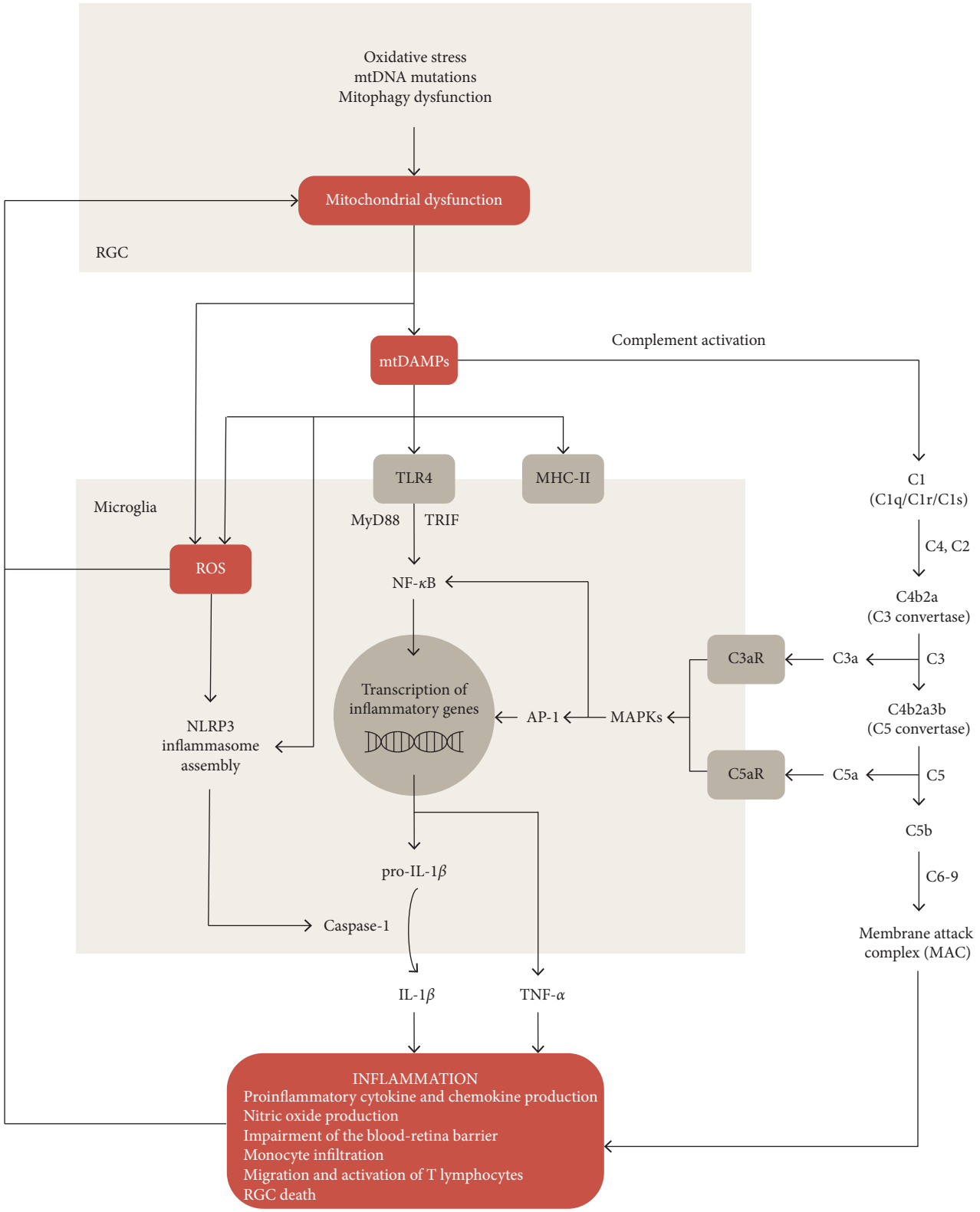


Figure 2: Neuronal mitochondrial damage-associated molecular patterns (mtDAMPs) can act as inducers of chronic inflammation in glaucoma. Mitochondrial-derived components from retinal ganglion cells (RGCs) can trigger inflammatory responses when recognized by complement molecules (classical pathway) and microglial pattern-recognition receptors, such as toll-like receptors (TLRs). TLR signaling induces the transcription of proinflammatory cytokines and chemokines (e.g., pro-IL-1β and TNF-α) through NF-κB. Inflammation triggered by mtDAMPs can further induce mitochondrial dysfunction, thereby amplifying a vicious cycle of inflammation. In addition to TLR signaling, the presentation of DAMPs through Class II MHC (MHC-II) molecules can further promote the induction of inflammation through T-cell activation. Mitochondrial products can also function directly as NLRP3 activators, which allows caspase-1-dependent release of IL-1β. AP-1: activator protein-1; MAPKs: mitogen-activated protein kinases; C3aR: C3a receptor; C5aR: C5a receptor.

Astroglia and microglia are known to participate in early stages of glaucoma but, to date, it is not known which deleterious events contribute the most to the pathogenesis of the disease. A possibility is that stressed or damaged RGCs in the ONH are the seminal source of DAMPs. Heat shock proteins (HSPs), a type of DAMPs, were shown to be upregulated in response to an elevation in IOP and increased in human glaucomatous retinas [20, 138, 176]. More recently, Chen et al. identified both bacterial and host HSPs as possible key natural antigens and demonstrated that commensal microflora induces HSP-specific memory T cells, which are then activated by host HSPs released in the retina after IOP elevation [177]. A second and still valid possibility is that astrocytes and/or microglia initiate gliosis and produce DAMPs independently of RGCs or preceding neuronal dysfunction [178]. Experimental models of glaucoma have identified reactive morphologic changes that precede axonopathy [179–181]. Along with morphologic changes derived from biomechanic causes, neuronal insult in glaucoma may be initiated or aggravated by the absence of the critical glial support, such as in astroglial metabolic substrate transfers (e.g., lactate), neurotrophin secretion, and transforming growth factor-beta (TGF-β) production, combined with a detrimental production of TNF-α [178, 182]. Speculatively, early glial responses may also constitute a glial effort to guarantee their own survival, with consequential detrimental effects on neuronal support. The identification of initiators of inflammatory events is a relevant area of study in glaucoma.

4.2. Activation of Pattern-Recognition Receptors and Inflammatory Signaling. Once stress products are recognized by PRRs such as TLRs, TNF receptor, and inflammasome, specific pathways initiate cascades of events that involve NF-κB (Figure 2) [151]. NF-κB, one of the key regulators of inflammatory immune responses, has been shown to be activated in human glaucoma and in animal models of glaucoma [20, 160].

TLR signaling pathways are among the first to be upregulated in the retinas of patients with glaucoma [19, 20, 183]. Different TLRs display increased expression on astroglia and microglia in human glaucoma, experimental ocular hypertension, and DBA/2J mice with hereditary glaucoma [19, 20]. TLR2, TLR3, and TLR4 were shown to be expressed in microglia and astrocytes of DBA/2J mice retinas, and 11 of 13 TLRs were upregulated in the ONH in early stages of the disease [183]. From these, TLR4 is the most studied Toll-like receptor and is widely expressed in the CNS. Upon DAMPs recognition, TLRs recruit adaptor proteins such as myeloid differentiation primary-response protein 88 (MyD88) and/or Toll/interleukin-1 receptor domain-containing adaptor including interferon-β (TRIF) to activate downstream transcription factors. These transcription factors include members of the NF-κB, AP-1, and interferon regulator factor families, which allow the initiation of the transcription of amplifiers and effectors such as TNF-α, IL-1β, IL-6, and an array of chemokines (e.g., CCL2, CXCL1, and CXCL10) [138, 171, 184].

Another group of PRRs that can modulate microglial inflammatory response is the NOD-like receptors, which form protein complexes commonly known as inflammasomes. Cooperative downstream cross-talks between TLRs and NOD-like receptors can lead to the maturation and release of cytokines like IL-1β and IL-18 [185–187]. Mitochondria can directly activate inflammasome signaling. Mitochondria-derived or DAMPs-induced ROS activate the NLR family pyrin domain-containing 3 (NLRP3) inflammasome pathway [188]. NLRP3 is normally associated with the endoplasmic reticulum membrane but, upon activation, is redistributed to nuclear and mitochondrial membranes, where it oligomerizes with apoptosis-associated speck-like protein containing a CARD (ASC) and procaspase-1 to form the NLRP3 inflammasome (Figure 2) [185–187]. NLRP3 inflammasome activation leads to caspase-1-dependent secretion of IL-1β and IL-18 and an inflammatory form of cell death termed as pyroptosis [188]. Studies in neurodegeneration in the CNS revealed that mitochondrial dysfunction, including the blockade of mitophagy, is sensed by the NLRP3 inflammasome [188, 189]. Several molecules were specifically identified as DAMPs by these complexes, namely, mtDNA, ATP, and cytochrome C [114, 188]. Despite this evidence, it has not been identified yet how the various aspects of mitochondrial dysfunction converge to a common pathway to activate NLRP3 inflammasome [188, 189]. On the other hand, in bone marrow-derived macrophages, caspase-1 was shown to increase mitochondrial disassembly through the activation of multiple deleterious pathways that amplify ROS production, as well as through the inhibition mitophagy mediated by cleavage of Parkin [190]. NLRP1 and NLRP3 inflammasomes seem to be involved in the pathogenesis of glaucoma in models of acute glaucoma, and, to date, only one study has demonstrated the presence of NLRP3 inflammasome in human glaucomatous eyes [160, 191, 192]. More studies are required to better understand the role of inflammasomes in the progression of glaucoma.

In addition to stress/damage sensors (e.g., TLRs and NLRP3) and inflammatory transducers (e.g., MyD88 and NF-κB), effectors and amplifiers of inflammation are also upregulated in glaucoma. Proteomic analysis of human and experimental glaucomatous retinas revealed an upregulation of kinases that are involved in the activation of the NF-κB pathway, such as RIPK, NIK, and IκK [19, 160]. Activation of NF-κB results in the transcription of IL-1 cytokine family, which can further amplify inflammation by inducing a secondary release of cytokines in microglia and astrocytes. IL-1 cytokines were shown to be upregulated in the ONH at early stages of DBA/2J glaucoma in mice, and antioxidant treatment could afford the downregulation of proinflammatory cytokines and NF-κB [183, 193].

The proinflammatory imbalance seen in glaucomatous tissues is characterized by a marked increase of TNF-α production, which is also linked to RGC death [23, 151, 161, 194, 195]. TNF-α release can itself affect mitochondrial function by impairing the function of mitochondrial components, reducing ATP production, increasing ROS, and depolarizing the mitochondrial

membrane potential [39]. Increased ROS can then have further detrimental effects by maintaining the activation of NF-κB and the production of proinflammatory signaling. The stimulation of neuroinflammation can therefore also have a damaging effect on RGC mitochondrial function itself, thus creating a vicious cycle.

4.3. Activation of the Complement System. The complement system is part of the innate immune defense, and it consists of a number of small proteins that provide immune surveillance both peripherally and in the CNS. Complement proteins are expressed in normal physiological processes of the retina and become increased in pathological conditions like inflammation, aging, and trauma and in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and glaucoma [138, 196]. In addition to its role in pathogen recognition and removal, the complement system is also involved in homeostasis by synapse elimination and clearance of potential mediators of damage or injury [197, 198]. Thus, the decisive element that tilts the balance between a homeostatic or a proinflammatory complement-mediated response appears to be the presence of specific danger signals associated with chronic inflammation.

The complement system comprises three distinct and tightly regulated activatory cascades known as the classical, alternative, and lectin pathways [197]. All pathways, which converge in the activation of C3 convertase, contribute to the opsonization of foreign pathogens or apoptotic cell/cellular compartments, the release of inflammatory signaling through anaphylatoxins, and the formation of the membrane attack complex (MAC) [199, 200]. The classical pathway (Figure 2) is initiated when the C1 complex recognizes, among others, antigen-antibody complexes, mitochondrial membrane components, apoptotic cells, or amyloid fibrils [201]. This recognition leads to the formation of C3 convertase and cleavage of C3 protein into C3a and C3b. C3a is an anaphylatoxin that attracts phagocytes to sites of inflammation, and C3b acts as opsonin, which further amplifies complement activation by inducing the cleavage of C5 into C5a and C5b by C5 convertase. C5a is also an anaphylatoxin, and C5b leads to the formation of a lysis-inducing channel in the targeted cell together with C6, C7, C8, and C9 [138, 200].

Increased complement activation has been linked to decreased RGC survival in humans and in animal models of glaucoma, suggesting an involvement of the complement system in the progression of the disease [24, 183, 202–204]. Studies in rodents and nonhuman primates indicated an increased expression of multiple components of the classical complement cascade, which seem to constitute one of the earliest signaling responses to high IOP in the retina and ONH [183, 205, 206]. In DBA/2J glaucomatous mice, an increment of the C1 complex protein C1QA was observed in the ONH before the detection of axonal damage, indicating a potential causal contribution [183]. Deposition of C1QA was also observed in RGC dendrites in glaucomatous retinas of DBA/2J mice, nonhuman primates, and humans, suggesting an involvement of the complement cascade in pathological synapse elimination and/or dendrite remodeling in glaucoma [198, 202–204]. Ablation of C1QA in DBA/2J mice or the viral overexpression of *Crry*, a C3 inhibitor, were both effective in decreasing RGC loss [183, 207, 208].

Downstream components of the complement cascade that are necessary for the formation of MAC seem to also contribute to glaucoma [197]. In human glaucomatous retinas and in experimental models of glaucoma, RGCs were shown to have a marked deposition of MAC [202, 208, 209]. Drug inhibition of complement activation or knockout of the C5 gene reduced MAC deposition as well as RGC loss in rodent models of ocular hypertension and glaucoma [208, 209]. More recently, the intravitreal administration of a monoclonal antibody against the C5 could also afford the preservation of RGCs in an experimental autoimmune model of glaucoma [210]. Together these findings indicate that the complement system can be activated in early stages of RGC injury, which can result in a net increase of retinal damage. Although poorly defined, astrocytes and Müller cells are also able to produce complement proteins and therefore may be conducive to degeneration in a similar fashion [203, 211].

4.4. Leukocyte Infiltration. Leukocyte infiltration is a common event in CNS that occurs after injury, disease, and chronic stress [18, 138]. Although early immune responses are likely to be natural attempts to minimize damage after an injury, later immune responses are prone to evolve into chronicity and become more detrimental. In some cases, these beneficial and detrimental events involve molecules in the same pathways [138].

Leukocyte infiltration comprises a cascade of sequential steps that are initiated by the production of cytokines and chemokines in glial cells [23, 138, 212]. The molecular markers involved in leukocyte infiltration include various classes of adhesion/activation/migration proteins expressed on the blood-retina barrier and migrating leukocytes, such as selectins (e.g., P-selectin) and integrin ligands (e.g., VCAM-1 and ICAM-1) on endothelial cells, and selectin ligands (e.g., PSGL1) and integrins (e.g., LFA1) on the surface of leukocytes [18, 138]. These interactions between endothelial cells and leukocytes result in the loosening of endothelial tight junctions to grant access of leukocytes to the CNS [18, 213]. The mechanisms of transendothelial migration in the CNS during injury and disease are very complex, and recent work has shown that the molecules involved in this process are more abundant than previously thought [213].

In the DBA/2J glaucoma model, aberrant upregulation of P-selectin and VCAM-1 was observed in early stages of the disease, suggesting that transendothelial migration of leukocytes takes part in the initiation of glaucomatous damage [17]. Reactive gliosis seems to be able to weaken the perivascular barriers and facilitate access of circulating immune cells and other components such as autoantibodies into the retina and ONH [214]. Breaches in the blood-retina barrier may become visible and result in small optic disc hemorrhages or parapapillary chorioretinal atrophy areas,

both of which are commonly detectable in glaucomatous eyes [215, 216]. In line with these results, a longitudinal study revealed a gradual increment of serum autoantibodies in glaucoma patients with optic disc hemorrhages and no detectable change in patients with no optic disc hemorrhages [217]. In addition, the transfer of mononuclear cells from patients with optic disc hemorrhages into immune-deficient mice resulted in increased RGC loss [145, 217]. The tracing of circulating monocytes with an inflammatory profile in the DBA/2J model confirmed the entry of these cells into the ONH and its contribution to glaucomatous damage [17]. These observations are consistent with the higher titers of proinflammatory cytokines found in the blood of patients with glaucoma, as well as the reduction of monocyte infiltration when DBA/2J mice were treated with radiation [17, 218, 219].

Recent findings shed light on the involvement of T-cell-mediated mechanisms in the pathogenesis of glaucoma. This notion is supported by the observation that local inflammation in the retina and ONH is required for T cell crossing of the blood-retina barrier and T cell accumulation is more prominent in inflammatory areas that are more susceptible to high IOP [177, 220–222]. Mice deficient in T cells, but not B cells, displayed a dramatically attenuated RGC and axon damage [177]. One report, however, pointed out that neurodegeneration could be induced in mice, with IOP being within normal or elevated range, after the adoptive transfer of T cells isolated from genetic mouse models of glaucoma [177, 223]. These results suggest that activated T cells of glaucomatous mice are also capable of entering the retina with an intact blood-retina barrier, although probably at a much slower rate or under certain conditions [177, 223]. Studies of blood samples from patients with glaucoma have also detected a shift in the T lymphocyte signature towards a proinflammatory Th1 phenotype and an imbalance of regulatory T cells, indicating a possible lack of efficient T-cell suppression and a minor role of B cells in the disease process [218, 219]. Despite these observations, it remains unclear which subsets of T cells predominate as effector cells or act as initiators of glaucomatous neurodegeneration.

5. Conclusion

Numerous forms of endogenous and environmental stressors may disrupt mitochondrial function by impacting mitochondrial homeostasis. Mitochondrial function in RGCs may decline progressively in association with physiologic aging, leading to the release of multiple mitochondrial DAMPs. These misplaced or altered mitochondria-derived molecules may subsequently trigger innate immune responses and result in the onset or progression of inflammatory neurodegenerative diseases, such as in glaucoma. However, the precise mechanism of how mitochondrial DAMPs lead to glaucomatous neurodegeneration is yet to be fully dissected.

There are also mechanisms of glial dysfunction that contribute to neurodegeneration in the early stages of glaucoma. Despite the evident role of inflammation in the disease, to date, it is still not known which exact inflammatory signals lead to activation of glia during the disease progression and how intricate these processes are to neuronal dysfunction, retina-blood barrier impairment, or systemic inflammation. Several experimental challenges hinder a comprehensive understanding of glaucoma. Unlike neurons, microglia and astrocytes are challenging to study in vitro as these cells acquire different phenotypes that hardly resemble in vivo conditions. Several important questions, therefore, remain open, such as, which and how mitochondria-derived molecules contribute to neuroinflammation, and how upstream or downstream this process is in the progression of the disease.

An answer to these questions will facilitate the design of better therapeutic options that are not merely supportive. As glaucoma has many subphenotypes, finding a common therapeutic solution for all poses many challenges. Nonetheless, considering that RGC death is the final common pathway for a very complex pathology, identifying and targeting the key events of neuronal, glial, and immune dysfunction may help halt the neurodegenerative process in the early stages of the disease.

Conflicts of Interest

The author declares no conflicts of interest.

Acknowledgments

The author apologizes for any studies that may have been omitted in this review. This work was supported by the Lundbeck Foundation (grant R266-2017-3984) and Novo Nordisk Foundation (grant NNF18SA0034956).

References

[1] H. A. Quigley, G. R. Dunkelberger, and W. R. Green, "Chronic human glaucoma causing selectively greater loss of large optic nerve fibers," *Ophthalmology*, vol. 95, no. 3, pp. 357–363, 1988.

[2] H. A. Quigley, "The number of people with glaucoma worldwide in 2010 and 2020," *British Journal of Ophthalmology*, vol. 90, no. 3, pp. 262–267, 2006.

[3] S. Kingman, "Glaucoma is second leading cause of blindness globally," *Bulletin of the World Health Organization*, vol. 82, no. 11, pp. 887-888, 2004.

[4] R. W. Nickells, "From ocular hypertension to ganglion cell death: a theoretical sequence of events leading to glaucoma," *Canadian Journal of Ophthalmology*, vol. 42, no. 2, pp. 278–287, 2007.

[5] J. Hirt, K. Porter, A. Dixon, S. McKinnon, and P. B. Liton, "Contribution of autophagy to ocular hypertension and neurodegeneration in the DBA/2J spontaneous glaucoma mouse model," *Cell Death Discovery*, vol. 4, p. 14, 2018.

[6] S. D. Crish, R. M. Sappington, D. M. Inman, P. J. Horner, and D. J. Calkins, "Distal axonopathy with structural persistence in glaucomatous neurodegeneration," *Proceedings of the National Academy of Sciences*, vol. 107, no. 11, pp. 5196–5201, 2010.

[7] P. Rakic and K. Riley, "Overproduction and elimination of retinal axons in the fetal rhesus monkey," *Science*, vol. 219, no. 4591, pp. 1441–1444, 1983.

[8] H. A. Quigley, S. J. McKinnon, D. J. Zack et al., "Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats," *Investigative Ophthalmology & Visual Science*, vol. 41, no. 11, pp. 3460–3466, 2000.

[9] M. Almasieh, A. M. Wilson, B. Morquette, J. L. Cueva Vargas, and A. Di Polo, "The molecular basis of retinal ganglion cell death in glaucoma," *Progress in Retinal and Eye Research*, vol. 31, no. 2, pp. 152–181, 2012.

[10] J. V. Nguyen, I. Soto, K.-Y. Kim et al., "Myelination transition zone astrocytes are constitutively phagocytic and have synuclein dependent reactivity in glaucoma," *Proceedings of the National Academy of Sciences*, vol. 108, no. 3, pp. 1176–1181, 2011.

[11] G. Chidlow, A. Ebneter, J. P. M. Wood, and R. J. Casson, "The optic nerve head is the site of axonal transport disruption, axonal cytoskeleton damage and putative axonal regeneration failure in a rat model of glaucoma," *Acta Neuropathologica*, vol. 121, no. 6, pp. 737–751, 2011.

[12] M. Iester, F. De Feo, and G. R. Douglas, "Visual field loss morphology in high- and normal-tension glaucoma," *Journal of Ophthalmology*, vol. 2012, Article ID 327326, 8 pages, 2012.

[13] M. B. Shields, "Normal-tension glaucoma: is it different from primary open-angle glaucoma?" *Current Opinion in Ophthalmology*, vol. 19, no. 2, pp. 85–88, 2008.

[14] M. C. Leske, A. Heijl, L. Hyman, B. Bengtsson, L. Dong, and Z. Yang, "Predictors of long-term progression in the early manifest glaucoma trial," *Ophthalmology*, vol. 114, no. 11, pp. 1965–1972, 2007.

[15] G. Tezel, "Immune regulation toward immunomodulation for neuroprotection in glaucoma," *Current Opinion in Pharmacology*, vol. 13, no. 1, pp. 23–31, 2013.

[16] M. I. Rizzo, A. Greco, A. De Virgilio et al., "Glaucoma: recent advances in the involvement of autoimmunity," *Immunologic Research*, vol. 65, no. 1, pp. 207–217, 2017.

[17] G. R. Howell, I. Soto, X. Zhu et al., "Radiation treatment inhibits monocyte entry into the optic nerve head and prevents neuronal damage in a mouse model of glaucoma," *Journal of Clinical Investigation*, vol. 122, no. 4, pp. 1246–1261, 2012.

[18] R. M. Ransohoff and M. A. Brown, "Innate immunity in the central nervous system," *Journal of Clinical Investigation*, vol. 122, no. 4, pp. 1164–1171, 2012.

[19] G. Tezel, X. Yang, C. Luo, J. Cai, and D. W. Powell, "An astrocyte-specific proteomic approach to inflammatory responses in experimental rat glaucoma," *Investigative Opthalmology & Visual Science*, vol. 53, no. 7, pp. 4220–4233, 2012.

[20] C. Luo, X. Yang, A. D. Kain, D. W. Powell, M. H. Kuehn, and G. Tezel, "Glaucomatous tissue stress and the regulation of immune response through glial Toll-like receptor signaling," *Investigative Opthalmology & Visual Science*, vol. 51, no. 11, pp. 5697–5707, 2010.

[21] J. Chua, M. Vania, C. M. Cheung et al., "Expression profile of inflammatory cytokines in aqueous from glaucomatous eyes," *Molecular Vision*, vol. 18, pp. 431–438, 2012.

[22] M. H. Madeira, R. Boia, P. F. Santos, A. F. Ambrósio, and A. R. Santiago, "Contribution of microglia-mediated neuroinflammation to retinal degenerative diseases," *Mediators of Inflammation*, vol. 2015, Article ID 673090, 15 pages, 2015.

[23] T. Nakazawa, C. Nakazawa, A. Matsubara et al., "Tumor necrosis factor- mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma," *Journal of Neuroscience*, vol. 26, no. 49, pp. 12633–12641, 2006.

[24] F. Ahmed, K. M. Brown, D. A. Stephan, J. C. Morrison, E. C. Johnson, and S. I. Tomarev, "Microarray analysis of changes in mRNA levels in the rat retina after experimental elevation of intraocular pressure," *Investigative Opthalmology & Visual Science*, vol. 45, no. 4, pp. 1247–1258, 2004.

[25] Z. Yang, H. A. Quigley, M. E. Pease et al., "Changes in gene expression in experimental glaucoma and optic nerve transection: the equilibrium between protective and detrimental mechanisms," *Investigative Opthalmology & Visual Science*, vol. 48, no. 12, pp. 5539–5548, 2007.

[26] J. Tombran-Tink, C. J. Barnstable, and M. Bruce Shields, *Mechanisms of the Glaucomas: Disease Processes and Therapeutic Modalities*, Springer Science & Business Media, Berlin, Germany, 2008.

[27] A. I. Ramirez, R. de Hoz, E. Salobrar-Garcia, J. J. Salazar, B. Rojas, and D. Ajoy, "The role of microglia in retinal neurodegeneration: Alzheimer's disease, Parkinson, and glaucoma," *Frontiers in Aging Neuroscience*, vol. 9, p. 214, 2017.

[28] A. K. Toft-Kehler, D. M. Skytt, A. Svare et al., "Mitochondrial function in Müller cells-does it matter?" *Mitochondrion*, vol. 36, pp. 43–51, 2017.

[29] D.-Y. Yu and S. J. Cringle, "Retinal degeneration and local oxygen metabolism," *Experimental Eye Research*, vol. 80, no. 6, pp. 745–751, 2005.

[30] Y. A. Ito and A. Di Polo, "Mitochondrial dynamics, transport, and quality control: a bottleneck for retinal ganglion cell viability in optic neuropathies," *Mitochondrion*, vol. 36, pp. 186–192, 2017.

[31] J. E. Morgan, "Circulation and axonal transport in the optic nerve," *Eye*, vol. 18, no. 11, pp. 1089–1095, 2004.

[32] M. J. Barron, P. Griffiths, D. M. Turnbull, D. Bates, and P. Nichols, "The distributions of mitochondria and sodium channels reflect the specific energy requirements and conduction properties of the human optic nerve head," *British Journal of Ophthalmology*, vol. 88, no. 2, pp. 286–290, 2004.

[33] R. M. Andrews, P. G. Griffiths, M. A. Johnson, and D. M. Turnbull, "Histochemical localisation of mitochondrial enzyme activity in human optic nerve and retina," *British Journal of Ophthalmology*, vol. 83, no. 2, pp. 231–235, 1999.

[34] E. A. Bristow, P. G. Griffiths, R. M. Andrews, M. A. Johnson, and D. M. Turnbull, "The distribution of mitochondrial activity in relation to optic nerve structure," *Archives of Ophthalmology*, vol. 120, no. 6, pp. 791–796, 2002.

[35] L. Wang, J. Dong, G. Cull, B. Fortune, and G. A. Cioffi, "Varicosities of intraretinal ganglion cell axons in human and nonhuman primates," *Investigative Opthalmology & Visual Science*, vol. 44, no. 1, pp. 2–9, 2003.

[36] A. S. Rambold, B. Kostelecky, N. Elia, and J. Lippincott-Schwartz, "Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation," *Proceedings of the National Academy of Sciences*, vol. 108, no. 25, pp. 10190–10195, 2011.

[37] L. C. Gomes, G. D. Benedetto, and L. Scorrano, "During autophagy mitochondria elongate, are spared from degradation and sustain cell viability," *Nature Cell Biology*, vol. 13, no. 5, pp. 589–598, 2011.

[38] H. M. Wilkins, S. M. Carl, A. C. S. Greenlief, B. W. Festoff, and R. H. Swerdlow, "Bioenergetic dysfunction and inflammation in Alzheimer's disease: a possible connection," *Frontiers in Aging Neuroscience*, vol. 6, 2014.

[39] H. M. Wilkins, I. W. Weidling, Y. Ji, and R. H. Swerdlow, "Mitochondria-derived damage-associated molecular

patterns in neurodegeneration," *Frontiers in Immunology*, vol. 8, p. 508, 2017.

[40] A. P. West and G. S. Shadel, "Mitochondrial DNA in innate immune responses and inflammatory pathology," *Nature Reviews Immunology*, vol. 17, no. 6, pp. 363–375, 2017.

[41] B. Tang, S. Li, W. Cao, and X. Sun, "The association of oxidative stress status with open-angle glaucoma and exfoliation glaucoma: a systematic review and meta-analysis," *Journal of Ophthalmology*, vol. 2019, Article ID 1803619, 14 pages, 2019.

[42] A. Kanamori, M.-M. Catrinescu, N. Kanamori, K. A. Mears, R. Beaubien, and L. A. Levin, "Superoxide is an associated signal for apoptosis in axonal injury," *Brain*, vol. 133, no. 9, pp. 2612–2625, 2010.

[43] S. Alqawlaq, J. G. Flanagan, and J. M. Sivak, "All roads lead to glaucoma: induced retinal injury cascades contribute to a common neurodegenerative outcome," *Experimental Eye Research*, vol. 183, pp. 88–97, 2019.

[44] V. Chrysostomou, F. Rezania, I. A. Trounce, and J. G. Crowston, "Oxidative stress and mitochondrial dysfunction in glaucoma," *Current Opinion in Pharmacology*, vol. 13, no. 1, pp. 12–15, 2013.

[45] G. L N. Tezel and X. Yang, "Caspase-independent component of retinal ganglion cell death, in vitro," *Investigative Opthalmology & Visual Science*, vol. 45, no. 11, pp. 4049–4059, 2004.

[46] G.-Y. Li and N. N. Osborne, "Oxidative-induced apoptosis to an immortalized ganglion cell line is caspase independent but involves the activation of poly(ADP-ribose)polymerase and apoptosis-inducing factor," *Brain Research*, vol. 1188, pp. 35–43, 2008.

[47] E. N. Iomdina, I. P. Khoroshilova-Maslova, O. V. Robustova et al., "Mitochondria-targeted antioxidant SkQ1 reverses glaucomatous lesions in rabbits," *Frontiers in Bioscience (Landmark Edition)*, vol. 20, pp. 892–901, 2015.

[48] D. M. Inman, W. S. Lambert, D. J. Calkins, and P. J. Horner, "α-Lipoic acid antioxidant treatment limits glaucoma-related retinal ganglion cell death and dysfunction," *PLoS One*, vol. 8, Article ID e65389, 2013.

[49] M. C. Moreno, J. Campanelli, P. Sande, D. A. Sáenz, M. I. Keller Sarmiento, and R. E. Rosenstein, "Retinal oxidative stress induced by high intraocular pressure," *Free Radical Biology and Medicine*, vol. 37, no. 6, pp. 803–812, 2004.

[50] G. L. N. Tezel, X. Yang, and J. Cai, "Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma," *Investigative Opthalmology & Visual Science*, vol. 46, no. 9, p. 3177, 2005.

[51] K. R. Martin, H. Levkovitch-Verbin, D. Valenta, L. Baumrind, M. E. Pease, and H. A. Quigley, "Retinal glutamate transporter changes in experimental glaucoma and after optic nerve transection in the rat," *Investigative Ophthalmology & Visual Science*, vol. 43, no. 7, pp. 2236–2243, 2002.

[52] W.-J. Lin and H.-Y. Kuang, "Oxidative stress induces autophagy in response to multiple noxious stimuli in retinal ganglion cells," *Autophagy*, vol. 10, no. 10, pp. 1692–1701, 2014.

[53] R. Scherz-Shouval, E. Shvets, E. Fass, H. Shorer, L. Gil, and Z. Elazar, "Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4," *The EMBO Journal*, vol. 26, no. 7, pp. 1749–1760, 2007.

[54] K. Yoshimura, M. Shibata, M. Koike et al., "Effects of RNA interference of Atg4B on the limited proteolysis of LC3 in PC12 cells and expression of Atg4B in various rat tissues," *Autophagy*, vol. 2, no. 3, pp. 200–208, 2006.

[55] M. Ü. C. Satilmis, S. OrgÜl, B. Doubler, and J. Flammer, "Rate of progression of glaucoma correlates with retrobulbar circulation and intraocular pressure," *American Journal of Ophthalmology*, vol. 135, no. 5, pp. 664–669, 2003.

[56] M. Yanagi, R. Kawasaki, J. J. Wang, T. Y. Wong, J. Crowston, and Y. Kiuchi, "Vascular risk factors in glaucoma: a review," *Clinical & Experimental Ophthalmology*, vol. 39, no. 3, pp. 252–258, 2011.

[57] P. E. Malone and M. R. Hernandez, "4-Hydroxynonenal, a product of oxidative stress, leads to an antioxidant response in optic nerve head astrocytes," *Experimental Eye Research*, vol. 84, no. 3, pp. 444–454, 2007.

[58] E. M. McElnea, B. Quill, N. G. Docherty et al., "Oxidative stress, mitochondrial dysfunction and calcium overload in human lamina cribrosa cells from glaucoma donors," *Molecular Vision*, vol. 17, pp. 1182–1191, 2011.

[59] R. Vohra, L. M. Dalgaard, J. Vibæk et al., "Potential metabolic markers in glaucoma and their regulation in response to hypoxia," *Acta Ophthalmologica*, vol. 97, no. 6, pp. 567–576, 2019.

[60] S. Lee, L. Sheck, J. G. Crowston et al., "Impaired complex-I-linked respiration and ATP synthesis in primary open-angle glaucoma patient lymphoblasts," *Investigative Opthalmology & Visual Science*, vol. 53, no. 4, pp. 2431–2437, 2012.

[61] N. J. Van Bergen, J. G. Crowston, J. E. Craig, K. P. Burdon, L. S. Kearns, and S. Sharma, "Measurement of systemic mitochondrial function in advanced primary open-angle glaucoma and leber hereditary optic neuropathy," *PLoS One*, vol. 10, Article ID e0140919, 2015.

[62] A. Chevrollier, V. Guillet, D. Loiseau et al., "Hereditary optic neuropathies share a common mitochondrial coupling defect," *Annals of Neurology*, vol. 63, no. 6, pp. 794–798, 2008.

[63] A. H. V. Schapira, "Complex I: inhibitors, inhibition and neurodegeneration," *Experimental Neurology*, vol. 224, no. 2, pp. 331–335, 2010.

[64] G. Chidlow, J. P. M. Wood, and R. J. Casson, "Investigations into hypoxia and oxidative stress at the optic nerve head in a rat model of glaucoma," *Frontiers in Neuroscience*, vol. 11, p. 478, 2017.

[65] G. Tezel and M. B. Wax, "Hypoxia-inducible factor 1α in the glaucomatous retina and OpticNerve head," *Archives of Ophthalmology*, vol. 122, no. 9, pp. 1348–1356, 2004.

[66] D. Guo, H. Bi, D. Wang, and Q. Wu, "Zinc oxide nanoparticles decrease the expression and activity of plasma membrane calcium ATPase, disrupt the intracellular calcium homeostasis in rat retinal ganglion cells," *The International Journal of Biochemistry & Cell Biology*, vol. 45, no. 8, pp. 1849–1859, 2013.

[67] L. Rohowetz, J. Kraus, and P. Koulen, "Reactive oxygen species-mediated damage of retinal neurons: Drug development targets for therapies of chronic neurodegeneration of the retina," *International Journal of Molecular Sciences*, vol. 19, no. 11, p. 3362, 2018.

[68] A. Zaidi and M. L. Michaelis, "Effects of reactive oxygen species on brain synaptic plasma membrane Ca2+-ATPase," *Free Radical Biology and Medicine*, vol. 27, no. 7-8, pp. 810–821, 1999.

[69] T. Yokota, N. Kamimura, T. Igarashi, H. Takahashi, S. Ohta, and H. Oharazawa, "Protective effect of molecular hydrogen against oxidative stress caused by peroxynitrite derived from nitric oxide in rat retina," *Clinical & Experimental Ophthalmology*, vol. 43, no. 6, pp. 568–577, 2015.

[70] J. Alvarado, C. Murphy, J. Polansky, and R. Juster, "Age-related changes in trabecular meshwork cellularity," *Investigative Ophthalmology & Visual Science*, vol. 21, no. 5, pp. 714–727, 1981.

[71] S. C. Saccà, "Oxidative DNA damage in the human trabecular meshwork," *Archives of Ophthalmology*, vol. 123, no. 4, p. 458, 2005.

[72] M. K. Joe and S. I. Tomarev, "Expression of myocilin mutants sensitizes cells to oxidative stress-induced apoptosis," *The American Journal of Pathology*, vol. 176, no. 6, pp. 2880–2890, 2010.

[73] Y. He, K. W. Leung, Y. H. Zhuo, and J. Ge, "Pro370Leu mutant myocilin impairs mitochondrial functions in human trabecular meshwork cells," *Molecular Vision*, vol. 15, pp. 815–825, 2009.

[74] S. M. Ferreira, S. F. Lerner, R. Brunzini, C. G. Reides, P. A. Evelson, and S. F. Llesuy, "Time course changes of oxidative stress markers in a rat experimental glaucoma model," *Investigative Opthalmology & Visual Science*, vol. 51, no. 9, pp. 4635–4640, 2010.

[75] M.-L. Ko, P.-H. Peng, M.-C. Ma, R. Ritch, and C.-F. Chen, "Dynamic changes in reactive oxygen species and antioxidant levels in retinas in experimental glaucoma," *Free Radical Biology and Medicine*, vol. 39, no. 3, pp. 365–373, 2005.

[76] Y. X. G. Kong, N. van Bergen, B. V. Bui, V. Chrysostomou, A. J. Vingrys, and I. A. Trounce, "Impact of aging and diet restriction on retinal function during and after acute intraocular pressure injury," *Neurobiology of Aging*, vol. 33, p. 1126, 2012.

[77] A. Kawasaki, Y. Otori, and C. J. Barnstable, "Müller cell protection of rat retinal ganglion cells from glutamate and nitric oxide neurotoxicity," *Investigative Ophthalmology & Visual Science*, vol. 41, pp. 3444–3450, 2000.

[78] D. M. Skytt, A. M. Klawonn, M. H. Stridh et al., "siRNA knock down of glutamate dehydrogenase in astrocytes affects glutamate metabolism leading to extensive accumulation of the neuroactive amino acids glutamate and aspartate," *Neurochemistry International*, vol. 61, no. 4, pp. 490–497, 2012.

[79] R. H. Farkas, I. Chowers, A. S. Hackam, M. Kageyama, R. W. Nickells, and D. C. Otteson, "Increased expression of iron-regulating genes in monkey and human glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 45, no. 5, pp. 1410–1417, 2004.

[80] C. Benoist d'Azy, B. Pereira, F. Chiambaretta, and F. Dutheil, "Oxidative and anti-oxidative stress markers in chronic glaucoma: a systematic review and meta-analysis," *PLoS One*, vol. 11, Article ID e0166915, 2016.

[81] K. Yuki, D. Murat, I. Kimura, and K. Tsubota, "Increased serum total antioxidant status and decreased urinary 8-hydroxy-2′-deoxyguanosine levels in patients with normal-tension glaucoma," *Acta Ophthalmologica*, vol. 88, no. 7, pp. e259–e264, 2010.

[82] W. D. Ramdas, R. C. W. Wolfs, J. C. Kiefte-de Jong et al., "Nutrient intake and risk of open-angle glaucoma: the Rotterdam study," *European Journal of Epidemiology*, vol. 27, no. 5, pp. 385–393, 2012.

[83] M.-L. Ko, P.-H. Peng, S.-Y. Hsu, and C.-F. Chen, "Dietary deficiency of vitamin E aggravates retinal ganglion cell death in experimental glaucoma of rats," *Current Eye Research*, vol. 35, no. 9, pp. 842–849, 2010.

[84] G. Ozdemir, F. I. Tolun, M. Gul, and S. Imrek, "Retinal oxidative stress induced by intraocular hypertension in rats may be ameliorated by brimonidine treatment and N-acetyl cysteine supplementation," *Journal of Glaucoma*, vol. 18, no. 9, pp. 662–665, 2009.

[85] K. Y. C. Lee, M. Nakayama, M. Aihara, Y.-N. Chen, and M. Araie, "Brimonidine is neuroprotective against glutamate-induced neurotoxicity, oxidative stress, and hypoxia in purified rat retinal ganglion cells," *Molecular Vision*, vol. 16, pp. 246–251, 2010.

[86] E. Yoles, L. A. Wheeler, and M. Schwartz, "Alpha2-adrenoreceptor agonists are neuroprotective in a rat model of optic nerve degeneration," *Investigative Ophthalmology & Visual Science*, vol. 40, no. 1, pp. 65–73, 1999.

[87] B. Lv, T. Chen, Z. Xu, F. Huo, Y. Wei, and X. Yang, "Crocin protects retinal ganglion cells against H_2O_2-induced damage through the mitochondrial pathway and activation of NF-κB," *International Journal of Molecular Medicine*, vol. 37, no. 1, pp. 225–232, 2016.

[88] R. Russo, F. Cavaliere, L. Rombolà et al., "Rational basis for the development of coenzyme Q10 as a neurotherapeutic agent for retinal protection," *Progress in Brain Research*, vol. 173, pp. 575–582, 2008.

[89] H. R. Ahn, H. J. Lee, K.-A. Kim et al., "Hydroxycinnamic acids in Crepidiastrum denticulatum protect oxidative stress-induced retinal damage," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 6, pp. 1310–1323, 2014.

[90] A. Piotrowska-Nowak, E. Kosior-Jarecka, A. Schab et al., "Investigation of whole mitochondrial genome variation in normal tension glaucoma," *Experimental Eye Research*, vol. 178, pp. 186–197, 2019.

[91] J. L. Wiggs and L. R. Pasquale, "Genetics of glaucoma," *Human Molecular Genetics*, vol. 26, no. R1, pp. R21–R27, 2017.

[92] J. N. C. Bailey, S. J. Loomis, S. J. Loomis et al., "Genome-wide association analysis identifies TXNRD2, ATXN2 and FOXC1 as susceptibility loci for primary open-angle glaucoma," *Nature Genetics*, vol. 48, no. 2, pp. 189–194, 2016.

[93] J. Caprioli, Y. Munemasa, J. M. Kwong, and N. Piri, "Overexpression of thioredoxins 1 and 2 increases retinal ganglion cell survival after pharmacologically induced oxidative stress, optic nerve transection, and in experimental glaucoma," *Transactions of the American Ophthalmological Society*, vol. 107, pp. 161–165, 2009.

[94] T. Rezaie, A. Child, R. Hitchings, G. Brice, L. Miller, and M. Coca-Prados, "Adult-onset primary open-angle glaucoma caused by mutations in optineurin," *Science*, vol. 295, no. 5557, pp. 1077–1079, 2002.

[95] E. M. Stone, J. H. Fingert, W. L. Alward, T. D. Nguyen, J. R. Polansky, and S. L. Sunden, "Identification of a gene that causes primary open angle glaucoma," *Science*, vol. 275, no. 5300, pp. 668–670, 1997.

[96] V. C. Sheffield, E. M. Stone, W. L. M. Alward et al., "Genetic linkage of familial open angle glaucoma to chromosome 1q21-q31," *Nature Genetics*, vol. 4, no. 1, pp. 47–50, 1993.

[97] G. Charliat, D. Jolly, and F. Blanchard, "Genetic risk factor in primary open-angle glaucoma: a case-control study," *Ophthalmic Epidemiology*, vol. 1, no. 3, pp. 131–138, 1994.

[98] J. H. Fingert, A. L. Robin, J. L. Stone et al., "Copy number variations on chromosome 12q14 in patients with normal tension glaucoma," *Human Molecular Genetics*, vol. 20, no. 12, pp. 2482–2494, 2011.

[99] N. C. Sears, E. A. Boese, M. A. Miller, and J. H. Fingert, "Mendelian genes in primary open angle glaucoma," *Experimental Eye Research*, vol. 186, Article ID 107702, 2019.

[100] D. W. Collins, H. V. Gudiseva, B. T. Trachtman, M. Jerrehian, T. Gorry, and W. T. Merritt III,

"Mitochondrial sequence variation in African-American primary open-angle glaucoma patients," *PLoS One*, vol. 8, Article ID e76627, 2013.

[101] J. W. Jeoung, M.-W. Seong, S. S. Park, D. M. Kim, S. H. Kim, and K. H. Park, "Mitochondrial DNA variant discovery in normal-tension glaucoma patients by next-generation sequencing," *Investigative Opthalmology & Visual Science*, vol. 55, no. 2, pp. 986–992, 2014.

[102] P. Sundaresan, D. A. Simpson, C. Sambare et al., "Whole-mitochondrial genome sequencing in primary open-angle glaucoma using massively parallel sequencing identifies novel and known pathogenic variants," *Genetics in Medicine*, vol. 17, no. 4, pp. 279–284, 2015.

[103] N. B. Larsen, M. Rasmussen, and L. J. Rasmussen, "Nuclear and mitochondrial DNA repair: similar pathways?" *Mitochondrion*, vol. 5, no. 2, pp. 89–108, 2005.

[104] G. Lascaratos, D. F. Garway-Heath, C. E. Willoughby, K.-Y. Chau, and A. H. V. Schapira, "Mitochondrial dysfunction in glaucoma: understanding genetic influences," *Mitochondrion*, vol. 12, no. 2, pp. 202–212, 2012.

[105] A. P. Khawaja, J. N. Cooke Bailey, J. H. Kang et al., "Assessing the association of mitochondrial genetic variation with primary open-angle glaucoma using gene-set analyses," *Investigative Opthalmology & Visual Science*, vol. 57, no. 11, pp. 5046–5052, 2016.

[106] M. F. Beal and M. Flint Beal, "Mitochondria take center stage in aging and neurodegeneration," *Annals of Neurology*, vol. 58, no. 4, pp. 495–505, 2005.

[107] A. G. Tsilis, K. K. Tsilidis, S. H. Pelidou, and G. Kitsos, "Systematic review of the association between Alzheimer's disease and chronic glaucoma," *Clinical Ophthalmology*, vol. 8, pp. 2095–2104, 2014.

[108] N.-G. Larsson, "Somatic mitochondrial DNA mutations in mammalian aging," *Annual Review of Biochemistry*, vol. 79, no. 1, pp. 683–706, 2010.

[109] A. Bender, K. J. Krishnan, C. M. Morris et al., "High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease," *Nature Genetics*, vol. 38, no. 5, pp. 515–517, 2006.

[110] A. Mathew, T. A. Lindsley, A. Sheridan et al., "Degraded mitochondrial DNA is a newly identified subtype of the damage associated molecular pattern (DAMP) family and possible trigger of neurodegeneration," *Journal of Alzheimer's Disease*, vol. 30, no. 3, pp. 617–627, 2012.

[111] N. E. Abramova, K. J. A. Davies, and D. R. Crawford, "Polynucleotide degradation during early stage response to oxidative stress is specific to mitochondria," *Free Radical Biology and Medicine*, vol. 28, no. 2, pp. 281–288, 2000.

[112] D. Matheoud, A. Sugiura, A. Bellemare-Pelletier, A. Laplante, C. Rondeau, and M. Chemali, "Parkinson's Disease-Related Proteins PINK1 and Parkin Repress Mitochondrial Antigen Presentation," *Cell*, vol. 166, no. 2, pp. 314–327, 2016.

[113] W. Wu, H. Xu, Z. Wang, Y. Mao, L. Yuan, and W. Luo, "PINK1-Parkin-Mediated mitophagy protects mitochondrial integrity and prevents metabolic stress-induced endothelial injury," *PLoS One*, vol. 10, Article ID e0132499, 2015.

[114] K. Cowan, O. Anichtchik, and S. Luo, "Mitochondrial integrity in neurodegeneration," *CNS Neuroscience & Therapeutics*, vol. 25, no. 7, pp. 825–836, 2019.

[115] C. Vives-Bauza, C. Zhou, Y. Huang et al., "PINK1-dependent recruitment of Parkin to mitochondria in mitophagy," *Proceedings of the National Academy of Sciences*, vol. 107, no. 1, pp. 378–383, 2010.

[116] Y. Wang, Y. Nartiss, B. Steipe, G. A. McQuibban, and P. K. Kim, "ROS-induced mitochondrial depolarization initiates PARK2/PARKIN-dependent mitochondrial degradation by autophagy," *Autophagy*, vol. 8, no. 10, pp. 1462–1476, 2012.

[117] K. Chernyshova, K. Inoue, S.-I. Yamashita, T. Fukuchi, and T. Kanki, "Glaucoma-associated mutations in the optineurin gene have limited impact on parkin-dependent mitophagy," *Investigative Opthalmology & Visual Science*, vol. 60, no. 10, pp. 3625–3635, 2019.

[118] M. S. Shim, Y. Takihara, K.-Y. Kim, T. Iwata, B. Y. J. T. Yue, and M. Inatani, "Mitochondrial pathogenic mechanism and degradation in optineurin E50K mutation-mediated retinal ganglion cell degeneration," *Scientific Reports*, vol. 6, p. 33830, 2016.

[119] S. Morton, L. Hesson, M. Peggie, and P. Cohen, "Enhanced binding of TBK1 by an optineurin mutant that causes a familial form of primary open angle glaucoma," *FEBS Letters*, vol. 582, no. 6, pp. 997–1002, 2008.

[120] K. Sirohi, A. Kumari, V. Radha, and G. Swarup, "A glaucoma-associated variant of optineurin, M98K, activates Tbk1 to enhance autophagosome formation and retinal cell death dependent on Ser177 phosphorylation of optineurin," *PLoS One*, vol. 10, Article ID e0138289, 2015.

[121] Y. Minegishi, D. Iejima, H. Kobayashi et al., "Enhanced optineurin E50K-TBK1 interaction evokes protein insolubility and initiates familial primary open-angle glaucoma," *Human Molecular Genetics*, vol. 22, no. 17, pp. 3559–3567, 2013.

[122] J. H. Fingert, K. Miller, A. Hedberg-Buenz et al., "Transgenic TBK1 mice have features of normal tension glaucoma," *Human Molecular Genetics*, vol. 26, no. 1, pp. 124–132, 2017.

[123] G. Matsumoto, T. Shimogori, N. Hattori, and N. Nukina, "TBK1 controls autophagosomal engulfment of polyubiquitinated mitochondria through p62/SQSTM1 phosphorylation," *Human Molecular Genetics*, vol. 24, no. 15, pp. 4429–4442, 2015.

[124] Y. Li, J. Kang, and M. S. Horwitz, "Interaction of an adenovirus E3 14.7-kilodalton protein with a novel tumor necrosis factor alpha-inducible cellular protein containing leucine zipper domains," *Molecular and Cellular Biology*, vol. 18, no. 3, pp. 1601–1610, 1998.

[125] R. P. Toth and J. D. Atkin, "Dysfunction of optineurin in amyotrophic lateral sclerosis and glaucoma," *Frontiers in Immunology*, vol. 9, p. 1017, 2018.

[126] G. Swarup and Z. Sayyad, "Altered functions and interactions of glaucoma-associated mutants of optineurin," *Frontiers in Immunology*, vol. 9, p. 1287, 2018.

[127] G. Zhu, C.-J. Wu, Y. Zhao, and J. D. Ashwell, "Optineurin negatively regulates TNFα- induced NF-κB activation by competing with NEMO for ubiquitinated RIP," *Current Biology*, vol. 17, no. 16, pp. 1438–1443, 2007.

[128] M. Tanishima, S. Takashima, A. Honda et al., "Identification of optineurin as an interleukin-1 receptor-associated kinase 1-binding protein and its role in regulation of MyD88-dependent signaling," *Journal of Biological Chemistry*, vol. 292, no. 42, pp. 17250–17257, 2017.

[129] A. Nagabhushana, M. Bansal, and G. Swarup, "Optineurin is required for CYLD-dependent inhibition of TNFα-induced NF-κB activation," *PLoS One*, vol. 6, Article ID e17477, 2011.

[130] C. Sudhakar, A. Nagabhushana, N. Jain, and G. Swarup, "NF-κB mediates tumor necrosis factor α-induced expression of optineurin, a negative regulator of NF-κB," *PLoS One*, vol. 4, no. 4, p. e5114, 2009.

[131] C. Louis, C. Burns, and I. Wicks, "TANK-binding kinase 1-dependent responses in health and autoimmunity," *Frontiers in Immunology*, vol. 9, p. 434, 2018.

[132] J. L. Pomerantz and D. Baltimore, "NF-kappa B activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase," *The EMBO Journal*, vol. 18, no. 23, pp. 6694–6704, 1999.

[133] S. A. Liddelow, K. A. Guttenplan, L. E. Clarke et al., "Neurotoxic reactive astrocytes are induced by activated microglia," *Nature*, vol. 541, no. 7638, pp. 481–487, 2017.

[134] E. Vecino, F. D. Rodriguez, N. Ruzafa, X. Pereiro, and S. C. Sharma, "Glia-neuron interactions in the mammalian retina," *Progress in Retinal and Eye Research*, vol. 51, pp. 1–40, 2016.

[135] C. Iadecola and M. Nedergaard, "Glial regulation of the cerebral microvasculature," *Nature Neuroscience*, vol. 10, no. 11, pp. 1369–1376, 2007.

[136] E. M. Ullian, S. K. Sapperstein, K. S. Christopherson, and B. A. Barres, "Control of synapse number by glia," *Science*, vol. 291, no. 5504, pp. 657–661, 2001.

[137] R. Vohra, J. C. Tsai, and M. Kolko, "The role of inflammation in the pathogenesis of glaucoma," *Survey of Ophthalmology*, vol. 58, no. 4, pp. 311–320, 2013.

[138] I. Soto and G. R. Howell, "The complex role of neuroinflammation in glaucoma," *Cold Spring Harbor Perspectives in Medicine*, vol. 4, no. 8, Article ID a017269, 2014.

[139] L. Wang, G. A. Cioffi, G. Cull, J. Dong, and B. Fortune, "Immunohistologic evidence for retinal glial cell changes in human glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 43, no. 4, pp. 1088–1094, 2002.

[140] D. M. Inman and P. J. Horner, "Reactive nonproliferative gliosis predominates in a chronic mouse model of glaucoma," *Glia*, vol. 55, no. 9, pp. 942–953, 2007.

[141] G. L. N. Tezel, B. C. Chauhan, R. P. LeBlanc, and M. B. Wax, "Immunohistochemical assessment of the glial mitogen-activated protein kinase activation in glaucoma," *Investigative Opthalmology & Visual Science*, vol. 44, no. 7, pp. 3025–3033, 2003.

[142] M. A. Fard, S. Moghimi, A. Sahraian, and R. Ritch, "Optic nerve head cupping in glaucomatous and non-glaucomatous optic neuropathy," *British Journal of Ophthalmology*, vol. 103, no. 3, pp. 374–378, 2019.

[143] C. Dai, P. T. Khaw, Z. Q. Yin, D. Li, G. Raisman, and Y. Li, "Structural basis of glaucoma: the fortified astrocytes of the optic nerve head are the target of raised intraocular pressure," *Glia*, vol. 60, no. 1, pp. 13–28, 2012.

[144] D. Sun, J. Qu, and T. C. Jakobs, "Reversible reactivity by optic nerve astrocytes," *Glia*, vol. 61, no. 8, pp. 1218–1235, 2013.

[145] M. Bariş and G. Tezel, "Immunomodulation as a neuroprotective strategy for glaucoma treatment," *Current Opthalmology Reports*, vol. 7, no. 2, pp. 160–169, 2019.

[146] T. Nikolskaya, Y. Nikolsky, T. Serebryiskaya, S. Zvereva, E. Sviridov, and Z. Dezso, "Network analysis of human glaucomatous optic nerve head astrocytes," *BMC Medical Genomics*, vol. 2, p. 24, 2009.

[147] K. S. Kompass, O. A. Agapova, W. Li, P. L. Kaufman, C. A. Rasmussen, and M. R. Hernandez, "Bioinformatic and statistical analysis of the optic nerve head in a primate model of ocular hypertension," *BMC Neuroscience*, vol. 9, p. 93, 2008.

[148] F. Ginhoux, M. Greter, M. Leboeuf et al., "Fate mapping analysis reveals that adult microglia derive from primitive macrophages," *Science*, vol. 330, no. 6005, pp. 841–845, 2010.

[149] A. H. Neufeld, "Microglia in the optic nerve head and the region of parapapillary chorioretinal atrophy in glaucoma," *Archives of Ophthalmology*, vol. 117, no. 8, pp. 1050–1056, 1999.

[150] L. Yuan and A. H. Neufeld, "Activated microglia in the human glaucomatous optic nerve head," *Journal of Neuroscience Research*, vol. 64, no. 5, pp. 523–532, 2001.

[151] G. Tezel and M. B. Wax, "Increased production of tumor necrosis factor-α by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells," *The Journal of Neuroscience*, vol. 20, no. 23, pp. 8693–8700, 2000.

[152] A. Bosco, C. O. Romero, K. T. Breen et al., "Neurodegeneration severity can be predicted from early microglia alterations monitored in vivo in a mouse model of chronic glaucoma," *Disease Models & Mechanisms*, vol. 8, no. 5, pp. 443–455, 2015.

[153] A. Bosco, D. M. Inman, M. R. Steele et al., "Reduced retina microglial activation and improved optic nerve integrity with minocycline treatment in the DBA/2J mouse model of glaucoma," *Investigative Opthalmology & Visual Science*, vol. 49, no. 4, pp. 1437–1446, 2008.

[154] M. K. Jha, M. Seo, J.-H. Kim, B.-G. Kim, J.-Y. Cho, and K. Suk, "The secretome signature of reactive glial cells and its pathological implications," *Biochimica et Biophysica Acta (BBA)—Proteins and Proteomics*, vol. 1834, no. 11, pp. 2418–2428, 2013.

[155] A. H. Neufeld, M. R. Hernandez, and M. Gonzalez, "Nitric oxide synthase in the human glaucomatous optic nerve head," *Archives of Ophthalmology*, vol. 115, no. 4, pp. 497–503, 1997.

[156] K. J. Cho, J. H. Kim, H.-Y. L. Park, and C. K. Park, "Glial cell response and iNOS expression in the optic nerve head and retina of the rat following acute high IOP ischemia-reperfusion," *Brain Research*, vol. 1403, pp. 67–77, 2011.

[157] L. Vidal, F. Díaz, A. Villena, M. Moreno, J. G. Campos, and I. P. d. Vargas, "Nitric oxide synthase in retina and optic nerve head of rat with increased intraocular pressure and effect of timolol," *Brain Research Bulletin*, vol. 70, no. 4–6, pp. 406–413, 2006.

[158] S. Shareef, A. Sawada, and A. H. Neufeld, "Isoforms of nitric oxide synthase in the optic nerves of rat eyes with chronic moderately elevated intraocular pressure," *Investigative Ophthalmology & Visual Science*, vol. 40, no. 12, pp. 2884–2891, 1999.

[159] A. H. Neufeld, A. Sawada, and B. Becker, "Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma," *Proceedings of the National Academy of Sciences*, vol. 96, no. 17, pp. 9944–9948, 1999.

[160] X. Yang, C. Luo, J. Cai et al., "Neurodegenerative and inflammatory pathway components linked to TNF-α/TNFR1 signaling in the glaucomatous human retina," *Investigative Opthalmology & Visual Science*, vol. 52, no. 11, pp. 8442–8454, 2011.

[161] G. L. N. Tezel, X. Yang, C. Luo, Y. Peng, S. L. Sun, and D. Sun, "Mechanisms of immune system activation in glaucoma: oxidative stress-stimulated antigen presentation by the retina and optic nerve head glia," *Investigative Opthalmology & Visual Science*, vol. 48, no. 2, pp. 705–714, 2007.

[162] G. Tezel, I. L. Thornton, M. G. Tong et al., "Immunoproteomic analysis of potential serum biomarker candidates in human glaucoma," *Investigative Opthalmology & Visual Science*, vol. 53, no. 13, p. 8222, 2012.

[163] G. Tezel, R. Hernandez, and M. B. Wax, "Immunostaining of heat shock proteins in the retina and optic nerve head of normal and glaucomatous eyes," *Archives of Ophthalmology*, vol. 118, no. 4, pp. 511–518, 2000.

[164] M. R. Steele, D. M. Inman, D. J. Calkins, P. J. Horner, and M. L. Vetter, "Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma," *Investigative Opthalmology & Visual Science*, vol. 47, no. 3, pp. 977–985, 2006.

[165] L. Panagis, X. Zhao, Y. Ge, L. Ren, T. W. Mittag, and J. Danias, "Gene expression changes in areas of focal loss of retinal ganglion cells in the retina of DBA/2J mice," *Investigative Opthalmology & Visual Science*, vol. 51, no. 4, pp. 2024–2034, 2010.

[166] E. C. Johnson, L. Jia, W. O. Cepurna, T. A. Doser, and J. C. Morrison, "Global changes in optic nerve head gene expression after exposure to elevated intraocular pressure in a rat glaucoma model," *Investigative Opthalmology & Visual Science*, vol. 48, no. 7, p. 3161, 2007.

[167] J. Yang, P. Yang, G. Tezel, R. V. Patil, M. R. Hernandez, and M. B. Wax, "Induction of HLA-DR expression in human lamina cribrosa astrocytes by cytokines and simulated ischemia," *Investigative Ophthalmology & Visual Science*, vol. 42, no. 2, pp. 365–371, 2001.

[168] G. Chidlow, A. Ebneter, J. P. M. Wood, and R. J. Casson, "Evidence supporting an association between expression of major histocompatibility complex II by microglia and optic nerve degeneration during experimental glaucoma," *Journal of Glaucoma*, vol. 25, no. 8, pp. 681–691, 2016.

[169] J. N. Duarte, J. J. Cragnolini, L. K. Swee et al., "Generation of immunity against pathogens via single-domain antibody-antigen constructs," *The Journal of Immunology*, vol. 197, no. 12, pp. 4838–4847, 2016.

[170] A. P. West, G. S. Shadel, S. Ghosh, and S. Ghosh, "Mitochondria in innate immune responses," *Nature Reviews Immunology*, vol. 11, no. 6, pp. 389–402, 2011.

[171] T. Kawasaki and T. Kawai, "Toll-like receptor signaling pathways," *Frontiers in Immunology*, vol. 5, p. 461, 2014.

[172] O. Takeuchi and S. Akira, "Innate immunity to virus infection," *Immunological Reviews*, vol. 227, no. 1, pp. 75–86, 2009.

[173] D. Tang, R. Kang, C. B. Coyne, H. J. Zeh, and M. T. Lotze, "PAMPs and DAMPs: signal 0 s that spur autophagy and immunity," *Immunological Reviews*, vol. 249, no. 1, pp. 158–175, 2012.

[174] A. P. West, "Mitochondrial dysfunction as a trigger of innate immune responses and inflammation," *Toxicology*, vol. 391, pp. 54–63, 2017.

[175] K. Nakahira, S. Hisata, and A. M. K. Choi, "The roles of mitochondrial damage-associated molecular patterns in diseases," *Antioxidants & Redox Signaling*, vol. 23, no. 17, pp. 1329–1350, 2015.

[176] G. Tezel, J. Yang, and M. B. Wax, "Heat shock proteins, immunity and glaucoma," *Brain Research Bulletin*, vol. 62, no. 6, pp. 473–480, 2004.

[177] H. Chen, K.-S. Cho, T. H. K. Vu, C.-H. Shen, M. Kaur, and G. Chen, "Commensal microflora-induced T cell responses mediate progressive neurodegeneration in glaucoma," *Nature Communications*, vol. 9, p. 3209, 2018.

[178] E. C. Johnson and J. C. Morrison, "Friend or foe? Resolving the impact of glial responses in glaucoma," *Journal of Glaucoma*, vol. 18, no. 5, pp. 341–353, 2009.

[179] M. L. Cooper, S. D. Crish, D. M. Inman, P. J. Horner, and D. J. Calkins, "Early astrocyte redistribution in the optic nerve precedes axonopathy in the DBA/2J mouse model of glaucoma," *Experimental Eye Research*, vol. 150, pp. 22–33, 2016.

[180] J. L. Son, I. Soto, E. Oglesby et al., "Glaucomatous optic nerve injury involves early astrocyte reactivity and late oligodendrocyte loss," *Glia*, vol. 58, no. 7, pp. 780–789, 2010.

[181] R. Wang, P. Seifert, and T. C. Jakobs, "Astrocytes in the optic nerve head of glaucomatous mice display a characteristic reactive phenotype," *Investigative Opthalmology & Visual Science*, vol. 58, no. 2, pp. 924–932, 2017.

[182] D. M. Inman and M. Harun-Or-Rashid, "Metabolic vulnerability in the neurodegenerative disease glaucoma," *Frontiers in Neuroscience*, vol. 11, p. 146, 2017.

[183] G. R. Howell, D. G. Macalinao, G. L. Sousa et al., "Molecular clustering identifies complement and endothelin induction as early events in a mouse model of glaucoma," *Journal of Clinical Investigation*, vol. 121, no. 4, pp. 1429–1444, 2011.

[184] C. K. Glass, K. Saijo, B. Winner, M. C. Marchetto, and F. H. Gage, "Mechanisms underlying inflammation in neurodegeneration," *Cell*, vol. 140, no. 6, pp. 918–934, 2010.

[185] M. E. Heid, P. A. Keyel, C. Kamga, S. Shiva, S. C. Watkins, and R. D. Salter, "Mitochondrial reactive oxygen species induces NLRP3-dependent lysosomal damage and inflammasome activation," *The Journal of Immunology*, vol. 191, no. 10, pp. 5230–5238, 2013.

[186] R. Zhou, A. S. Yazdi, P. Menu, and J. Tschopp, "A role for mitochondria in NLRP3 inflammasome activation," *Nature*, vol. 469, no. 7329, pp. 221–225, 2011.

[187] P. Gurung, J. R. Lukens, and T.-D. Kanneganti, "Mitochondria: diversity in the regulation of the NLRP3 inflammasome," *Trends in Molecular Medicine*, vol. 21, no. 3, pp. 193–201, 2015.

[188] Q. Liu, D. Zhang, D. Hu, X. Zhou, and Y. Zhou, "The role of mitochondria in NLRP3 inflammasome activation," *Molecular Immunology*, vol. 103, pp. 115–124, 2018.

[189] C. S. Dela Cruz and M.-J. Kang, "Mitochondrial dysfunction and damage associated molecular patterns (DAMPs) in chronic inflammatory diseases," *Mitochondrion*, vol. 41, pp. 37–44, 2018.

[190] J. Yu, H. Nagasu, T. Murakami et al., "Inflammasome activation leads to Caspase-1-dependent mitochondrial damage and block of mitophagy," *Proceedings of the National Academy of Sciences*, vol. 111, no. 43, pp. 15514–15519, 2014.

[191] W. Chi, F. Li, H. Chen et al., "Caspase-8 promotes NLRP1/NLRP3 inflammasome activation and IL-1 production in acute glaucoma," *Proceedings of the National Academy of Sciences*, vol. 111, no. 30, pp. 11181–11186, 2014.

[192] Z. Puyang, L. Feng, H. Chen, P. Liang, J. B. Troy, and X. Liu, "Retinal ganglion cell loss is delayed following optic nerve crush in NLRP3 knockout mice," *Scientific Reports*, vol. 6, p. 20998, 2016.

[193] X. Yang, G. Hondur, and G. Tezel, "Antioxidant treatment limits neuroinflammation in experimental glaucoma," *Investigative Opthalmology & Visual Science*, vol. 57, no. 4, p. 2344, 2016.

[194] X. Yan, G. Tezel, M. B. Wax, and D. P. Edward, "Matrix metalloproteinases and tumor necrosis factor α in glaucomatous optic nerve head," *Archives of Ophthalmology*, vol. 118, no. 5, pp. 666–673, 2000.

[195] G. Tezel, L. Y. Li, R. V. Patil, and M. B. Wax, "TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes," *Investigative Ophthalmology & Visual Science*, vol. 42, no. 8, pp. 1787–1794, 2001.

[196] D. Bonifati and U. Kishore, "Role of complement in neurodegeneration and neuroinflammation," *Molecular Immunology*, vol. 44, no. 5, pp. 999–1010, 2007.

[197] D. Ricklin, G. Hajishengallis, K. Yang, and J. D. Lambris, "Complement: a key system for immune surveillance and homeostasis," *Nature Immunology*, vol. 11, no. 9, pp. 785–797, 2010.

[198] B. Stevens, N. J. Allen, L. E. Vazquez et al., "The classical complement cascade mediates CNS synapse elimination," *Cell*, vol. 131, no. 6, pp. 1164–1178, 2007.

[199] S. B. Storrs, W. P. Kolb, and M. S. Olson, "C1q binding and C1 activation by various isolated cellular membranes," *Journal of Immunology*, vol. 131, no. 1, pp. 416–422, 1983.

[200] J. Kohl, "The role of complement in danger sensing and transmission," *Immunologic Research*, vol. 34, no. 2, pp. 157–176, 2006.

[201] E. S. Reis, D. C. Mastellos, G. Hajishengallis, and J. D. Lambris, "New insights into the immune functions of complement," *Nature Reviews Immunology*, vol. 19, no. 8, pp. 503–516, 2019.

[202] M. H. Kuehn, C. Y. Kim, J. Ostojic et al., "Retinal synthesis and deposition of complement components induced by ocular hypertension," *Experimental Eye Research*, vol. 83, no. 3, pp. 620–628, 2006.

[203] K. Stasi, D. Nagel, X. Yang et al., "Complement component 1Q (C1Q) upregulation in retina of murine, primate, and human glaucomatous eyes," *Investigative Opthalmology & Visual Science*, vol. 47, no. 3, pp. 1024–1029, 2006.

[204] G. Tezel, X. Yang, C. Luo et al., "Oxidative stress and the regulation of complement activation in human glaucoma," *Investigative Opthalmology & Visual Science*, vol. 51, no. 10, pp. 5071–5082, 2010.

[205] E. C. Johnson, T. A. Doser, W. O. Cepurna et al., "Cell proliferation and interleukin-6-type cytokine signaling are implicated by gene expression responses in early optic nerve head injury in rat glaucoma," *Investigative Opthalmology & Visual Science*, vol. 52, no. 1, pp. 504–518, 2011.

[206] T. Miyahara, T. Kikuchi, M. Akimoto, T. Kurokawa, H. Shibuki, and N. Yoshimura, "Gene microarray analysis of experimental glaucomatous retina from cynomologous monkey," *Investigative Opthalmology & Visual Science*, vol. 44, no. 10, pp. 4347–4356, 2003.

[207] A. Bosco, S. R. Anderson, K. T. Breen et al., "Complement C3-targeted gene therapy restricts onset and progression of neurodegeneration in chronic mouse glaucoma," *Molecular Therapy*, vol. 26, no. 10, pp. 2379–2396, 2018.

[208] P. Jha, H. Banda, R. Tytarenko, P. S. Bora, and N. S. Bora, "Complement mediated apoptosis leads to the loss of retinal ganglion cells in animal model of glaucoma," *Molecular Immunology*, vol. 48, no. 15-16, pp. 2151–2158, 2011.

[209] G. R. Howell, I. Soto, M. Ryan, L. C. Graham, R. S. Smith, and S. W. M. John, "Deficiency of complement component 5 ameliorates glaucoma in DBA/2J mice," *Journal of Neuroinflammation*, vol. 10, p. 76, 2013.

[210] S. Reinehr, S. C. Gomes, C. J. Gassel et al., "Intravitreal therapy against the complement factor C5 prevents retinal degeneration in an experimental autoimmune glaucoma model," *Frontiers in Pharmacology*, vol. 10, 2019.

[211] P. Gasque, Y. D. Dean, E. P. McGreal, J. VanBeek, and B. P. Morgan, "Complement components of the innate immune system in health and disease in the CNS," *Immunopharmacology*, vol. 49, no. 1-2, pp. 171–186, 2000.

[212] L. Piccio, B. Rossi, E. Scarpini et al., "Molecular mechanisms involved in lymphocyte recruitment in inflamed brain microvessels: critical roles for P-selectin glycoprotein ligand-1 and heterotrimeric gi-linked receptors," *The Journal of Immunology*, vol. 168, no. 4, pp. 1940–1949, 2002.

[213] M. Charabati, J.-M. Rabanel, C. Ramassamy, and A. Prat, "Overcoming the brain barriers: from immune cells to nanoparticles," *Trends in Pharmacological Sciences*, vol. 41, no. 1, pp. 42–54, 2020.

[214] J. Flammer and M. Mozaffarieh, "What is the present pathogenetic concept of glaucomatous optic neuropathy?" *Survey of Ophthalmology*, vol. 52, no. Suppl 2, pp. S162–S173, 2007.

[215] T. A. Uhler and J. Piltz-Seymour, "Optic disc hemorrhages in glaucoma and ocular hypertension: implications and recommendations," *Current Opinion in Ophthalmology*, vol. 19, no. 2, pp. 89–94, 2008.

[216] G. Tezel, K. D. Siegmund, K. Trinkaus, M. B. Wax, M. A. Kass, and A. E. Kolker, "Clinical factors associated with progression of glaucomatous optic disc damage in treated patients," *Archives of Ophthalmology*, vol. 119, no. 6, pp. 813–818, 2001.

[217] K. Lorenz, S. Beck, M. M. Keilani, J. Wasielica-Poslednik, N. Pfeiffer, and F. H. Grus, "Longitudinal analysis of serum autoantibody-reactivities in patients with primary open angle glaucoma and optic disc hemorrhage," *PLoS One*, vol. 11, Article ID e0166813, 2016.

[218] C. Guo, N. Wu, X. Niu, Y. Wu, D. Chen, and W. Guo, "Comparison of T Helper cell patterns in primary open-angle glaucoma and normal-pressure glaucoma," *Medical Science Monitor*, vol. 24, pp. 1988–1996, 2018.

[219] J. Yang, R. V. Patil, H. Yu, M. Gordon, and M. B. Wax, "T cell subsets and sIL-2R/IL-2 levels in patients with glaucoma," *American Journal of Ophthalmology*, vol. 131, no. 4, pp. 421–426, 2001.

[220] M. B. Wax, G. Tezel, J. Yang et al., "Induced autoimmunity to heat shock proteins elicits glaucomatous loss of retinal ganglion cell neurons via activated T-cell-derived fas-ligand," *Journal of Neuroscience*, vol. 28, no. 46, pp. 12085–12096, 2008.

[221] S. C. Joachim, O. W. Gramlich, P. Laspas, H. Schmid, S. Beck, and H. D. von Pein, "Retinal ganglion cell loss is accompanied by antibody depositions and increased levels of microglia after immunization with retinal antigens," *PLoS One*, vol. 7, Article ID e40616, 2012.

[222] O. W. Gramlich, S. Beck, N. von Thun und Hohenstein-Blaul et al., "Enhanced insight into the autoimmune component of glaucoma: IgG autoantibody accumulation and pro-inflammatory conditions in human glaucomatous retina," *PLoS One*, vol. 8, no. 2, Article ID e57557, 2013.

[223] O. W. Gramlich, Q. J. Ding, W. Zhu, A. Cook, M. G. Anderson, and M. H. Kuehn, "Adoptive transfer of immune cells from glaucomatous mice provokes retinal ganglion cell loss in recipients," *Acta Neuropathologica Communications*, vol. 3, p. 56, 2015.

Hindawi
Journal of Ophthalmology
Volume 2021, Article ID 5013529, 10 pages
https://doi.org/10.1155/2021/5013529

Review Article

What Is New in Glaucoma: From Treatment to Biological Perspectives

Raffaele Nuzzi,[1] **Paola Marolo**,[1] **and Alessia Nuzzi**[2]

[1]*Eye Clinic Section, Department of Surgical Sciences, University of Turin, Turin, Italy*
[2]*Department of Clinical Sciences and Community Health, Eye Clinic San Giuseppe Hospital,IRCCS Multimedica, University of Milan, Milan, Italy*

Correspondence should be addressed to Raffaele Nuzzi; prof.nuzzi_raffaele@hotmail.it

Received 3 January 2020; Revised 24 February 2021; Accepted 24 March 2021; Published 14 April 2021

Academic Editor: Steffen Heegaard

Glaucoma is a chronic silent disease and an irreversible cause of blindness worldwide. Research has made many efforts to improve disease control and especially to anticipate both early diagnosis and treatment of advanced stages of glaucoma. In terms of prevention, networking between professionals and nonprofessionals is an important goal to disseminate information and help diagnose the disease early. On the other hand, the most recent approaches to treat glaucoma outcomes in its advanced stages include electrical stimulation, stem cells, exosomes, extracellular vesicles, and growth factors. Finally, neuronal plasticity-based rehabilitation methods are being studied to reeducate patients in order to stimulate their residual visual capacity. This review provides an overview of new approaches to future possible glaucoma treatment modalities and gives insight into the perspectives available nowadays in this field.

1. Introduction

Glaucoma is one of the leading causes of blindness in the world, second only to cataracts [1, 2]. It is a chronic, degenerative disease affecting the optic nerve, but insidious: in fact, when the etiopathogenetic process has started and has already damaged the nerve fibers, the symptomatology is almost silent [3, 4]. When the patient becomes aware of the visual impairment, the neural function is already compromised and the chances of recovery are significantly reduced. There is no scientific evidence of the field of visual recovery once its defect has been documented. However, a perimetric learning effect among a percentage of patients could occur, very unlikely to represent a real improvement. For these reasons, glaucoma is a disease of enormous social impact, both from the human point of view, because it is highly disabling and compromises the quality of life and autonomy of those affected [5], and from an economic point of view: for its clinical-therapeutic management, in fact, a substantial percentage of public healthcare expenditure is invested [6]. The term "glaucoma" actually encompasses several forms of optical neuropathies with still partly obscure etiopathogenesis associated with typical visual field alterations and increased intraocular pressure [7, 8]. In reality, this last characteristic is not the rule: in recent years, the number of cases of "normotensive glaucoma," which is not associated with an increment in IOP, has increased dramatically, especially in relation to the lengthening of life expectancy [9, 10]. In fact, it has long been known that only about half of the glaucoma cases have intraocular pressure above reference values [11–13]. In any case, the most accredited etiopathogenetic hypothesis would be the death of retinal ganglion cells due to mechanical stress and apoptosis following ischemic and/or chemical mechanisms, which would seem to have glutamate and NMDA receptor activation as protagonists, which would cause an exponential increase in intracellular calcium concentration, thus triggering irreversible damage to DNA and cell death [14, 15]. In light of this, it is clear that it is of great importance to study the phases of this disorder and to continually seek new preventive and therapeutic strategies [16]. According to the current scientific panorama, the therapeutic possibilities are

aimed at acting both on the initial stages of the disease and on the final outcomes, i.e., on the advanced stages of glaucoma for which the lesions are considerable and no longer reversible, but in which it is possible to intervene by enhancing the residual functions at the highest level. In this context, the aim of our manuscript is to provide a comprehensive review of the recently investigated new approaches to treat early and late stages of the disease.

This literature search was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [17]. Studies reporting novel treatment strategies of glaucoma were systematically reviewed. PubMed, MEDLINE, Web of Science, and Embase databases (from inception up to 1 January, 2020) were searched. The electronic search method included the terms 'glaucoma', 'novel therapies', 'electrical stimulation', 'micropulse', 'stem cells', 'exosomes', 'optic nerve regeneration', 'growth factors', 'rehabilitation', which were connected in various combinations by 'or'/'and'. The last search was carried out on 1 January, 2020. Either prospective or retrospective, both randomized and not randomized studies were considered for eligibility. No restrictions in terms of follow-up were applied. Eligible papers must have been published in peer-reviewed journals and in English, with no publication date or publication status limitations. Titles and abstracts of all identified studies were independently reviewed by two researchers (P.M. and A.N.) to assess eligibility. A full-text evaluation of all potential studies was performed later. Once studies have been selected and included, data were extracted by two independent investigators (P.M. and A.N.). When discrepancies were found, a third author (R.N.) was involved to achieve consensus.

2. Overview of the Current Evolution of Treatment Strategies

In addition to the well-known hypotensive drugs, in recent years, research has focused on the development of new local and systemic drugs to be used to reduce intraocular pressure. The main innovative drugs discovered are latrunculin derivatives, ROCK inhibitors, cannabinoids, local calcium channel blockers, and A1 receptor agents. Latrunculin derivatives have mild hypotonic properties and low solubility [18], while ROCK inhibitors have been shown to be effective in reducing pressure in animal models, also acting as neuroprotectors and vasoregulators [19, 20]. These agents seem to interfere with the healing that generally occurs after filtering surgery [21]. Cannabinoids increase the outflow of aqueous humor that acts on ciliary processes with a vasodilator effect and increase PGE2 levels [22–24]. A1 receptor agonists [25] and selective calcium channel blockers improve watery mood drainage, but the latter can lead to serious systemic consequences, such as severe bradycardia and arterial hypotension [26].

Moreover, in the last years, great interest has been devoted to the design of modern devices for instilling drugs in situ: one of the main disadvantages of eye drops treatment is, in fact, the poor compliance and fluctuating adherence of patients to the therapy [27]. These new delivery systems include eye inserts, surgical implants, soft medicated contact lens, and nanospheres. Ocular inserts are projected to provide medication for several days, while surgical implants can perform their function for months but require surgical intervention [28]. Another product in the design phase is the medicated soft contact lens, which, however, must be worn constantly and does not always allow drugs to pass through the surface of the eye in adequate quantities [27, 29]. Nanospheres are another type of device that ensures good drug penetration but does not prevent the patient's from poor adherence to therapy because these microspheres are administered through eye drops [30]. To date, the experimental data in favor of the application of these delivery systems are promising but still limited, and further studies are needed to confirm such evidence [31].

Concerning laser therapy, diode laser trabeculoplasty (DLT) uses lower energy spots than SLT (selective laser trabeculoplasty) and ALT (argon laser trabeculoplasty) for the same performance, and micropulse diode laser trabeculoplasty (MDLT) emits microspots to limit heat-induced damage to adjacent structures [32]. Ab intero excimer laser trabeculectomy is based on the creation of microperforation connecting the anterior chamber to the Schlemm's channel, theoretically not causing heat-induced damage and healing [33].

However, its effectiveness is still uncertain. Despite the advantages of laser technology, research is still focusing on finding the best way to minimize tissue rupture and subsequent healing and to achieve better effectiveness in terms of lowering IOP.

Regarding surgery, the main goal of the latest research has been to improve its risk/benefit ratio, trying to overcome the traditional trabeculectomy technique, which still remains the gold standard of treatment. Minimally invasive glaucoma surgery (MIGS) [34] has been developed in an attempt to obtain a better efficacy/safety ratio in eyes with mild or medium-mild grade glaucoma. The efficacy of MIGS is lower in IOP reduction compared to standard surgery, and its costs are elevated; nevertheless, it is a safe technique and can play a role in a subgroup of patients who are not willing to undergo regular surgery or when patients with a moderate level of pressure lowering do not tolerate drops or do not respond to laser treatment.

3. Novel Treatment Strategies for Glaucoma Outcomes

Despite the numerous therapeutic efforts described above, glaucoma is often diagnosed late and blocking the natural evolution of the disease is still the main obstacle in its management. Delaying therapies to the more advanced stages of glaucoma leads to its evolution towards irreversible optic nerve damage and blindness. In this respect, numerous studies have examined the action of new molecules and techniques to improve the control of the disease and restore lost nerve function and protect its anatomical and functional residuals. It is possible to intervene both separately and concurrently on four levels: the trabecular meshwork, the ciliary body, the retinal ganglion cells (RGCs), and the optic

nerve. The main approaches concern the use of stem cells, exosomes or extracellular vesicles, neuroprotection, and rehabilitation therapy. As far as the optic nerve is concerned, the most encouraging instruments are growth factors and chitosan sheaths.

In this section, we will focus our attention on the latest discoveries on the treatment of glaucoma, explaining for each strategy which aspects of the disease it targeted, its underlying mechanisms and/or molecules, its development phase, and the main obstacles to be overcome in order to bring it to the clinic.

3.1. Electrical Stimulation of the Trabecular Meshwork. Electrical stimulation has recently been proposed as a novel approach to decrease IOP in open-angle glaucoma [35]. The target of this technique is the trabecular meshwork (TM), which is not just a passive way of drainage of the aqueous humor but also has an active role in the resistance to the passage of AH through mechanisms that are not fully understood [36]. Early transcorneal electrical stimulation (TcES) [37] has been shown to have a positive IOP lowering effect in preclinical studies. RGCs in the eyes of gerbil prone to retinal lesions related to acute ocular hypertension have been protected from damage by TcES. The implicit mechanism of action was the modulation of the inflammatory response activated by microglial cells [37]. Transpalpebral electrical stimulation (TES) performed on human eyes with open-angle glaucoma has been shown to have a significant effect in lowering IOP [35]. The purpose of TES is to reproduce the role of tyrosine kinase inhibitors by stimulating the reactivation of calcium-activated potassium channels in TM cells. The hyperpolarization induced by the efflux of potassium to TM promotes its relaxation and thus facilitates the outflow of aqueous humor to Schlemm's channel. The progressive functional damage of the TM in glaucoma is inversely proportional to the effectiveness of electrical stimulation. When the ion channel dysfunction is too advanced and both the volume and the elasticity of TM cells are affected, it is more difficult to obtain a good response. Less trabecular function in more advanced glaucoma results in reduced efficacy of the procedure and increased need to replicate it. Therefore, it is our opinion that electrical stimulation may be more useful in the early stages of the disease. Additional studies are needed to further investigate this new technique and to evaluate the maintenance of the IOP lowering effect in time after treatment.

3.2. Micropulse Cyclophotocoagulation and Ultrasound Cyclomodification. Cilioablation is a well-known procedure that has undergone a drastic evolution in basic technology in recent years. While prostaglandin analogues activate the receptors of the smooth muscles of the ciliary body and increase the uveoscleral outflow, surgical ablation of a ciliary body part can decrease the secretory activity of the ciliary epithelium, thus reducing IOP. Diode laser cyclophotocoagulation (CPC) has shown an encouraging risk-benefit profile, with a much more tolerable side effect profile than previous cyclocryotherapy and has led to the development of transscleral diode CPC and endoscopic diode CPC.

Advances in the study of diode technology have allowed the development of the new transscleral Micropulse Diode laser CPC. Its diode laser emits a series of short (microsecond), repetitive bursts of energy, so that the thermal effect is limited to the absorbing tissue with minimal heat diffusion to adjacent structures. During the cooling period, the tissue has time to relax and return to the base temperature. Micropulse diode laser technology has been successfully used for the treatment of diabetic retinopathy and maculopathy, and the expectation on glaucoma is to achieve the same IOP lowering effect as traditional CPC diode, with fewer associated side effects. Tan et al. [38] conducted a study in which good rates of IOP and med reduction were detected and about one-third of the patients reported suffering pain during the procedure, while none described the discomfort as moderate or severe. Although the initial results are encouraging, further studies with longer follow-up are required to better assess the actual benefits of CPC micropulse compared to traditional CPC. Efforts in the search for an alternative to cyclodestructive procedures to further reduce tissue damage have led to the introduction of high-intensity ultrasound (HIFU) for the treatment of glaucoma. The device, firstly proposed in 1991 [39], has recently been redesigned into a compact and easy-to-use device (EyeOP1, EyeTechCare, Rillieux-la-Pape, France). The two essential components of the system are the generator, which gives power to piezoelectric transducers, and the pressure reduction system, which modulates the suction of the probe with its ultrasonic beam. The device uses what is known as circular ultrasound cyclocoagulation and simultaneously treats the entire ciliary body through the release of a titratable dose of six distinct ultrasonic energy beams. In the first clinical study on this procedure [40], a good response to IOP lowering was obtained in the treated group with a duration of four seconds of ultrasound exposure per shot and the complications were three cases of superficial punctate epitheliopathy and one of central ulcer, with no reports of chronic pain hypotony or phthisis bulbs.

Considering that cyclodestructive procedures have been used to treat the later stages of glaucoma, particularly neovascular glaucoma, it is not surprising that the results are mostly poor in the literature. Recently, some studies have evaluated the use of the new technologies described to treat earlier stages of glaucoma, and this has been possible thanks to their good safety profile [41, 42]. With the increase in supporting evidence, more and more surgeons are now considering these new technologies in the treatment of early stages of glaucoma rather than more advanced cases.

3.3. Stem Cells Therapy. Stem cells are an important resource for the maintenance, repair, and possible regeneration of anatomical structures such as the optic nerve [43]. The scientific interest in these cells is due to their unique properties, including the capacity to divide themselves an infinite number of times and the ability to differentiate into many types of cells. However, ethical concerns and technical

barriers are still implied. Promising results have been reported in the literature, but further research is required in order to bring its application to the clinic. To date, the most used cell lines are adult limbic stem cells to restore the corneal epithelium [44], those situated at the location of Schwalbe's ring (the transitional zone between the corneal endothelium and the TM) [45, 46] and those of the ciliary epithelium, which seem to be able to differentiate into various retinal cell strains [47–49]. With regard to differentiation into neural and retinal cells, embryonic/progenitors retinal stem cells have demonstrated successful differentiation into retinal cell types, either in vitro [50] or in vivo [51]. Nevertheless, their use for ex vivo cell therapy still presents barriers: insufficient availability of stem cells/progenitors, immune rejection, and clinical issues related to embryonic and fetal origins. To overcome this hurdle, Parameswaran et al. [48] have demonstrated that mouse fibroblast induced pluripotent stem cells (iPSCs) were also able to generate RGCs, rods, cones, and photoreceptors. The iPSCs were stimulated by a simulated microenvironment of late retinal histogenesis and finally expressed retinal cell type-specific regulators. Anyway, the clinical application of iPSCs for cell therapy in glaucoma is still unknown, and in order to consider the use of stem cells as a replacement for RGCs, some challenges should be addressed. First, the stimulation method of transplanted stem cells is not yet fully understood and further studies are needed in order to bring functional results to the damaged optic nerve in glaucomatous eyes. In addition, RGCs have a heterogeneous nature with different morphological and molecular criteria, making the induced differentiation progress even more challenging. Other types of stem cells that can be employed are mesenchymal stem cells (MSCs) from bone marrow and adipose tissue [52]. The main advantages of MSCs are their pluripotency, their ease of extraction, and their availability for autologous transplantation. The neuroprotective effects of MCSs in experimental glaucoma are now gaining more and more evidence in experimental glaucoma models [52, 53]. MSCs have also shown the ability to produce neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF), with a 28% reduction of cell death after 1 month from glaucoma onset [54]. Connick et al. [55] have suggested a promising application of MSCs that demonstrates positive effects in patients with multiple sclerosis after their intravenous administration. Visual acuity and visual evoked potential latency have improved. It would be interesting to evaluate the possibility of reintegrating trabecular cells by transplanting MSCs into the anterior chamber. The hydrodynamics of aqueous humor should allow the cells to settle at the iridocorneal level, with phenomena of differentiation and intratissue migration. However, experimental data supporting their application in glaucoma are few, except for the production of neurotrophic factors by transplanted stem cells that stimulate ganglion cells survival.

3.4. Exosomes or Extracellular Vesicles. Recent studies have demonstrated the paracrine capacity of MSCs to secrete exosomes [56–58]. Exosomes small extracellular vesicles of endocytic origin of 30–100 nm diameter, with a membrane enclosure and containing proteins, as well as mRNA and miRNA, which can be delivered to the nearby cells. The advantages of exosomes are many. First, they can be easily isolated and purified; second, they are cell-free and do not proliferate, thus avoiding ethical issues related to stem cells. They can also be easily stored and the application of specific doses is easier. Since they are really small, they can migrate into the ganglion cell layer from the vitreous, and this is not possible with transplanted cells. Furthermore, and most importantly, exosomes are immunologically inert.

It has been proven that the content of exosomes is translated to other cells when the exosome blends with the nearby cell membranes and this leads to the translation of new proteins [59]. Once the exosomes deliver their content to cells, this is shuttled inside endocytic vesicles and delivered to endoplasmic reticule and lysosomes [60]. Mead and Tomarev isolated the exosomes from stem cells derived from bone marrow MSC (BMSC) and tested them into a murine model [61]. They performed the first study in which RGC were treated with exosomes and, for the first time, BMSC-derived exosomes were delivered into an eye. In their study, a significant neuroprotective effect was shown when the rapt optic nerve model was analyzed with optical coherence tomography, electroretinography, and immunohistochemistry. RGC survival, regeneration of axons, and partial prevention of RGC axon loss (measured as RNFL thickness) and preservation of RGC function (measured with electroretinogram) were possible thanks to intravitreal injections of BMSC-derived exosomes and were associated with miRNA-dependent mechanisms.

However, the miRNA content and its targets have to be better characterized, and it is not clear which dose of intravitreal exosomes should be injected in order to have a therapeutic effect (weekly, biweekly, or monthly). Further research is needed to bring these promising new results into the clinic.

3.5. Optic Nerve Axonal Regeneration. Irreversible lesions related to the progression of glaucoma lead to optic nerve atrophy and loss of visual functions up to blindness. It is not necessary to emphasize how important it is to avoid such an eventuality and how much effort and energy research have invested for years to discover therapeutic strategies to be adopted in this context.

One of the most investigated therapeutic approaches is optic nerve transplantation, which can be achieved with a peripheral nerve graft, but currently, the most promising resource seems to be the realization of polymeric membranes. The optic nerve transplantation is not yet ready to be applied in human models, and further studies are needed; anyway, many researchers are now focusing on the development of this topic.

After any kind of optic nerve injury, the regeneration of the optic nerve is blocked by major obstacles. The most considerable ones are the following: apoptosis of RGC, the difficulty in triggering the axonal growth, and the presence

of inhibitory factors in the microenvironment of the central nervous system. Concerning optic nerve regeneration, a promising strategy is the creation of conduits that damaged neurons and axons, which can be utilized as a guide for nerve repair and regeneration; such conduits can be of various kinds, and many studies are now focusing on chitosan, a promising derivative of chitin extracted from shellfish [62–64]. Peripheral nerve grafts demonstrated an effect on the restoration of the pupillary reflex in mice with the damaged optic nerve. In addition, other substrates are under investigation. A peptide nanofiber scaffold has been studied in hamsters, with good recovery of visual function. Negishi et al. [65] applied a silicone tube graft enriched with purified Schwann cells, extracellular matrix, and growth factors in mice subjected to axotomy, showing regeneration of blood vessels, RGC, and axons. Concerning chitosan, studies demonstrated their utility in neural regeneration of either the peripheral or the central nervous system [63]. In order to facilitate neural regeneration, chitosan can be enriched with adhesion molecules, MSCs, and neurotrophic factors. Polyglycolic acid- (PGA-) chitosan scaffolds [66] and cationic chitosan-graft-poly(ε-caprolactone)/polycaprolactone (CS-PCL/PCL) scaffolds have been studied with promising results concerning their potential in stimulation and regeneration of damaged nerve fibers.

Triggering the neuronal growth implies the simultaneous action on different intracellular signals [67]. One of the most interesting pathways of triggering involves the ROCK inhibitors, which have a negative effect on the ROCK signaling cascade (which is itself a negative regulator of neural growth). In addition, alpha-crystallin proteins, which are components of the ocular lens, showed antiapoptotic properties thanks to their structural homology with heat shock proteins with chaperone-like features [68]. Piri et al. [69] demonstrated decreased levels of these proteins in glaucoma models, leading to the thought that down-regulation of these proteins may reduce RGC survival. Other studies [68] supported this theory and further research is ongoing in order to give some clinical application to this interesting finding.

Molecular targets have been investigated in the field of new glaucoma treatment strategies [70]. Death from RGC is related to neurotrophic factor deprivation, hypoxia, excitotoxicity, gene dysregulation, and activation of apoptosis. Therefore, studies have focused on the possibility of enhancing the BDNF-TrkB signal (brain-derived neurotrophic factor-tyrosine protein kinase) and on the chance of pharmacologically modulating TrKB. Endogenous phosphatase Shp2 was also studied, considering its role in regulating TrKB. Recent findings have shown that stress-induced protein aggregation could cause the formation of unfolded proteins in the ER (endoplasmic reticulum) and apoptosis. In order to modulate the equilibrium between the apoptotic and the survival pathways, also proapoptotic Bcl2 and antiapoptotic Bax molecules are under investigation.

Furthermore, many studies focused on the beneficial effect of local, controlled inflammation. Vitreal inflammation induces the activation of retinal astrocytes and Müller cells and the secretion of many glial-derived growth factors, including BDNF, CNTF, leukemia inhibitory factor (LIF), and bone morphogenetic proteins (BMP) [71]. This event promotes neuroprotection of the RGCs and axonal growth by interacting with macrophage-derived factors (MDF), suggesting that immunomodulatory treatments may promote optic nerve regeneration. Intravitreal injections of zymosan and other immunoregulatory molecules, as well as the release of β/γ-crystallins from the injured lens [72], proved a proregenerative effect on the optic nerve. Zymosan is a yeast cell wall carbohydrate and it is a toll-like receptor 2 (TLR-2) ligand, which stimulates the ingress of the macrophages into the vitreous body and the MDF production when injected inside the vitreous [73]. Optic nerve regeneration was also shown using another TLR-2 ligand, the Pam_3Cys, which is a water-soluble bisacyl-lipopeptide and a selective TLR-2 agonist. Its intravitreal application can induce glial activation, transform RGCs into a regenerative state, and stimulate axon regeneration [74].

3.6. Neurotrophic Growth Factors. As previously mentioned, particular attention has been dedicated to oxidative stress and its etiopathogenetic role in glaucoma. In fact, it has been observed that ocular hypertension establishes a stress condition that stimulates oxygen-free radicals production, which harms both directly and indirectly the retinal cells [75]. The use of molecular agents capable of arresting this oxidative burst would therefore be desirable. Currently, only brimonidine seems to possess neuroprotective properties and it has been shown that in patients treated with this substance, there is a slowdown in campimetric damage compared to those treated with timolol [76]. Another promising molecule is citicoline, already approved in Italy, i.e., a molecule previously involved in other neurodegenerative diseases, which could be used as a therapeutic tool in addition to hypotonic pharmacological treatment. Other interesting molecules are EPO (erythropoietin), BDNF, and CNTF, which appear to be involved in the growth and survival of RGCs: they are administered intravitreally, currently, their action is transient, and their use may cause teratogenic ocular effects. The most desirable resource remains gene therapy, which would directly induce endogenous production of these neurotrophic factors without the need to inject them externally [70]. Gene therapy can also take advantage of siRNA and polysaccharide or liposomal nanoparticles that act as vectors for placing a particular gene at a specific site [77]. The approach via viral vectors has already shown promising results [78]. Recent human clinical trials focused their attention mainly on Leber's Hereditary Optic Neuropathy (LHON); nevertheless, many animal studies about other optic neuropathies and RGCs neuroprotection have been conducted. Animal studies showed promising results about regeneration and neuroprotection. Concerning LHON, it has been shown that intravitreal injections of AAV2-ND4 (adeno-associated viruses type 2 carrying NADH dehydrogenase, subunit 4 gene) viral vector are safe and feasible [79]. However, long-term efficacy and risks such as tumors are concerns still to be better considered.

Table 1: Summary of the new treatment strategies under development.

	Anatomic target	Mechanism of action	Expected effect	Advantages	Disadvantages
Electrical stimulation	Trabecular meshwork (TM)	(i) Relaxation of TM, with less resistance to aqueous humor outflow	(i) Significant effect on lowering IOP	(i) Early stages of glaucoma (ii) Facilitates the physiological pathway of the aqueous humor through TM towards Schlemm's canal (SC)	(i) Not in advanced stages of glaucoma (ii) Needs to be repeated
Micropulse cyclophotocoagulation (CPC) and ultrasound cyclomodification	Ciliary body	(i) Decrease the secretory activity of the ciliary epithelium	(i) Same IOP lowering effect as traditional CPC diode	(i) Advanced stages of glaucoma (ii) Minimal heat diffusion (iii) Less pain than traditional CPC	(i) Not in the early stages of glaucoma
Mesenchymal stem cells (MSCs)	Retinal ganglion cells (RGCs)	(i) Differentiation into retinal cell types (RGC) (ii) production of growth factors	(i) Neuroprotective effects	(i) Pluripotency (ii) Ease of extraction (bone marrow, adipose tissue) (iii) Autologous transplantation	(i) Ethical issues
Exosomes	RGCs	(i) Translation of new proteins through mi-RNA-dependent mechanisms (ii) RGC survival and preservation of function	(i) Neuroprotective effects	(i) Easily isolated and purified (ii) Do not proliferate (iii) Easily stored (iv) Able to migrate (v) Immunologically inert	(i) Not clear which dose for a therapeutic effect (weekly, biweekly, or monthly)
Optic nerve scaffolds	Optic nerve	(i) Stimulation and regeneration of damaged nerve fibers	(i) Neural regeneration	(i) Potential restoration of neural function	(i) Obstacles to neural regeneration: apoptosis of RGC, difficulty in triggering the axonal growth, inhibitory factors
ROCK inhibitors	Optic nerve and retinal ganglion cells	(i) Positive regulation of neural growth triggers	(i) Neural growth	(i) Augmented RGC survival	(i) Few studies to support this evidence
Neurotrophic factors (NF)	Optic nerve and retinal ganglion cells	(i) Interaction with macrophage-derived factors with immunomodulation	(i) Neural regeneration and growth	(i) Autologous molecules	(i) Local inflammation is needed to induce secretion of NF (ii) Not selective (iii) Reduced half-life
Alternating current stimulation (ACS)	Visual cortex and neural vision pathways	(i) Weak current pulses delivered to the brain	(i) Improvement of brain excitability and resynchronization of neuronal oscillation	(i) Very advanced glaucoma stages	(i) Few studies to support this evidence
Epiretinal, subretinal, and transchoroidal electrode implants	Optic nerve and RGCs	(i) Weak current pulses delivered to the eye	(i) Improvement of the optic nerve and retinal ganglion cell excitability	(i) Very advanced glaucoma stages (ii) Low stimulation thresholds	(i) Invasive approach (ii) Gliosis over the implant over time (iii) Poor results described in the literature

The use of neurotrophic factors is promising, but limited, as there are not yet adequate techniques to selectively target these molecules at specific sites. Moreover, they may be beneficial for some types of cells, but toxic for others if they reach certain concentration values; they also have a reduced half-life. The solution to these challenges seems to be the design of carrier particles that preserve the neuroprotective molecules and direct them to the desired targets.

3.7. Rehabilitation Therapy. Rehabilitation treatment is a therapeutic method that aims to reeducate the patient to the use of residual vision through repeated visual stimulation. What this technique is based on is the neuronal plasticity of the visual system, as the damaged nerve fibers are able to reorganize themselves and repair the injury by creating new connections or rediscovering existing but little exploited networks. Therefore, if the optic nerve is completely damaged (e.g., very late stages of glaucoma), it is impossible to apply this strategy. An example of neuronal plasticity concerning the visual system is the phenomenon of blindsight, which occurs in some patients suffering from cortical blindness: Weiskrants et al. [80] described it as the capacity of some patients to respond to visual stimuli in the corresponding area of the visual field without perceiving it consciously. This phenomenon may be attributed to the recruitment of subcortical pathways in order to partially compensate for the loss of visual functionality.

Many methods have been studied and tested to improve vision in partially blind patients. These methods include vision training exercises such as computer-based vision restoration therapy [2], retinal implants, and noninvasive brain current stimulation. In the latter case, direct current or alternating current stimulation (ACS) can be used to improve brain excitability or resynchronize neuronal oscillations. ACS uses weak current pulses delivered through electrodes placed on the forehead for some minutes daily, for a period of 10 days on average. Electroencephalography and functional magnetic resonance demonstrated local activation of the visual cortex, reorganization of the neural pathways, and enhanced blood flow in the stimulated area. ACS showed proregenerative effects in controlled trials in patients with glaucoma and optic neuropathy.

The process of physiological plasticity can be enhanced by neurorehabilitation cycles, which can be further supported by the utilization of neurotrophic factors.

Other technologies indicated in this field are epiretinal electrode implants, which stimulate RGCs, subretinal electrodes, transchoroidal implants, devices acting on the optic nerve, and cortical implants, which target the brain areas responsible for vision. The latter could be the most suitable therapeutic strategy to target the latest stages of glaucoma, where no function of the optic nerve is left [81, 82]. However, although the results are promising, the ultimate goal of restoring good vision is still almost a mirage, and several limitations and problems still have to be overcome in order to give these new technologies a clinical application. Regarding epiretinal implants, all the studies described left the patients far below the limit of legal blindness (20/200) and all the stimuli were unable to maintain specific retinotopy. Moreover, this is an invasive approach, highly anatomically destructive, and without the possibility of recovery in case of device failure. Subretinal implants, when compared to the previous ones, are more stable as they are implanted beneath the retina, they do not require connection to external devices, and their stimulation thresholds are lower. Nevertheless, this technology is still invasive and provides a minimal or no visual recovery. Transchoroidal implants are less invasive but require a higher stimulation. The possibility of acting directly on the optic nerve should theoretically allow stimulating both the central and peripheral visual field with a lower intensity of the stimuli and lower invasiveness since the electrodes are localized in a smaller area. Several kinds of implants have been proposed [83], which permitted the perception of light and spatial orientation in a small number of cases. However, it has been shown that with time the intensity of the stimuli required becomes higher, and this is probably due to the development of gliosis surrounding the implant after time. Finally, cortical implants showed encouraging results in the study of Dobelle [84], but in order to decrease the intensity of stimulation thresholds, penetrative cortical implants should be used, which would lead to higher invasiveness, risk of infection, and inflammation with reactive gliosis and neuronal death.

Thus, subsequent studies are needed to fully understand the mechanisms that are implied and to refine these devices.

4. Conclusions

Glaucoma is an increasingly widespread social disease and many advances have been achieved to improve the diagnostic and therapeutic resources available (Table 1). However, new options need to be further enhanced and supported by significant experimental data on the biological responses of intraocular and brain tissues, in particular trabecular cells, RGCs, retinal fibers, and optical pathways. The union between clinic, biology, and biotechnology and their synchronous enforcement appears to be the winning strategy to defeat the "silent thief of sight": the challenge is still open.

Data Availability

All necessary data are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

References

[1] H. A. Quigley and A. T. Broman, "The number of people with glaucoma worldwide in 2010 and 2020," *British Journal of Ophthalmology*, vol. 90, no. 3, pp. 262–267, 2006.

[2] R. R. A. Bourne, G. A. Stevens, R. A. White et al., "Causes of vision loss worldwide, 1990–2010: a systematic analysis," *Lancet Global Health*, vol. 1, no. 6, pp. e339–e349, 2013.

[3] J. T. Whitson, "Glaucoma: a review of adjunctive therapy and new management strategies," *Expert Opinion on Pharmacotherapy*, vol. 8, no. 18, pp. 3237–3249, 2007.

[4] H. A. Quigley, "Glaucoma," *The Lancet*, vol. 377, no. 9774, pp. 1367–1377, 2011.

[5] L. Quaranta, I. Riva, C. Gerardi, F. Oddone, I. Floriano, and A. G. P. Konstas, "Quality of life in glaucoma: a review of the literature," *Advances in Therapy*, vol. 33, no. 6, pp. 959–981, 2016.

[6] R. Nuzzi and F. Tridico, "Glaucoma: biological trabecular and neuroretinal pathology with perspectives of therapy innovation and preventive diagnosis," *Frontiers in Neuroscience*, vol. 11, p. 494, 2017.

[7] A. K. Sawchyn and M. A. Slabaugh, "Innovations and adaptations in trabeculectomy," *Current Opinion in Ophthalmology*, vol. 27, no. 2, pp. 158–163, 2016.

[8] N. Gupta and Y. H. Yücel, "Glaucoma as a neurodegenerative disease," *Current Opinion in Ophthalmology*, vol. 18, no. 2, pp. 110–114, 2007.

[9] X. S. Mi, T. F. Yuan, and K. F. So, "The current research status of normal tension glaucoma," *Clinical Interventions in Aging*, vol. 9, pp. 1563–1571, 2014.

[10] H. E. Killer and A. Pircher, "Normal tension glaucoma: review of current understanding and mechanisms of the pathogenesis," *Eye*, vol. 32, no. 5, pp. 924–930, 2018.

[11] F. C. Hollows and P. A. Graham, "Intra-ocular pressure, glaucoma, and glaucoma suspects in a defined population," *British Journal of Ophthalmology*, vol. 50, no. 10, pp. 570–586, 1966.

[12] M. C. Leske, "The epidemiology of open-angle glaucoma: a review," *American Journal of Epidemiology*, vol. 118, no. 2, pp. 166–191, 1983.

[13] K. Grødum, A. Heijl, and B. Bengtsson, "A comparison of glaucoma patients identified through mass screening and in routine clinical practice," *Acta Ophthalmologica Scandinavica*, vol. 80, no. 6, pp. 627–631, 2002.

[14] H. A. Quigley, R. W. Nickells, L. A. Kerrigan, M. E. Pease, D. J. Thibault, and D. J. Zack, "Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis," *Investigative Ophthalmology & Visual Science*, vol. 36, no. 5, pp. 774–786, 1995, https://pubmed.ncbi.nlm.nih.gov/7706025/.

[15] L. Guo, T. E. Salt, A. Maass et al., "Assessment of neuroprotective effects of glutamate modulation on glaucoma-related retinal ganglion cell apoptosis in vivo," *Investigative Opthalmology & Visual Science*, vol. 47, no. 2, pp. 626–633, 2006.

[16] R. Nuzzi, P. Marolo, A. Nuzzi et al., "The hub-and-spoke management of glaucoma," *Frontiers in Neuroscience*, vol. 14, no. 80, 2020.

[17] A. Liberati, D. G. Altman, J. Tetzlaff et al., "The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration," *BMJ*, vol. 339, p. b2700, 2009.

[18] J. Chen, S. A. Runyan, and M. R. Robinson, "Novel ocular antihypertensive compounds in clinical trials," *Clinical Ophthalmology*, vol. 5, no. 1, pp. 667–677, 2011.

[19] H. Tokushige, M. Inatani, S. Nemoto et al., "Effects of topical administration of Y-39983, a selective Rho-associated protein kinase inhibitor, on ocular tissues in rabbits and monkeys," *Investigative Opthalmology & Visual Science*, vol. 48, no. 7, pp. 3216–3222, 2007.

[20] S. Van de Velde, T. Van Bergen, D. Sijnave et al., "AMA0076, a novel, locally acting rho kinase inhibitor, potently lowers intraocular pressure in New Zealand white rabbits with minimal hyperemia," *Investigative Opthalmology & Visual Science*, vol. 55, no. 2, pp. 1006–1016, 2014.

[21] S. Van de Velde, L. De Groef, I. Stalmans, L. Moons, and I. Van Hove, "Towards axonal regeneration and neuroprotection in glaucoma: Rho kinase inhibitors as promising therapeutics," *Progress in Neurobiology*, vol. 131, pp. 105–119, 2015.

[22] S. Rösch, R. Ramer, K. Brune, and B. Hinz, "R(+)-methanandamide and other cannabinoids induce the expression of cyclooxygenase-2 and matrix metalloproteinases in human nonpigmented ciliary epithelial cells," *Journal of Pharmacology and Experimental Therapeutics*, vol. 316, no. 3, pp. 1219–1228, 2006.

[23] E. A. Cairns, W. H. Baldridge, and M. E. M. Kelly, "The endocannabinoid system as a therapeutic target in glaucoma," *Neural Plasticity*, vol. 2016, Article ID 9364091, 1 page, 2016.

[24] C. Rapino, D. Tortolani, L. Scipioni, and M. Maccarrone, "Neuroprotection by (endo) Cannabinoids in glaucoma and retinal neurodegenerative diseases," *Current Neuropharmacology*, vol. 16, no. 7, pp. 959–970, 2018.

[25] Y. Zhong, Z. Yang, W.-C. Huang, and X. Luo, "Adenosine, adenosine receptors and glaucoma: an updated overview," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 1830, no. 4, pp. 2882–2890, 2013.

[26] K. A. Erickson, A. Schroeder, and P. A. Netland, "Verapamil increases outflow facility in the human eye," *Experimental Eye Research*, vol. 61, no. 5, pp. 565–567, 1995.

[27] N. Peppas, P. Bures, W. Leobandung, and H. Ichikawa, "Hydrogels in pharmaceutical formulations," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 50, no. 1, pp. 27–46, 2000.

[28] E. Lavik, M. H. Kuehn, and Y. H. Kwon, "Novel drug delivery systems for glaucoma," *Eye*, vol. 25, no. 5, pp. 578–586, 2011.

[29] S. Saati, R. Lo, P.-Y. Li, E. Meng, R. Varma, and M. S. Humayun, "Mini drug pump for ophthalmic use," *Current Eye Research*, vol. 35, no. 3, pp. 192–201, 2010.

[30] C.-H. Lee, Y.-J. Li, C.-C. Huang, and J.-Y. Lai, "Poly (ε-caprolactone) nanocapsule carriers with sustained drug release: single dose for long-term glaucoma treatment," *Nanoscale*, vol. 9, no. 32, pp. 11754–11764, 2017.

[31] H. A. Quigley, "21st century glaucoma care," *Eye*, vol. 33, no. 2, pp. 254–260, 2019.

[32] S. Sivaprasad, M. Elagouz, D. McHugh, O. Shona, and G. Dorin, "Micropulsed diode laser therapy: evolution and clinical applications," *Survey of Ophthalmology*, vol. 55, no. 6, pp. 516–530, 2010.

[33] S. Wilmsmeyer, H. Philippin, and J. Funk, "Excimer laser trabeculotomy: a new, minimally invasive procedure for patients with glaucoma," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 244, no. 6, pp. 670–676, 2006.

[34] P. Agrawal and S. E. Bradshaw, "Systematic literature review of clinical and economic outcomes of micro-invasive glaucoma surgery (MIGS) in primary open-angle glaucoma," *Ophthalmology and Therapy*, vol. 7, no. 1, pp. 49–73, 2018.

[35] F. Gil-Carrasco, D. Ochoa-Contreras, M. A. Torres et al., "Transpalpebral electrical stimulation as a novel therapeutic approach to decrease intraocular pressure for open-angle glaucoma: a pilot study," *Journal of Ophthalmology*, vol. 2018, Article ID 2930519, 1 page, 2018.

[36] W. D. Stamer and T. S. Acott, "Current understanding of conventional outflow dysfunction in glaucoma," *Current Opinion in Ophthalmology*, vol. 23, no. 2, pp. 135–143, 2012.

[37] L. Fu, F. Fung, A. C.-Y. Lo et al., "Transcorneal electrical stimulation inhibits retinal microglial activation and enhances retinal ganglion cell survival after acute ocular hypertensive injury," *Translational Vision Science & Technology*, vol. 7, no. 3, p. 2, 2018.

[38] A. M. Tan, M. Chockalingam, M. C. Aquino, Z. I. L. Lim, J. L. S. See, and P. T. Chew, "Micropulse transscleral diode laser cyclophotocoagulation in the treatment of refractory glaucoma," *Clinical & Experimental Ophthalmology*, vol. 38, no. 3, pp. 266–272, 2010.

[39] R. H. Silverman, B. Vogelsang, M. J. Rondeau, and D. J. Coleman, "Therapeutic ultrasound for the treatment of glaucoma," *American Journal of Ophthalmology*, vol. 111, no. 3, pp. 327–337, 1991.

[40] F. Aptel, T. Charrel, C. Lafon et al., "Miniaturized high-intensity focused ultrasound device in patients with glaucoma: a clinical pilot study," *Investigative Opthalmology & Visual Science*, vol. 52, no. 12, pp. 8747–8753, 2011.

[41] N. Yildirim, I. S. Yalvac, A. Sahin, A. Ozer, and T. Bozca, "A comparative study between diode laser cyclophotocoagulation and the Ahmed glaucoma valve implant in neovascular glaucoma: a long-term follow-up," *Journal of Glaucoma*, vol. 18, no. 3, pp. 192–196, 2009.

[42] K. Kramp, H.-P. Vick, and R. Guthoff, "Transscleral diode laser contact cyclophotocoagulation in the treatment of different glaucomas, also as primary surgery," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 240, no. 9, pp. 698–703, 2002.

[43] X. Chamling, V. M. Sluch, and D. J. Zack, "The potential of human stem cells for the study and treatment of glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 57, no. 5, 2016.

[44] G. Pellegrini, C. E. Traverso, A. T. Franzi, M. Zingirian, R. Cancedda, and M. De Luca, "Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium," *The Lancet*, vol. 349, no. 9057, pp. 990–993, 1997.

[45] M. J. Kelley, A. Y. Rose, K. E. Keller, H. Hessle, J. R. Samples, and T. S. Acott, "Stem cells in the trabecular meshwork: present and future promises," *Experimental Eye Research*, vol. 88, no. 4, pp. 747–751, 2009.

[46] W. Y. Yu, C. Sheridan, I. Grierson et al., "Progenitors for the corneal endothelium and trabecular meshwork: a potential source for personalized stem cell therapy in corneal endothelial diseases and glaucoma," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 412743, 1 page, 2011.

[47] D. I. Bettis, J. J. Whitehead, P. Farhi, and N. A. Zabriskie, "Intraocular pressure spike and corneal decompensation following selective laser trabeculoplasty in patients with exfoliation glaucoma," *Journal of Glaucoma*, vol. 25, no. 4, pp. e433–e437, 2016.

[48] S. Parameswaran, S. Balasubramanian, N. Babai et al., "Induced pluripotent stem cells generate both retinal ganglion cells and photoreceptors: therapeutic implications in degenerative changes in glaucoma and age-related macular degeneration," *Stem Cells*, vol. 28, no. 4, pp. 695–703, 2010.

[49] S. G. Giannelli, G. C. Demontis, G. Pertile, P. Rama, and V. Broccoli, "Adult human müller glia cells are a highly efficient source of rod photoreceptors," *Stem Cells*, vol. 29, no. 2, pp. 344–356, 2011.

[50] J. James, A. V. Das, S. Bhattacharya, D. M. Chacko, X. Zhao, and I. Ahmad, "In vitro generation of early-born neurons from late retinal progenitors," *The Journal of Neuroscience*, vol. 23, no. 23, pp. 8193–8203, 2003.

[51] D. M. Chacko, J. A. Rogers, J. E. Turner, and I. Ahmad, "Survival and differentiation of cultured retinal progenitors transplanted in the subretinal space of the rat," *Biochemical and Biophysical Research Communications*, vol. 268, no. 3, pp. 842–846, 2000.

[52] S. Yu, T. Tanabe, M. Dezawa, H. Ishikawa, and N. Yoshimura, "Effects of bone marrow stromal cell injection in an experimental glaucoma model," *Biochemical and Biophysical Research Communications*, vol. 344, no. 4, pp. 1071–1079, 2006.

[53] T. V. Johnson, N. D. Bull, D. P. Hunt, N. Marina, S. I. Tomarev, and K. R. Martin, "Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma," *Investigative Opthalmology & Visual Science*, vol. 51, no. 4, pp. 2051–2059, 2010.

[54] Y. Li, J. Chen, X. G. Chen et al., "Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery," *Neurology*, vol. 59, no. 4, pp. 514–523, 2002.

[55] P. Connick, M. Kolappan, C. Crawley et al., "Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study," *The Lancet Neurology*, vol. 11, no. 2, pp. 150–156, 2012.

[56] B. Mead, A. Logan, M. Berry, W. Leadbeater, and B. A. Scheven, "Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells," *PLoS One*, vol. 9, no. 10, p. e109305, 2014.

[57] H.-S. Kim, D.-Y. Choi, S. J. Yun et al., "Proteomic analysis of microvesicles derived from human mesenchymal stem cells," *Journal of Proteome Research*, vol. 11, no. 2, pp. 839–849, 2012.

[58] T. S. Chen, R. C. Lai, M. M. Lee, A. B. H. Choo, C. N. Lee, and S. K. Lim, "Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs," *Nucleic Acids Research*, vol. 38, no. 1, pp. 215–224, 2010.

[59] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, and J. O. Lötvall, "Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells," *Nature Cell Biology*, vol. 9, no. 6, pp. 654–659, 2007.

[60] W. Heusermann, J. Hean, D. Trojer et al., "Exosomes surf on filopodia to enter cells at endocytic hot spots, traffic within endosomes, and are targeted to the ER," *Journal of Cell Biology*, vol. 213, no. 2, pp. 173–184, 2016.

[61] B. Mead and S. Tomarev, "Bone marrow-derived mesenchymal stem cells-derived exosomes promote survival of retinal ganglion cells through mirna-dependent mechanisms," *Stem Cells Translational Medicine*, vol. 6, no. 4, pp. 1273–1285, 2017.

[62] X. Chen, Y. Yang, J. Yao et al., "Bone marrow stromal cells-loaded chitosan conduits promote repair of complete transection injury in rat spinal cord," *Journal of Materials Science: Materials in Medicine*, vol. 22, no. 10, pp. 2347–2356, 2011.

[63] S. Gnavi, C. Barwig, T. Freier, K. Haastert-Talini, C. Grothe, and S. Geuna, "The use of chitosan-based scaffolds to enhance regeneration in the nervous system," *International Review of Neurobiology*, vol. 109, pp. 1–62, 2013.

[64] C. Meyer, L. Stenberg, F. Gonzalez-Perez et al., "Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves," *Biomaterials*, vol. 76, pp. 33–51, 2016.

[65] H. Negishi, M. Dezawa, T. Oshitari, and E. Adachi-Usami, "Optic nerve regeneration within artificial Schwann cell graft

in the adult rat," *Brain Research Bulletin*, vol. 55, no. 3, pp. 409–419, 2001.

[66] G. Xu, D.-Y. Nie, W.-Z. Wang et al., "Optic nerve regeneration in polyglycolic acid-chitosan conduits coated with recombinant L1-Fc," *Neuroreport*, vol. 15, no. 14, pp. 2167–2172, 2004.

[67] D. Fischer and M. Leibinger, "Promoting optic nerve regeneration," *Progress in Retinal and Eye Research*, vol. 31, no. 6, pp. 688–701, 2012.

[68] J.-P. Liu, R. Schlosser, W.-Y. Ma et al., "Human alphaA- and alphaB-crystallins prevent UVA-induced apoptosis through regulation of PKCalpha, RAF/MEK/ERK and AKT signaling pathways," *Experimental Eye Research*, vol. 79, no. 3, pp. 393–403, 2004.

[69] N. Piri, M. Song, J. M. K. Kwong, and J. Caprioli, "Modulation of alpha and beta crystallin expression in rat retinas with ocular hypertension-induced ganglion cell degeneration," *Brain Research*, vol. 1141, no. 1, pp. 1–9, 2007.

[70] N. Chitranshi, Y. Dheer, M. Abbasi, Y. You, S. L. Graham, and V. Gupta, "Glaucoma pathogenesis and neurotrophins: focus on the molecular and genetic basis for therapeutic prospects," *Current Neuropharmacology*, vol. 16, no. 7, pp. 1018–1035, 2018.

[71] T. Harada, C. Harada, S. Kohsaka et al., "Microglia-müller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration," *The Journal of Neuroscience*, vol. 22, no. 21, pp. 9228–9236, 2002.

[72] D. Fischer, P. Heiduschka, and S. Thanos, "Lens-injury-stimulated axonal regeneration throughout the optic pathway of adult rats," *Experimental Neurology*, vol. 172, no. 2, pp. 257–272, 2001.

[73] Y. Yin, Q. Cui, Y. Li et al., "Macrophage-derived factors stimulate optic nerve regeneration," *The Journal of Neuroscience*, vol. 23, no. 6, pp. 2284–2293, 2003.

[74] T. G. Hauk, M. Leibinger, A. Müller, A. Andreadaki, U. Knippschild, and D. Fischer, "Stimulation of axon regeneration in the mature optic nerve by intravitreal application of the toll-like receptor 2 agonist Pam3Cys," *Investigative Opthalmology & Visual Science*, vol. 51, no. 1, pp. 459–464, 2010.

[75] C. McMonnies, "Especies reactivas de oxígeno, estrés oxidativo, glaucoma y terapia de oxígeno hiperbárico," *Journal of Optometry*, vol. 11, no. 1, pp. 3–9, 2018.

[76] T. Krupin, J. M. Liebmann, D. S. Greenfield, R. Ritch, and S. Gardiner, "A randomized trial of brimonidine versus timolol in preserving visual function: results from the low-pressure glaucoma treatment study," *American Journal of Ophthalmology*, vol. 151, no. 4, pp. 671–681, 2011.

[77] A. Guzman-Aranguez, P. Loma, and J. Pintor, "Small-interfering RNAs (siRNAs) as a promising tool for ocular therapy," *British Journal of Pharmacology*, vol. 170, no. 4, pp. 730–747, 2013.

[78] A. DeBusk and M. L. Moster, "Gene therapy in optic nerve disease," *Current Opinion in Ophthalmology*, vol. 29, no. 3, pp. 234–238, 2018.

[79] X. Wan, H. Pei, M.-J. Zhao et al., "Efficacy and safety of rAAV2-ND4 treatment for Leber's hereditary optic neuropathy," *Scientific Reports*, vol. 6, no. 1, 2016.

[80] L. Weiskrants, E. K. Warrington, M. D. Sanders, and J. Marshall, "Visual capacity in the hemianoptic field following a restricted occipital ablation," *Brain*, vol. 97, no. 4, pp. 709–728, 1974.

[81] H. Lorach, O. Marre, J.-A. Sahel, R. Benosman, and S. Picaud, "Neural stimulation for visual rehabilitation: advances and challenges," *Journal of Physiology-Paris*, vol. 107, no. 5, pp. 421–431, 2013.

[82] A. Sehic, S. Guo, K.-S. Cho, R. M. Corraya, D. F. Chen, and T. P. Utheim, "Electrical stimulation as a means for improving vision," *The American Journal of Pathology*, vol. 186, no. 11, pp. 2783–2797, 2016.

[83] M. E. Brelén, V. Vince, B. Gérard, C. Veraart, and J. Delbeke, "Measurement of evoked potentials after electrical stimulation of the human optic nerve," *Investigative Opthalmology & Visual Science*, vol. 51, no. 10, pp. 5351–5355, 2010.

[84] W. H. Dobelle, "Artificial vision for the blind by connecting a television camera to the visual cortex," *ASAIO Journal*, vol. 46, no. 1, pp. 3–9, 2000.

Hindawi
Journal of Ophthalmology
Volume 2021, Article ID 5826361, 9 pages
https://doi.org/10.1155/2021/5826361

Research Article

Enhanced Physiological Stress Response in Patients with Normal Tension Glaucoma during Hypoxia

Line Marie Dalgaard[1,2], **Jeppe Vibæk**[1,2], **Rupali Vohra**[1,3], **Lars Thorbjørn Jensen**[4], **Barbara Cvenkel**[5], **Niels H. Secher**[6], **Niels Vidiendal Olsen**[2,7] and **Miriam Kolko**[1,8]

[1]*Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark*
[2]*Department of Neuroanaesthesia, The Neuroscience Centre, Copenhagen University Hospital, Rigshospitalet-Glostrup, Copenhagen, Denmark*
[3]*Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark*
[4]*Department of Clinical Physiology and Nuclear Medicine, University Hospital of Herlev, Herlev, Denmark*
[5]*Department of Ophthalmology, University Medical Centre Ljubljana, Ljubljana, Slovenia*
[6]*Department of Anaesthesia, Copenhagen University Hospital, Rigshospitalet-Glostrup, Copenhagen, Denmark*
[7]*Department of Biomedical Science, University of Copenhagen, Copenhagen, Denmark*
[8]*Department of Ophthalmology, Copenhagen University Hospital, Rigshospitalet-Glostrup, Copenhagen, Denmark*

Correspondence should be addressed to Miriam Kolko; miriamk@sund.ku.dk

Received 20 July 2020; Revised 17 March 2021; Accepted 25 March 2021; Published 8 April 2021

Academic Editor: Biju B. Thomas

Purpose. To investigate whether patients with normal tension glaucoma (NTG) show an enhanced stress response to reduced oxygen supply compared to age-matched healthy controls, measured by serum adrenaline and endothelin-1 (ET-1) levels and changes in distal finger temperature. *Methods.* A thorough clinical characterization of patients with NTG and age-matched controls was performed prior to inclusion in the study. Twelve patients with NTG and eleven healthy controls met the inclusion criteria and were enrolled in the study. All subjects underwent a two-day investigation. Participants were randomly exposed to either hypoxia or normoxia during the first visit. Hypoxia or normoxia was induced for two hours through a tightly fitting face mask. In addition, the peripheral circulation was assessed with a thermographic camera. Blood samples were obtained before, during, and after hypoxia or normoxia to evaluate systemic stress molecules such as catecholamines and ET-1 levels. *Results.* In patients with NTG, reduced oxygen supply induced an increase in peripheral blood adrenaline ($p < 0.05$) and a decrease during recovery ($p < 0.01$). A difference in distal finger temperature was shown in patients with NTG under hypoxia compared to normoxia (exposure: $p < 0.05$; recovery: $p < 0.05$). Hypoxia induced an increase in peripheral blood ET-1 levels in both groups (NTG: $p < 0.01$; controls: $p < 0.05$). *Conclusion.* Patients with NTG had an enhanced physiological stress response as a consequence of hypoxia compared with age-matched controls. Although more studies are needed, the present study supports the involvement of vascular risk factors in the pathophysiology of NTG.

1. Introduction

Glaucoma is a progressive optic neuropathy characterized by loss of retinal ganglion cells (RGC) and their axons. Along with the loss of RGC, the disease is accompanied by a gradual loss of the peripheral visual field [1–4]. Glaucoma is the most frequent cause of incurable blindness and is estimated to affect approximately 111.8 million people by 2040 [5]. Together with aging, increased intraocular pressure (IOP) is recognized as the most important risk factor. However, patients may have an IOP in the normal range and still develop glaucomatous progression (normal tension glaucoma, NTG) [6]. Therefore, although the only existing treatments for glaucoma are IOP-lowering strategies, glaucoma is a multifactorial disease with many different risk factors [7]. Thus, identifying and characterizing other instigators are essential.

An increasing number of studies have suggested that glaucoma is a systemic disease that manifests in the inner retina, resulting in loss of RGC. The reason for this particular RGC vulnerability is the fact that these cells are especially dependent on a constant oxygen sand energy supply [8–12].

Consistent with this hypothesis, an increasing number of studies have shown that there is an association between vascular dysfunction and NTG [8–10, 12–14]. Vascular dysfunction is therefore thought to be an important risk factor for onset and progression of NTG [8–12, 15, 16].

Vascular dysfunction is defined as a condition in which the actual blood flow does not meet the requirements of the tissue for oxygen supply [13, 17]. This can result in overperfusion or underperfusion, which is basically due to an imbalance in the relationship between molecular vasoconstrictors and vasodilators. Dysregulated vascular constriction or dilation of the retinal blood flow may inevitably cause periods of hypo- and hyperperfusion [18–20], further escalating to hypoxic events and oxidative stress. As a result, such a cascade of reactions may contribute to glaucomatous neurodegeneration [11, 12, 21–25]. The body stabilizes blood flow by a highly regulated release of vasoconstrictive and vasodilative molecules [8–11]. This tight regulation of the vessel dynamics is termed autoregulation [18] and maintains flow by altering vessel diameter in response to changes in perfusion pressure [8, 12, 13]. Multiple factors influence autoregulation, such as CO_2 levels, temperature, low grade inflammatory molecules, catecholamines, and ATP production [8, 9, 13, 15, 26]. Many of these variables are influenced by hypoxia and have been explored in animal experimental models of glaucoma [27–29]. However, to our knowledge, the relationship between decreased oxygen supply and these molecular changes has not been studied in patients with glaucoma.

Thus, the present study aimed to provide novel insight into systemic effect and molecular changes in response to reduced oxygen supply in patients with glaucomatous neurodegeneration compared to controls. Since we assume that all patients with glaucoma have multiple risk factors and since we were particularly interested in studying IOP-independent factors, we examined glaucoma patients with IOP within the normal range, where IOP is hypothetically a less significant risk factor. We thus examined patients with NTG and compared their response to systemic hypoxia with age-matched control subjects. We have previously shown that hypoxia reduces oxygen saturation levels in healthy test subjects and in patients with NTG within six minutes when they breathe 10 % oxygen [30]. With this human experimental model, hypoxia is used as a universal oxygen stress model. In the present study, we aimed to investigate whether patients with NTG have an enhanced stress response compared to healthy controls when exposed to reduced oxygen availability, including measuring various vascular parameters such as serum levels of catecholamines and ET-1 as well as distal finger temperature.

2. Methods

A total of 23 eligible test subjects participated in the study between May 2015 and August 2016 [30]. The test subjects were assigned into two different groups: patients with NTG (12 participants) and age-matched healthy controls (11 participants) (Table 1 and Figure 1). Power and sample size calculations were based on an arterial vessel diameter from a previous study by Wong et al. [31]. In this study, the mean arterial vessel diameter was found to be 204.4 μm with a standard deviation of 18.6. With a power of 80%, a p value of 0.05, and an allowed variation in diameter of 12%, a total of 9 individuals were required in each group. Other published works that required gas inhalation of volunteers have used similar sample size to that in this study [32]. All participants were ≥50 years old and nonsmokers. Patients with NTG were recruited by a glaucoma specialist through the Department of Ophthalmology at Zealand University Hospital, Roskilde. Control subjects were recruited via opticians to the study "Detecting Visual Fields Defects With Damato Multifixation Campimetry Online" [33] and were invited to participate in the present study.

This interventional case-control study was performed in compliance with the Declaration of Helsinki approved by the National Committee on Health Research Ethics (ethical protocol: H-2-2014-060). All participants received written information about the study and had the study verbally explained and provided both oral and written consent prior to participation. Inclusion criteria and exclusion criteria are summarized in Tables 2 and 3.

All subjects underwent two days of investigation. In random order, the visits included either normobaric hypoxia or normobaric normoxia. Hypoxia/normoxia was induced for two hours through a tight fitting face mask. The mask was connected via a Y-piece to a Douglas bag which was filled with either atmospheric air or a mixture of 10% oxygen and 90% nitrogen. We chose two hours of hypoxia induction to ensure a sustained effect on the cardiovascular system. This has been shown to be evident two hours after initial exposure [34]. Furthermore, two hours of hypoxia has previously been used to investigate endothelial function in healthy adults [35]. Previous studies have verified that the acute effect of hypoxia is abolished after 15 min, and we added a safety margin of further 15 min [36], resulting in a defined recovery period of 30 min after terminated hypoxia.

To ensure the safety of our participants, they were continuously monitored on both days of investigations with a three-lead ECG (M1166A model 66S, Hewlett Packard, Palo Alto, California, USA) and noninvasive blood-pressure measurement and pulse oximeter by a Nexfin monitor (BMEYE B.V., Amsterdam, Netherlands). We also encouraged our participants to let us know if they felt any discomfort. As an additional safety measure, the investigations were carried out at the Department of Anesthesiology, Rigshospitalet, Denmark, where we had anesthesiologists on call.

The two days of investigation were at least three weeks apart. All investigations were preceded by 12 hours of fasting.

2.1. Blood Samples. Blood samples were collected from a peripheral vein on both days of investigation before, during,

Table 1: Characteristics of participants.

	NTG	Controls	p value
Age	70.3 ± SEM 1.5	65.6 ± SEM 2.3	0.09
Gender	Female: 6 (50%) Male: 6 (50%)	Female: 5 (45%) Male: 6 (55%)	0.83
BMI	23.6 ± SEM 1.0	25.8 ± SEM 0.8	0.11

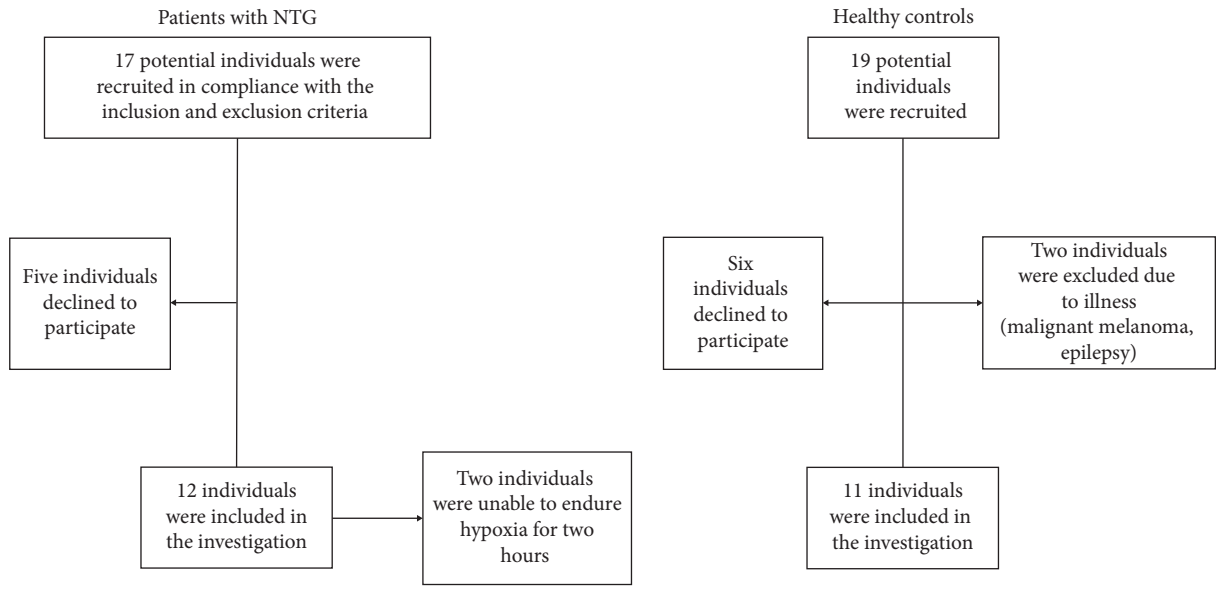


Figure 1: An overview of the inclusion of participants. A total of 12 patients with NTG were included, 10 of whom completed the study, in addition to 11 healthy controls. Excluded subjects did not meet the inclusion or did not want to participate.

Table 2: Inclusion criteria for patients with NTG.

Untreated intraocular pressure (IOP) never detected higher than 21 mmHg measured at different times of the day (8 AM–5 PM)
Open anterior chamber angles observed by gonioscopy
Glaucomatous cupping characterized by a violated ISNT rule (that normal eyes show a characteristic configuration for disc rim thickness of inferior ≥ superior ≥ nasal ≥ temporal)
Glaucomatous visual field loss by Humphrey perimetry or Octopus perimetry

Table 3: Exclusion criteria for patients with NTG and healthy age-matched controls.

Medical history including ocular trauma or eye conditions other than glaucoma involving the optic nerve
Significant systemic disease, e.g., hypertension, heart failure, hypercholesterolemia, diabetes mellitus, autoimmune diseases, and previous cerebral infract or bleeding
Individuals who were unable to cooperate during examination
Individuals below the age of 50 years
Individuals who smoke

and after hypoxia/normoxia as shown in Figure 2. At each collection, 3 × 6 mL EDTA glasses were taken and put on ice. The glasses were centrifuged for 10 minutes at 4000 rpm, after which the supernatant was pipetted to Eppendorf tubes and frozen at −80°C until further analyses.

2.1.1. Catecholamine Analysis. Catecholamine analyses were performed on serum from EDTA tubes at the Department of Clinical Biochemistry, Biolab, Rigshospitalet-Glostrup, Denmark, using a 2-CAT RIA kit according to the manufacturer's protocol (BA R-6500, Labor Diagnostika Nord GmbH & Co.KG, Nordhorn, Germany).

2.1.2. Endothelin-1 Analysis. ET-1 levels were determined using an ET-1 ELISA kit (ab133030, Abcam, Cambridge, United Kingdom) following the manufacturer's protocol. Serum from EDTA tubes was collected from the −80°C freezer, thawed on ice, and extracted prior to analysis through Sep-Pak columns (Sep-Pak Vac C18 3cc, Waters Corporation, Milford, Massachusetts, USA) according to the

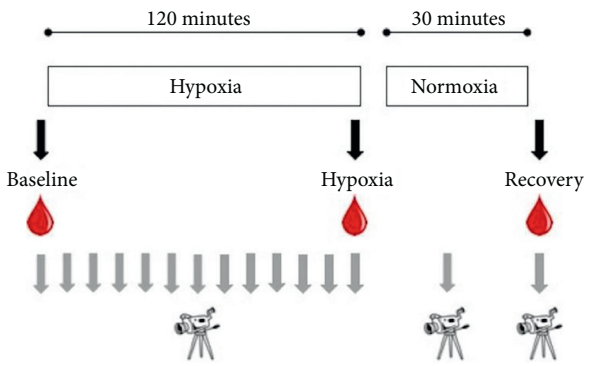


Figure 2: Blood samples were collected before, during, and after hypoxia. Thermographic images were obtained every tenth minute during hypoxia as well as 15 and 30 minutes after hypoxia.

manufacturer's protocol (ab133030, Abcam, Cambridge, United Kingdom). Nitrogen evaporation was used instead of a centrifugal concentrator under vacuum. The dried samples were stored at −20°C and reconstituted using 500 μL of Assay buffer.

2.2. Thermographic Imaging. Thermographic imaging was carried out using the FLIR SC660 camera (FLIR Systems Inc., Wilsonville, Oregon, USA). Thermographic pictures of the dorsum of the left hand including all fingers were taken every tenth minute throughout the two hours of hypoxia/normoxia. In addition, a picture was taken 15 minutes and 30 minutes after the end of gas inhalation. The thermographic camera was set in a fixed position approximately one meter above the hand of the subject.

All investigations were performed in the same temperature-controlled room (22.5–23.5°C). In order to eliminate the influence of changing outside temperatures as well as possible Raynaud's phenomenon, both days of investigations were preceded by 30 minutes of preparations within the temperature-controlled room.

The thermographic pictures were analyzed using the FLIR software in which a circle was added proximally to the cuticles of each finger (Figure 3) from which the average temperature was retrieved from the software. An average of the temperatures from the five fingers was set to the temperature of the hand at the according time.

2.3. Statistics. Statistical analyses were calculated using GraphPad software (GraphPad Prism version 7.0). Comparisons were analyzed by two-way ANOVA for comparing differences between baseline, hypoxia, and recovery among the two groups. One-way ANOVA with repeated measures taking pairing into account was used for comparing differences in time points within only one group (patients with NTG or age-matched healthy controls). In all analyses, $p < 0.05$ was considered statistically significant. Quantitative results are expressed as means ± SEM.

3. Results

3.1. Sustained Hypoxia in Patients with NTG and Healthy Controls. As we have previously reported, a significant decrease in pO_2 and saturation during hypoxia was seen in both NTG patients and healthy controls leading to sustained hypoxia in both groups within 6 minutes of breathing 10% oxygen. There were no significant differences between the two groups at any time. We found no significant differences or changes in blood pressure either in the same group or between groups. There were no significant differences between the two groups at any time. We found no significant differences or changes in blood pressure either in the same group or between groups [30].

3.2. Hypoxia Induces an Increase in Adrenaline in Peripheral Blood in Patients with NTG. Peripheral blood adrenaline levels increased during hypoxia in both groups but only significantly in patients with NTG (to 151.3% ± 19.2 during hypoxia ($p < 0.05$) (Figure 4). Likewise, a decrease during recovery was seen in both groups, but the decrease was only significant in patients with NTG (to 89.3 % ± 9.5 during recovery, $p < 0.01$). There were no significant differences in peripheral adrenaline levels between the two groups during baseline, hypoxia, or recovery (Figure 4(a)). No significant changes or differences were seen for peripheral noradrenaline levels (Figure 4(b)).

3.3. Hypoxia Induces Elevated Temperature in the Distal Finger of Patients with NTG. When comparing the two days of investigation (hypoxia versus normoxia), patients with NTG showed higher distal finger temperature after two hours of gas inhalation with 10 % oxygen compared to atmospheric air (31.07°C ± 0.87 for hypoxia and 29.57°C ± 1.08 for normoxia, $p < 0.05$). This difference was also present during the recovery phase (30.46°C ± 1.16 for hypoxia and 26.40°C ± 0.77 for normoxia, $p < 0.05$) in patients with NTG (Figure 5(a)). However, no difference in distal finger temperature was seen in the age-matched control group when comparing the two conditions (Figure 5(b)). Patients with NTG tended to have lower finger temperatures compared with controls throughout both investigations; however, the decreased temperatures in patients with NTG were not significant.

3.4. Hypoxia Leads to Increased Serum Endothelin-1 Levels. ET-1 levels increased during hypoxia in patients with NTG (from 6.68 mmol/L ± 0.82 at baseline to 11.05 mmol/L ± 1.14 during hypoxia, $p < 0.01$) (Figure 6(a)). Similarly, ET-1 levels also increased in healthy controls (from 8.23 mmol/L ± 0.87 to 13.27 mmol/L ± 1.61, $p < 0.05$) (Figure 6(b)). ET-1 levels only decreased slightly during recovery leading to a significant difference between baseline and recovery in both groups (NTG: 6.68 mmol/L ± 0.82 at baseline versus 10.81 mmol/L ± 1.15 during recovery, $p < 0.01$; controls: 8.23 mmol/L ± 0.87 at baseline versus 11.66 mmol/L ± 1.21

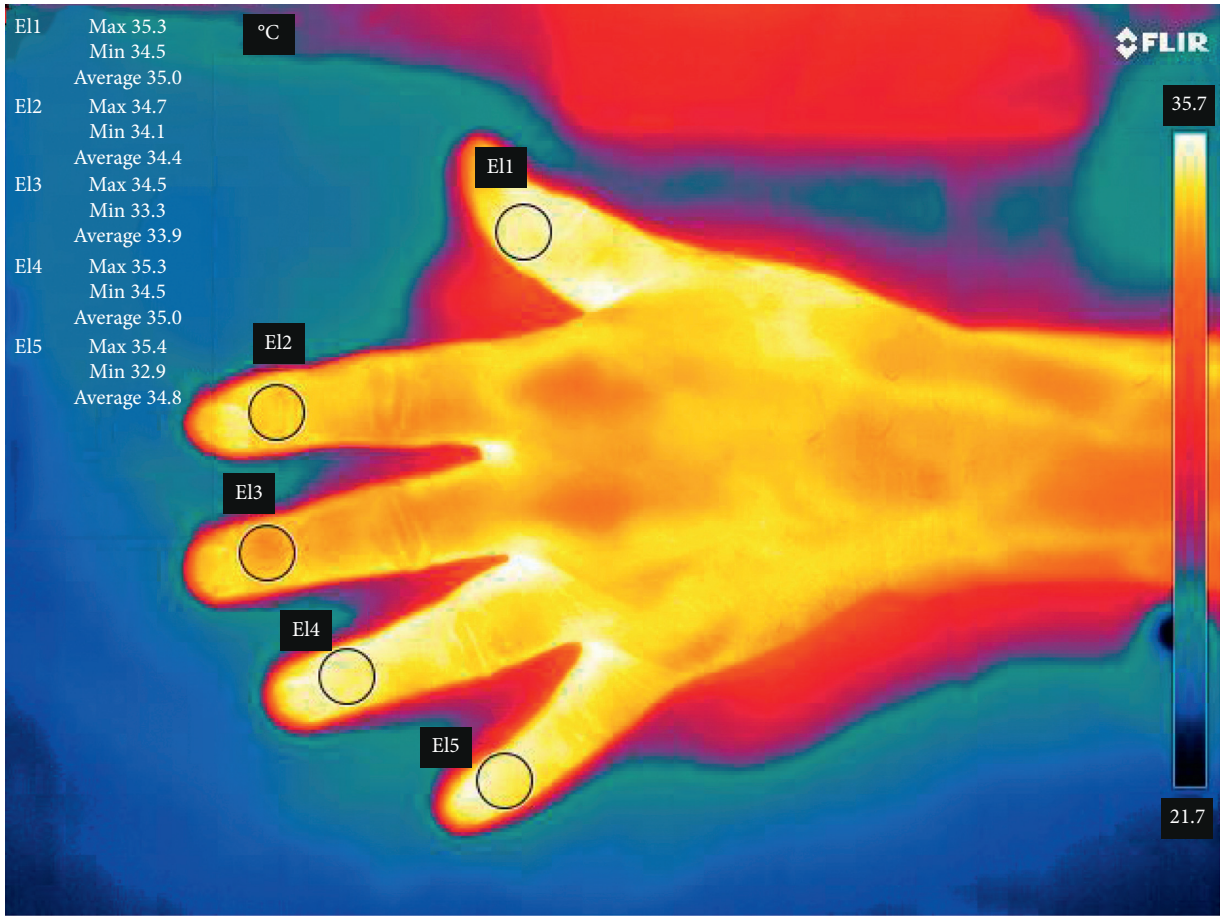


Figure 3: Thermographic image of a participant's hand. The circles represent the area in which the software calculates the mean temperature outlined in the left table. The circles were placed manually within the FLIR computer program. To the right, a scale of colors indicates that yellow and white symbolize warmer temperatures than blue and black.

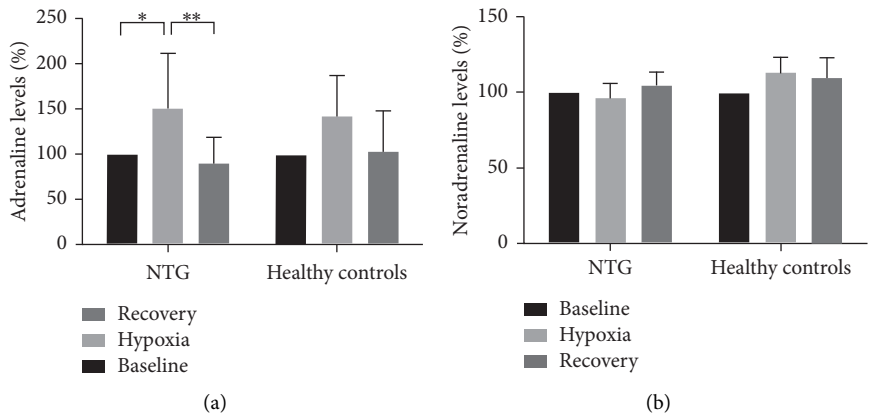


Figure 4: A relative increase in peripheral blood adrenaline was seen during hypoxia for patients with NTG. Patients with NTG also showed relative decrease in adrenaline during recovery. No significant changes were seen for age-matched healthy controls. No significant differences or changes were seen for noradrenaline (statistics: two-way ANOVA, Tukey's multiple comparisons test).

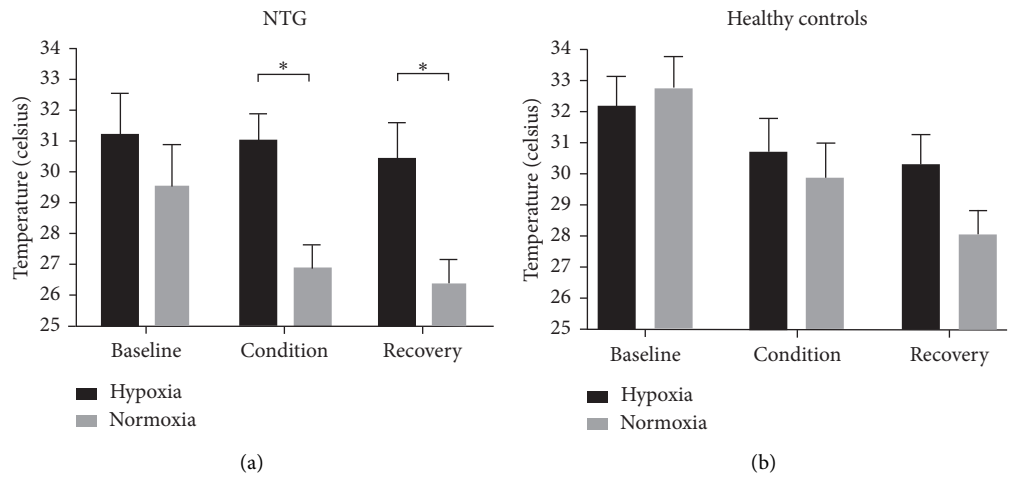


Figure 5: Overview of thermographic data. The distal finger temperature was significantly higher after two hours of hypoxia compared to two hours of normoxia, as well as during recovery, for patients with NTG. No significant differences were seen for age-matched healthy controls (statistics: two-way ANOVA, Sidak's multiple comparisons test).

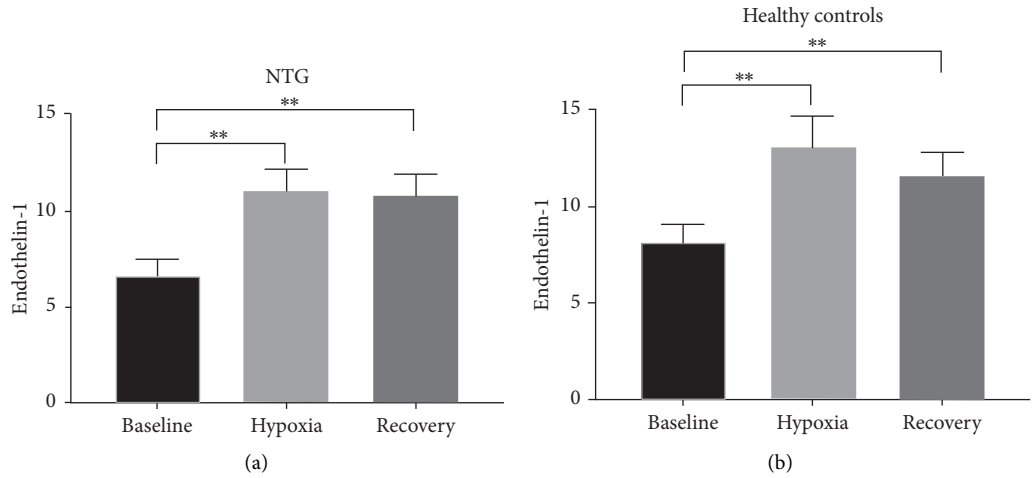


Figure 6: Overview of changes in serum levels of ET-1. ET-1 increased significantly during hypoxia in patients with NTG and in age-matched healthy controls. ET-1 levels remained high during recovery, creating a significant difference between baseline ET-1 levels and recovery in both groups (statistics: RM one-way ANOVA with Tukey's multiple comparisons test).

during recovery, $p < 0.01$). No significant differences were seen between the two groups.

4. Discussion

The vascular hypothesis, as a risk factor for glaucoma development and progression, suggests that RGC and their axons are prone to oxygen and nutrient insufficiency as a result of compromised blood flow. In support of this hypothesis, several studies have shown that hypoxia-related factors are upregulated in the eyes of glaucoma patients, indicating that levels of oxygen and nutritional supply fluctuate, which hypothetically will lead to oxidative stress and inflammation and ultimately glaucomatous neurodegeneration [11–13, 18–21, 27]. In this study, we have used a human experimental model to study how patients with glaucoma respond to systemic oxygen stress compared with age-matched test subjects. Although there is no significant difference in age between the two groups, the average age differs with 5 years between the two groups. The number of participants is small, and it is possible that this may have influenced the findings. With this model, we found that patients with NTG appear to have a higher systemic stress response in terms of greater changes in adrenaline levels during and after hypoxia compared with eye-healthy test subjects (Figure 4(a)) [8–12, 23, 24]. Furthermore, we found that temperature regulation is more fluctuating in patients with NTG compared to healthy controls. Our study suggests that patients with NTG are poorer at handling fluctuating oxygen availability, which may hypothetically result in increased levels of oxidative stress and inflammation which over time will lead to RGC neurodegeneration.

Previously, hypoxia has often been used as a metabolic stress model in *in vitro* studies of glaucoma [27–29], but, to the best of our knowledge, no previous studies have used an *in vivo* human model to study systemic changes that may be related to glaucomatous neurodegeneration. Whereas the present study verified increasing adrenaline in response to hypoxia in patients with NTG (Figure 4(a)), no significant increase was seen in the age-matched control subjects, implying that patients with NTG react more in response to metabolic stress compared to healthy control subjects.

Acute stress initiates a *fight-or-flight response* during harmful events, such as hypoxia, to promote survival. The response is facilitated through increase in adrenaline and manifests in increased blood flow and liberation of energy substrates for muscle action [37, 38]. In line with this, our recent study revealed a significant elevation in serum lactate from the patients with NTG [30], which has been attributed to being a prominent energy source in cells of the inner retina, including RGCs [39–41].

Adrenaline and noradrenaline are furthermore each known to stimulate both vasodilatory and vasoconstrictor responses in the same vascular bed, depending on their concentration and the distribution of adrenergic receptor subtypes in the vessel. There are two subtypes of receptors in blood vessels of clinical relevance: α_1 elicits vasoconstriction and β_2 induces vasodilation through the mechanism shown in Figure 7. Both adrenaline and noradrenaline have affinity for both receptors, but adrenaline has greater affinity for β_2, while noradrenaline has greater affinity for α_1. Although α_1 is the predominant receptor subtype in the blood vessels [42], we found only a significant increase in adrenaline in response to hypoxia, suggesting a net activation of β_2 receptors. Thus, our study suggests that hypoxia causes vasodilation, which may explain the increase in finger temperature in patients with NTG (Figure 5). Moreover, the increased finger temperature in patients with NTG indicates that patients with NTG are more prone to develop an acute stress response. In support of our study, Wierzbowska et al. have compared 24-hour ECG from NTG patients with healthy controls and found a higher ratio of low frequency to high frequency, indicating a shift towards sympathetic activity [43]. Furthermore, Flammer et al. demonstrated a link between primary vascular dysregulation syndrome (PVD) and NTG, highlighting lower hand temperature and an autonomic imbalance with sympathetic dominance as symptoms of PVD [15].

Despite the lack of autonomic innervation in the intraocular vascular system [44], it is likely that the systemic effects of adrenaline and noradrenaline play a role in the pathogenesis of glaucoma indirectly, if not directly [45–47]. In this context, Fitzgerald reports that systemic stress may lead to increased IOP [45]. In line with such systemic affection on retinal neurodegeneration, Horwitz et al. found that antiadrenergic antihypertensive drugs have a protective effect on the development of glaucoma [46, 47], which hypothetically may be explained by increased ocular perfusion. Thus, systemic levels of catecholamines may indirectly affect oxygen and energy supply to the inner retina and thereby play a role in the pathogenesis of glaucomatous neurodegeneration.

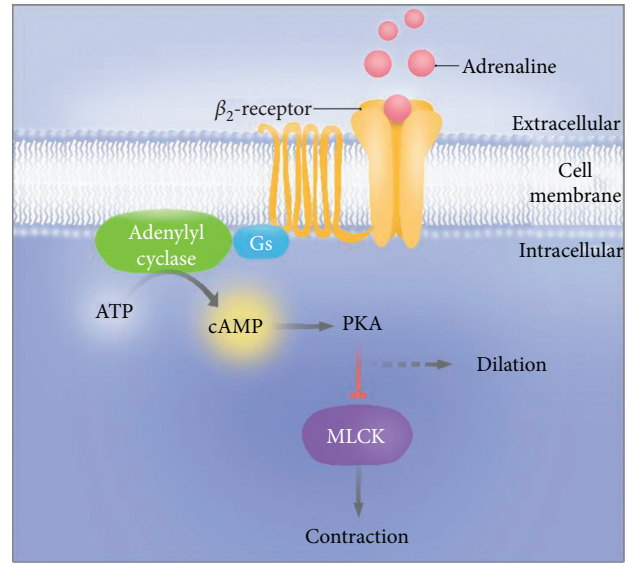


Figure 7: The β_2 adrenergic receptor is a G-protein coupled receptor. Activation of the receptor by adrenaline leads to peripheral vasodilation through the Gs signal pathway. Activation of adenylyl cyclase leads to conversion of ATP to cAMP, which activates phosphor kinase A (PKA). Activation of PKA leads to deactivation of myosin light chain kinase (MLCK), which, when activated, leads to contraction. Thus, activation of PKA leads to vasodilation (©EyeTRU).

Since retinal vessels are autoregulated by ET-1 amongst others [8, 9, 13, 15, 26], we measured ET-1 in peripheral blood as a surrogate for the retinal ET-1 concentrations. ET-1 is a well-known vasoconstrictor in the eye and has repeatedly been demonstrated to play a key role in the regulation of ocular perfusion and hypothetically in the overall pathogenesis of inner retinal diseases [15, 48–51]. Circulating ET-1 can reach vessels in the optic nerve head (ONH) in two ways: (1) diffusion from the fenestrated choriocapillaris bypassing the blood-brain-barrier (BBB)/blood retinal barrier (BRB) or (2) through disrupted BBB/BRB. Disrupted BBB/BRB occurs both physiologically with aging and in response to neurodegeneration [15, 52, 53]. As a consequence of disrupted BBB/BRB, ET-1 can potentially freely access the vasculature supplying the optic nerve, and an increase in ET-1 in peripheral blood will lead to an increase in ocular ET-1 [15]. Our study showed a significant hypoxia-induced increase in serum ET-1 levels in both patients with NTG and controls (Figure 6). Previous studies have identified differences in ET-1 levels, when comparing patients with NTG to healthy controls. Li et al. have analyzed seven studies in a meta study, which showed higher plasma levels of ET-1 in the NTG group (mean difference of 0.6 pg/mL [$p = 0.007$, 95% CI = 0.17–1.04]) [14]. However, the present study was not able to replicate these findings, possibly due to deviations in recruitment criteria concerning clinical phenotyping, ethnicity of the test subjects, and so forth.

In summary, the present study introduces the concept of an enhanced hypoxia-induced stress response in patients with NTG which may be correlated to glaucomatous neurodegeneration. However, future studies are required to evaluate retinal vessel diameter in response to hypoxia and correlate findings to other blood stress markers to elaborate on vascular dysfunction and hypoxia-mediated stress responses in patients with glaucoma.

Data Availability

The blood sample and thermographic data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Line Marie Dalgaard and Jeppe Vibæk contributed equally to this work.

Acknowledgments

The study was supported by Lions Prize, Fight for Sight Denmark, the Synoptik Foundation, the Michaelsen Foundation, the Danish Glaucoma Patient Organization, and the Novo Nordisk Foundation. Rupali Vohra is a part of the BRIDGE–Translational Excellence Programme (bridge.ku.dk) at the Faculty of Health and Medical Sciences, University of Copenhagen, funded by the Novo Nordisk Foundation (Grant no. NFF18SA0034956).

References

[1] P. J. Foster, R. Buhrmann, H. A. Quigley, and G. J. Johnson, "The definition and classification of glaucoma in prevalence surveys," *British Journal of Ophthalmology*, vol. 86, no. 2, pp. 238–242, 2002.

[2] A. T. Broman, H. A. Quigley, S. K. West et al., "Estimating the rate of progressive visual field damage in those with open-angle glaucoma, from cross-sectional data," *Investigative Opthalmology & Visual Science*, vol. 49, no. 1, pp. 66–76, 2008.

[3] J. M. Kim, H. Kyung, S. H. Shim, P. Azarbod, and J. Caprioli, "Location of initial visual field defects in glaucoma and their modes of deterioration," *Investigative Opthalmology & Visual Science*, vol. 56, no. 13, pp. 7956–7962, 2015.

[4] W. L. Membrey, D. P. Poinoosawmy, C. Bunce, F. W. Fitzke, and R. A. Hitchings, "Comparison of visual field progression in patients with normal pressure glaucoma between eyes with and without visual field loss that threatens fixation," *British Journal of Ophthalmology*, vol. 84, no. 10, pp. 1154–1158, 2000.

[5] Y.-C. Tham, X. Li, T. Y. Wong, H. A. Quigley, T. Aung, and C.-Y. Cheng, "Global prevalence of glaucoma and projections of glaucoma burden through 2040," *Ophthalmology*, vol. 121, no. 11, pp. 2081–2090, 2014.

[6] A. Sommer, J. M. Tielsch, J. Katz et al., "Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans," *The Baltimore Eye Survey. Arch Ophthalmol (Chicago, Ill 1960)*, vol. 109, no. 8, pp. 1090–1095, 1991.

[7] M. K. Kolko, "Present and new treatment strategies in the management of glaucoma," *The Open Ophthalmology Journal*, vol. 9, pp. 89–100, 2015.

[8] E. Henry, D. E. Newby, D. J. Webb, P. W. F. Hadoke, and C. J. O'Brien, "Altered endothelin-1 vasoreactivity in patients with untreated normal-pressure glaucoma," *Investigative Opthalmology & Visual Science*, vol. 47, no. 6, pp. 2528–2532, 2006.

[9] M. Pache and J. Flammer, "A sick eye in a sick body? Systemic findings in patients with primary open-angle glaucoma," *Survey of Ophthalmology*, vol. 51, no. 3, pp. 179–212, 2006.

[10] H. Resch, G. Garhofer, G. Fuchsjäger-Mayrl, A. Hommer, and L. Schmetterer, "Endothelial dysfunction in glaucoma," *Acta Ophthalmologica*, vol. 87, no. 1, pp. 4–12, 2009.

[11] N. Fan, P. Wang, L. Tang, and X. Liu, "Ocular blood flow and normal tension glaucoma," *Biomed Res Int*, vol. 2015, p. 308505, 2015.

[12] J. Choi and M. S. Kook, "Systemic and ocular hemodynamic risk factors in glaucoma," *Biomed Res Int*, vol. 2015, p. 141905, 2015.

[13] J. Flammer and M. Mozaffarieh, "Autoregulation, a balancing act between supply and demand," *Canadian Journal of Ophthalmology*, vol. 43, no. 3, pp. 317–321, 2008.

[14] S. Li, A. Zhang, W. Cao, and X. Sun, "Elevated plasma endothelin-1 levels in normal tension glaucoma and primary open-angle glaucoma: a meta-analysis," *Journal of Ophthalmology*, vol. 2016, Article ID 2678017, 6 pages, 2016.

[15] J. Flammer, K. Konieczka, and A. J. Flammer, "The primary vascular dysregulation syndrome: implications for eye diseases," *EPMA J*, vol. 4, no. 1, p. 14, 2013.

[16] M. T. Nicolela, "Clinical clues of vascular dysregulation and its association with glaucoma," *Canadian Journal of Ophthalmology*, vol. 43, no. 3, pp. 337–341, 2008.

[17] M. W. Country, "Retinal metabolism: a comparative look at energetics in the retina," *Brain Research*, vol. 1672, pp. 50–57, 2017.

[18] J. Flammer, S. Orgül, V. P. Costa et al., "The impact of ocular blood flow in glaucoma," *Progress in Retinal and Eye Research*, vol. 21, no. 4, pp. 359–393, 2002.

[19] L. Abegão Pinto, K. Willekens, K. Van Keer et al., "Ocular blood flow in glaucoma - the leuven eye study," *Acta Ophthalmologica*, vol. 94, no. 6, pp. 592–598, 2016.

[20] Y. Shiga, H. Kunikata, N. Aizawa et al., "Optic nerve head blood flow, as measured by laser speckle flowgraphy, is significantly reduced in preperimetric glaucoma," *Current Eye Research*, vol. 41, no. 11, pp. 1447–1453, 2016.

[21] R. Vohra, J. C. Tsai, and M. Kolko, "The role of inflammation in the pathogenesis of glaucoma," *Survey of Ophthalmology*, vol. 58, no. 4, pp. 311–320, 2013.

[22] C. Kaur, V. Sivakumar, W. S. Foulds, C. D. Luu, and E.-A. Ling, "Hypoxia-induced activation of N-methyl-D-aspartate receptors causes retinal ganglion cell death in the neonatal retina," *Journal of Neuropathology & Experimental Neurology*, vol. 71, no. 4, pp. 330–347, 2012.

[23] V. Sivakumar, W. S. Foulds, C. D. Luu, E.-A. Ling, and C. Kaur, "Retinal ganglion cell death is induced by microglia derived pro-inflammatory cytokines in the hypoxic neonatal retina," *The Journal of Pathology*, vol. 224, no. 2, pp. 245–260, 2011.

[24] C. Kaur, W. S. Foulds, and E.-A. Ling, "Hypoxia-ischemia and retinal ganglion cell damage," *Clinical Ophthalmology*, vol. 2, no. 4, pp. 879–889, 2008.

[25] M. Nakayama, M. Aihara, Y. N. Chen, M. Araie, K. Tomita-Yokotani, and T. Iwashina, "Neuroprotective effects of flavonoids on hypoxia-, glutamate-, and oxidative stress-induced retinal ganglion cell death," *Molecular Vision*, vol. 17, pp. 1784–1793, 2011.

[26] X. Luo, Y.-M. Shen, M.-N. Jiang, X.-F. Lou, and Y. Shen, "Ocular blood flow autoregulation mechanisms and methods," *Journal of Ophthalmology*, vol. 2015, Article ID 864871, 7 pages, 2015.

[27] G. Chidlow, J. P. M. Wood, and R. J. Casson, "Investigations into hypoxia and oxidative stress at the optic nerve head in a rat model of glaucoma," *Frontiers in Neuroscience*, vol. 11, p. 478, 2017.

[28] M. Griebsch, M. Klemm, J. Haueisen, and M. Hammer, "Hypoxia-induced redox signalling in Muller cells," *Acta Ophthalmologica. England*, vol. 95, pp. e337–e339, 2017.

[29] D. Z. Ellis, L. Li, Y. Park, S. He, B. Mueller, and T. Yorio, "Sigma-1 receptor regulates mitochondrial function in glucose- and oxygen-deprived retinal ganglion cells," *Investigative Opthalmology & Visual Science*, vol. 58, no. 5, pp. 2755–2764, may 2017.

[30] R. Vohra, L. M. Dalgaard, J. Vibaek et al., "Potential metabolic markers in glaucoma and their regulation in response to hypoxia," *Acta Ophthalmologica*, vol. 97, no. 6, pp. 567–576, Jan 2019.

[31] T. Y. Wong, R. Klein, B. E. K. Klein, S. M. Meuer, and L. D. Hubbard, "Retinal vessel diameters and their associations with age and blood pressure," *Investigative Opthalmology & Visual Science*, vol. 44, no. 11, pp. 4644–4650, Nov 2003.

[32] S. L. Hosking, A. Harris, H. S. Chung et al., "Ocular haemodynamic responses to induced hypercapnia and hyperoxia in glaucoma," *British Journal of Ophthalmology*, vol. 88, no. 3, pp. 406–411, Mar 2004.

[33] A. S. Olsen, M. Cour, B. Damato, and M. Kolko, "Detection of visual field defects by opticians—with Damato multifixation Campimetry online," *Acta Ophthalmologica*, vol. 97, no. 6, pp. 577–582, Sep 2019.

[34] N. Garcia, S. R. Hopkins, A. R. Elliott, E. A. Aaron, M. B. Weinger, and F. L. Powell, "Ventilatory response to 2-h sustained hypoxia in humans," *Respiration Physiology*, vol. 124, no. 1, pp. 11–22, 2001.

[35] P. I. Johansson, A. Bergström, N. J. Aachmann-Andersen et al., "Effect of acute hypobaric hypoxia on the endothelial glycocalyx and digital reactive hyperemia in humans," *Front Physiol*, vol. 5, p. 459, 2014.

[36] P. A. Easton, L. J. Slykerman, and N. R. Anthonisen, "Recovery of the ventilatory response to hypoxia in normal adults," *Journal of Applied Physiology*, vol. 64, no. 2, pp. 521–528, 1988.

[37] A. W. Tank and D. Lee Wong, "Peripheral and central effects of circulating catecholamines," *Comprehensive Physiology*, vol. 5, pp. 1–15, 2015.

[38] W. B. Cannon, "Studies ON the conditions OF activity IN endocrine glands," *American Journal of Physiology-Legacy Content*, vol. 50, no. 3, pp. 399–432, Dec 1919.

[39] R. Vohra, B. I. Aldana, D. M. Skytt et al., "Essential roles of lactate in müller cell survival and function," *Molecular Neurobiology*, vol. 55, no. 12, pp. 9108–9121, 2018.

[40] R. Vohra, B. I. Aldana, H. Waagepetersen, L. H. Bergersen, and M. Kolko, "Dual properties of lactate in müller cells: the effect of GPR81 activation," *Investigative Opthalmology & Visual Science*, vol. 60, no. 4, pp. 999–1008, 2019.

[41] R. Vohra, B. I. Aldana, G. Bulli et al., "Lactate-mediated protection of retinal ganglion cells," *Journal of Molecular Biology*, vol. 431, no. 9, pp. 1878–1888, 2019.

[42] W. F. Boron and E. L. Boulpaep, *Medical Physiology, a Cellular and Molecular Approach*, Saunders Elsevier, Philadelphia, PA, USA, 2012.

[43] J. Wierzbowska, R. Wierzbowski, A. Stankiewicz, B. Siesky, and A. Harris, "Cardiac autonomic dysfunction in patients with normal tension glaucoma: 24-h heart rate and blood pressure variability analysis," *British Journal of Ophthalmology*, vol. 96, no. 5, pp. 624–628, 2012 May.

[44] C. Delaey and J. Van De Voorde, "Regulatory mechanisms in the retinal and choroidal circulation," *Ophthalmic Research*, vol. 32, no. 6, pp. 249–256, 2000.

[45] P. J. Fitzgerald, "Is elevated noradrenaline an aetiological factor in a number of diseases?" *Autonomic and Autacoid Pharmacology*, vol. 29, no. 4, pp. 143–156, 2009.

[46] A. Horwitz, M. Klemp, J. Jeppesen, J. C. Tsai, C. Torp-Pedersen, and M. Kolko, "Antihypertensive medication postpones the onset of glaucoma: evidence from a nationwide study," *Hypertens (Dallas, Tex 1979)*, vol. 69, no. 2, pp. 202–210, 2017.

[47] A. Horwitz, B. E. Petrovski, C. Torp-Pedersen, and M. Kolko, "Danish nationwide data reveal a link between diabetes mellitus, diabetic retinopathy, and glaucoma," *ournal of Diabetes Research*, vol. 2016, Article ID 2684674, 10 pages, 2016.

[48] G. Wollensak, H.-E. Schaefer, and C. Ihling, "An immunohistochemical study of endothelin-1 in the human eye," *Current Eye Research*, vol. 17, no. 5, pp. 541–545, 1998.

[49] J. Lau, M. Dang, K. Hockmann, and A. K. Ball, "Effects of acute delivery of endothelin-1 on retinal ganglion cell loss in the rat," *Experimental Eye Research*, vol. 82, no. 1, pp. 132–145, 2006.

[50] M. E. Källberg, D. E. Brooks, K. N. Gelatt, G. A. Garcia-Sanchez, N. J. Szabo, and G. N. Lambrou, "Endothelin-1, nitric oxide, and glutamate in the normal and glaucomatous dog eye," *Veterinary Ophthalmology*, vol. 10, no. s1, pp. 46–52, 2007.

[51] J. Flammer and K. Konieczka, "Retinal venous pressure: the role of endothelin," *EPMA Journal*, vol. 6, p. 21, 2015.

[52] O. Arend, A. Remky, N. Plange, M. Kaup, and B. Schwartz, "Fluorescein leakage of the optic disc in glaucomatous optic neuropathy," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 243, no. 7, pp. 659–664, 2005.

[53] M. S. Thomsen, L. J. Routhe, and T. Moos, "The vascular basement membrane in the healthy and pathological brain," *Journal of Cerebral Blood Flow & Metabolism*, vol. 37, no. 10, pp. 3300–3317, 2017.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 8071280, 8 pages
https://doi.org/10.1155/2020/8071280

Review Article

Vitamin D and Glaucoma: A Critical Review of the Literature

Hana Abouzeid [1,2] **and Caroline Flora Samer** [3,4]

[1]*Ophthalmology Division, Geneva University Hospitals-HUG, Geneva, Switzerland*
[2]*Clinical Eye Research Centre Memorial Adolphe de Rothschild, Geneva, Switzerland*
[3]*Clinical Pharmacology and Toxicology Division, Geneva University Hospitals-HUG, Geneva, Switzerland*
[4]*Faculty of Medicine, Geneva University, Geneva, Switzerland*

Correspondence should be addressed to Hana Abouzeid; hana.abouzeid@hcuge.ch

Received 31 January 2020; Revised 18 August 2020; Accepted 24 September 2020; Published 16 November 2020

Academic Editor: Steffen Heegaard

Primary open-angle glaucoma is a progressive optic neuropathy which can lead to irreversible blindness if untreated. A number of studies have been published suggesting a correlation between the level of serum vitamin D3 and glaucoma or intraocular pressure (IOP). The latter is known to be a major risk factor for glaucoma and is the main target of glaucoma treatment. We give a critical review of the literature, exploring what is known about this matter. While some studies report an inverse association between serum vitamin D3 and IOP, others do not confirm this finding. Similar divergent conclusions came from studies regarding the association between serum vitamin D3 and the presence or severity of glaucoma. The effect of vitamin D3 on IOP decrease has been attributed to both aqueous humor production and trabecular meshwork outflow pathway increase. Vitamin D3 has been shown to play a major role in reducing inflammation, modulating the immune response, and decreasing angiogenesis in the eye and in other organs. It has been suggested that, through its neuroprotective effect, vitamin D3 could be a protective factor for glaucoma and that vitamin D3 deficiency could explain glaucoma occurrence or severity in some patients. Other neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis have been similarly related to vitamin D3 deficiency. 1α,25$(OH)_2$ vitamin D3 (calcitriol) supplementation has been shown to be beneficial for lowering IOP in monkeys. Although the studies highlighted in this review show interesting results, their limitations underscore the need for both population-based studies and larger randomized controlled trial with vitamin D3 supplementation. The specific role of vitamin D3 in the pathology of glaucoma remains to be elucidated, together with the possible therapeutic benefit of vitamin D3 supplementation.

1. Introduction

Glaucoma is an acquired and progressive optic neuropathy, which can lead to irreversible blindness. Its burden in the world population is growing as the population ages and no causative treatment has yet been identified [1]. Some of the main risk factors of glaucoma are advanced age, positive family history, higher cup-to-disk ratio, central corneal thickness, and elevated intraocular pressure (IOP), the latter being the only one which can be modified [2]. Lowering IOP has been proven to be effective in reducing glaucoma progression [3], hence the importance of identifying and understanding the IOP modifiers. Among numerous factors which have been reported to influence IOP, one can mention systemic blood pressure; time of day; supine position; caffeine, alcohol, nicotine, or cannabis consumption; steroid medication; age; genetic background; ethnicity; body mass index; diabetes [4]; and more recently vitamin D3 levels.

An inverse association between vitamin D3 levels in its hydroxylated form (25-hydroxyvitamin D3 or calcifediol, abbreviated 25(OH)D3) and IOP, and hence glaucoma, has recently been claimed in several studies [5–10]. Indeed, direct measurement of serum/plasma vitamin D levels is very difficult for clinical laboratories. 25(OH)D levels are measured because most vitamin D produced in the body is converted to 25(OH)D and its determination is feasible. Higher serum 25(OH)D levels have been described in fair-skinned Americans compared to Mexican Americans and to

non-Hispanic dark-skinned Americans, the latter having the lowest serum levels [11]. It is well known that fair-skinned people need less UVB exposure to produce the same amount of vitamin D3 compared with dark-skinned people, a fact which however remains poorly explained to date. Based on the prevalence of glaucoma [12] and the observation that the mean IOP is higher among African Americans compared to non-African populations [13], one might expect the vitamin D3-IOP interaction to shed light on this matter. Several studies have been published in the literature about vitamin D3 and IOP or glaucoma in humans [5–10], with different methodology and results.

In this context, we present a critical review of the literature in order to summarize our current knowledge on the interaction between IOP or glaucoma and vitamin D3.

1.1. Pharmacokinetics and Pharmacodynamics of Vitamin D. Vitamin D is a group of fat-soluble prohormones including vitamin D3 (cholecalciferol) and vitamin D2 (Figure 1). Vitamin D can either be produced in the skin from 7-dehydrocholesterol under the influence of UV light, so-called vitamin D3 (cholecalciferol), or directly absorbed from the diet as vitamin D3 and vitamin D2 (ergocalciferol) [14]. Either form is then hydroxylated to 25-hydroxycholecalciferol (calcidiol or calcifediol, 25(OH)D) in the liver by the cytochrome P450 (CYP) 2R1 mainly and 27A1. It is then transformed mainly in the kidney by 25-hydroxyvitamin D3-1α-hydroxylase (CYP27B1) to 1α,25-dihydroxyvitamin D ($1,25(OH)_2D$ or calcitriol), the active form of vitamin D3. CYP27B1 is expressed to a lesser degree in extrarenal tissues such as the epidermis, brain, pancreas, colon, breast, ovary, muscle, and immune cells/macrophages, as well as nonparenchymal hepatic cells [15]. Therefore a local production of $1,25(OH)_2D$ in these tissues is possible [16]. The excess of active vitamin D ($1,25(OH)_2D$) is converted into inactive metabolites ($1,24,25(OH)_3D$) by 24-hydroxylase (CYP24A1). The induction of CYP24A1 by $1,25(OH)_2D$ is a feed forward mechanism. The feedback mechanism is $1,25(OH)_2D$ inhibition of its own production by CYP27B1. CYP24A1 also converts 25(OH)D to another inactive metabolite, 24,25-dihydroxyvitamin D3, which is an additional mechanism to prevent excessive circulating levels of moderate or highly active vitamin D metabolites. Disproportionate administration of the hormonal form of vitamin D ($1,25(OH)_2D$) can cause vitamin D deficiency, that is through stimulating the degradation of 25(OH)D. This is important because most assays used to measure circulating 25(OH)D to estimate vitamin D status have a 100% cross reactivity with 24,25-dihydroxyvitamin D2.

Parathyroid hormone (PTH) stimulates CYP27B1 expression and decreases CYP24A1, to produce more active calcitriol [17].

$1,25(OH)_2D$ is the ligand for the vitamin D receptor (VDR), a transcription factor with hormone and DNA-binding domains. $1,25(OH)_2D$ binds to the vitamin D binding protein to reach its target cell, which then binds to cytoplasmic VDR to form a complex after entering the cell [18]. This complex then enters the nucleus where it heterodimerizes with the retinoid X receptor (RXR) and that heterodimer binds to DNA and thus regulates DNA transcription of hundreds of vitamin D dependent genes and largely influences gene expression in humans [19, 20]. $1,25(OH)_2D$ activates or represses transcription depending on the target gene. VDR is encoded by a gene located on chromosome 12 and over 63 polymorphisms have been reported. Most VDR polymorphisms only mildly modify the affinity of 1,25(OH)2D for the VDR, but some are associated with the inability for 1,25(OH)2D to bind VDR and may influence bone and mineral homeostasis. Only VDR mutations significantly compromise binding causing severe defects in bone and mineral homeostasis. The most studied VDR polymorphisms are rs10735810, rs1544410, rs731236, and rs7975232 [21].

The active form of vitamin D3 has many physiological roles such as the regulation of calcium homeostasis and bone mineralization, but it is now recognized to have antiproliferative and immunomodulatory properties [22–24]. It is reported to affect a wide range of human diseases, including notably cancer, cardiovascular, infectious, and autoimmune diseases [25].

Ergocalciferol, calcidiol and, calcitriol vary in their PK parameters in terms of onset of action and half-lives [26] (see Table 1).

2. Association of Vitamin D and Glaucoma

2.1. Clinical Studies. Table 2 summarizes the findings of the clinical studies discussed below.

In a study performed in Norway, Krefting at al. [8] divided healthy Caucasians with high or low serum 25(OH)D levels, recruited from a population-based study, into two groups. The IOP in the 87 participants with low serum 25(OH)D levels (mean 40.1 ± 12.9 nmol/l) did not differ from the IOP in the 42 participants with high serum 25(OH)D levels (mean 85.1 ± 14.0 nmol/l) (15.9 ± 3.3 mmHg versus 15.6 ± 3.1 mmHg, $p = 0.56$, independent t-test). The authors then performed a randomized clinical trial and measured the change in IOP after administration to the group with low serum 25(OH)D levels, twice a week over a period of 6 months, of either high dose vitamin D (one capsule of vitamin D3 20 000 IU-Dekristol; Mibe, Brehna, Germany) or placebo. At 6 months, the levels achieved upon the supplementation provided for 6 months to these individuals with low 25(OH)D at baseline were significantly higher than those of the placebo group (142.7 ± 25.3 nmol/l versus 41.7 ± 14.2 nmol/l, $p < 0.01$ for difference in delta values between groups). Although IOP slightly decreased after vitamin D administration, no statistical difference in IOP was reported with or without 25(OH)D administration ($p = 0.92$, independent t-test). However, the small size of each treatment arm ($n = 39$) and the selection bias inherent to the case-control methodology limit the conclusion one can draw from this study. Moreover, the most important data missing in the study is whether the vitamin D supplementation provided effectively corrected vitamin D deficiency and if there were significant differences in the decreases in IOP among those

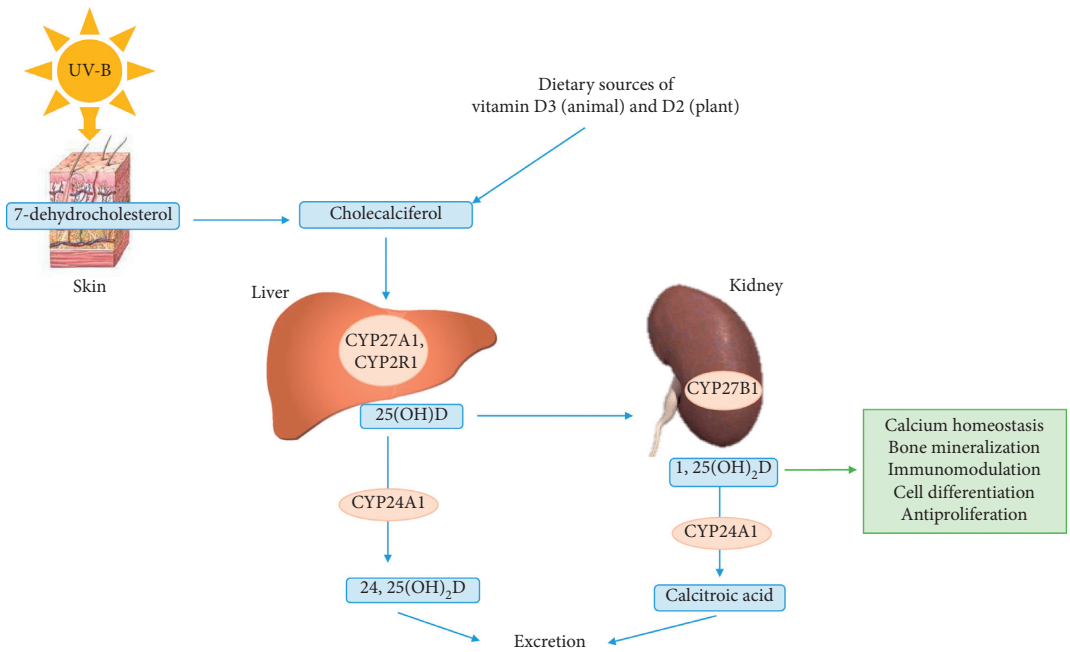


Figure 1: Normal vitamin D metabolism. Vitamin D can either be produced in the skin from 7-dehydrocholesterol under the influence of UV light, so-called vitamin D3 (cholecalciferol), or from ergosterol or directly absorbed from the diet as vitamin D3 and vitamin D2 (ergocalciferol). Either form is then hydroxylated to 25-hydroxycholecalciferol (calcidiol or calcifediol, 25(OH)D) in the liver by the cytochrome P450 (CYP) 27A1. It is then transformed mainly in the kidney by 25-hydroxyvitamin D3-1α-hydroxylase (CYP27B1) into 1α,25-dihydroxyvitamin D (1,25$(OH)_2$D or calcitriol).

Table 1: Vitamin D agents and half-lifes.

Agent	Half-life
Vitamin D2 (ergocalciferol) Vitamin D3 (cholecalciferol)	3–4 days
25(OH)D2 (ercalcidiol)	14 days
25(OH) D3 (calcifediol/calcidiol)	30 days
1,25$(OH)_2$D (calcitriol)	7–10 h

individuals who corrected the deficiency and those who remained insufficient or deficient.

Yoo et al. [10] reported in 2014 an inverse association between serum 25(OH)D concentration and primary open-angle glaucoma (POAG) ($n = 290$) in males only (OR = 0.98 with $p = 0.04$ for 1 ng/mL increase in 25(OH)D). The same inverse association with IOP was described in all three categories of participants which they considered. Their results were obtained in a cross-sectional study of 6,094 South Korean participants, including 290 open-angle glaucoma, 410 glaucoma suspect, and 5,394 healthy controls. However, and among other bias factors, the nonconsensual glaucoma definition used, the limited clinical expertise in glaucoma of the examiners (nonglaucoma specialists or residents), and the cross-sectional design of the study limit the conclusions which can be drawn from this study.

A larger retrospective cross-sectional study of 123,331 South Korean participants [7] found that lower 25(OH)D levels were significantly associated with an elevated risk of glaucoma in females only, found no association between IOP and vitamin D, and suggested that vitamin D may thus have a role in glaucoma pathogenesis independently of IOP levels. However, their study population had a large difference in the numbers of female and male subjects, and the group with high IOP was significantly smaller than the group with normal IOP. In addition, the subjects were diagnosed with glaucoma based only on fundus photographs, without any gonioscopic description of the angle. This limits the reliability of the assumed patient categorization and thus the conclusions on the association between vitamin D and the risk of glaucoma. Since angle closure is known to have an effect on IOP, it is difficult to hypothesize on the effect of vitamin D without a detailed gonioscopic description. In particular, angle-closure glaucoma should be excluded from vitamin D-glaucoma studies. In addition to the limitations in glaucoma definition mentioned, the cross-sectional design of the study and the fact that vitamin D supplementation was not taken into account preclude the determination of a direct association between vitamin D and open-angle glaucoma. There should be clear specification on exclusion or inclusion of vitamin D supplementation in published studies and data about whether the vitamin D supplementation provided effectively corrected vitamin D deficiency and if there were significant differences in the decreases of the studied outcome among those individuals who corrected the deficiency and those who remained insufficient or deficient. If there is a significant inverse association between glaucoma or IOP and low vitamin D levels, even if there were supplemented patients included in the study, this would strengthen the conclusions of an inverse association between vitamin D levels and glaucoma or IOP.

A French population case-control study [6] reported an association between the presence, but not the severity, of

Table 2: Summary of the reviewed clinical studies and their findings.

		Inverse association with 1. IOP 2. Glaucoma presence 3. Glaucoma severity	No. of patients	Type of study	Studied population	Tested serum vit D
1	[8]	1. N 2. NA 3. NA	129 healthy 87 low serum vit D 42 high serum vit D	Nested case-control and RCT	Norway	25(OH)
2	[10]	1. Y 2. Y 3. NA	POAG 290 Suspect 410 Healthy 5394	Cross-sectional	South Korea	25(OH)
3	[6]	1. N 2. Y 3. N	POAG 150 Severe 99 Moderate 51	Case-control	France	25(OH)
4	[7]	1. N 2. Y (in males only) 3. NA	Controls 164 Glaucoma 1627 Healthy 121,704	Cross-sectional	South Korea	25(OH)
5	[9]	1. NA 2. Y 3. NA VDR polymorphisms BsmI 'B' and TaqI 't'	POAG 79* Healthy 71	Case-control	China	1α,25(OH)2D3
6	[5]	1. Y 2. N 3. Y	POAG 357 Healthy 178	Case-control	African descent	25(OH)

*Unspecified gonioscopic status.

primary open-angle glaucoma and 25(OH)D serum levels. The 150 glaucoma patients had fifteen percent lower serum 25(OH)D concentration than the 164 controls (42.9 ± 25.7 nmol/L versus 49.4 ± 29.5 nmol/L, $p = 0.039$), as well as a greater prevalence of vitamin D insufficiency (90.7% of the glaucoma patients versus 82.3% of the controls, $p = 0.032$). Meanwhile, vitamin D insufficiency was found in 86.3% of all study participants. There was no difference regarding 25(OH)D serum levels or vitamin D insufficiency between the 99 patients with severe POAG and the 51 with moderate POAG. The serum concentration of 25(OH)D did not correlate with either intraocular pressure or visual field mean deviation, while POAG patient had treatment for controlling IOP, which significantly hinders the analysis of potential IOP lowering effect of 25(OH)D. The regular use of vitamin D supplementation by patients and controls was reported by direct inquiry, whatever the dosage, schedule, or route of administration and regardless of the date of commencement. Vitamin D supplementation was then added as a covariable in the statistical analysis. Despite the fact that the results were statistically adjusted for the presence of a vitamin D supplementation, the details of this supplementation are missing, limiting any conclusions about its efficacy and influence on the studied outcomes. The case-control design of this study and the small number of participants limit its robustness as well.

An American case-control study [5] reported an association between 25(OH)D serum level with the severity of glaucoma in a cohort of 357 POAG patients of African descent, but not with its presence. The mean (95% confidence interval [CI]) levels of vitamin D of the subjects in the control (8.02 ± 6.19 pg/ml) and early phenotype (7.56 ± 5.74 pg/ml) groups were significantly or marginally significantly different from the levels observed in subjects with the advanced phenotype (6.35 ± 4.76 pg/ml; $p = 0.0117$ and 0.0543, respectively). However, it should be noted that the serum levels of the 178 normal subjects were not significantly different from those of early glaucoma patients ($p = 0.8508$). In the latter study [5], the authors found a small yet significantly different from zero association between serum levels of 25(OH)D and IOP (correlation coefficient: −0.0819, p value: 0.0018). This study confirmed, in a population of African descent, the hypothesis of the role played by vitamin D in explaining the higher IOP observed among African Americans compared to non-African populations [13]. However, it relates this possible role to the severity of an existing glaucoma rather than to the occurrence of glaucoma. It should be noted that both studies [5, 6] used different definitions for glaucoma severity and included patients with different glaucoma severity. This may explain the difference found in their results. In the study of Ayyagari et al. [5], the authors compared 25(OH)D levels in early (better than −4 dB) and advanced primary open-angle glaucoma (worse than −10 dB) patients, while the French study [6] compared 25(OH)D levels in moderate (better than −12 dB) and advanced glaucoma (worse than −12 dB) patients. Other aspects which might explain the different results are on the one hand the fact that the populations studied were very different and on the other hand the glaucoma classifications used in these studies. At least in an

(unreported) number of cases, glaucoma classification was based on clinical appreciation only. These aspects weaken the reliability of the results.

Indeed, selection bias is inherent to case-control studies, especially when the study groups are relatively small, as in both cases discussed here, so the conclusions reached by the authors should be interpreted carefully. However, the suggestion by Ayyagari et al. [5] that, as a result of increased inflammation and neurodegeneration, the absence of the neuroprotective effect of vitamin D could explain glaucoma severity rather than occurrence is certainly interesting. In this context, it is worth mentioning the work of Uro et al. [27], which report serum 25(OH)D deficiency associated with a reduced ganglion cell complex (GCC) thickness in a cohort of 85 elderly French participants, as measured by high-definition optical coherence tomography ($72.1 \pm 7.4\,\mu$m versus $77.5 \pm 7.5\,\mu$m, $p = 0.028$). Participants were separated into 2 groups according to serum 25(OH)D levels. Only 11 participants were in the deficient group (deficient 25 nmol/l or sufficient > 25 nmol/l); open-angle glaucoma and age-related macular degeneration patients were excluded; the mean age was $71.13\,y \pm 4.71$. Of note is that vitamin D deficiency was not associated with reduced retinal fiber layer (RNLF) thickness. The authors speculated that they detected a neuronal loss affecting the GCC, possibly at an early stage preceding the thinning of the RNFL, which usually occurs in any optic neuropathy. The same group had previously published a study of reduced optical coherence tomography macular thickness in association with vitamin D deficiency [28]. These observations are in line with the speculative theory attributing a neurodegenerative effect of vitamin D insufficiency [5, 6, 27].

In a case-control study of 73 POAG, and 71 controls in the Han population of China, Lv et al. [9] reported $1\alpha,25(OH)_2D_3$ serum levels in POAG patients ($p < 0.001$) fifteen percent lower than in healthy controls (26.37 ng/ml ± 5.83 versus 30.43 ng/ml ± 3.91). Of note is that these levels are within the normal range. Interestingly, they also found a higher frequency of the vitamin D receptor BsmI 'B' (rs1544410) and TaqI 't' (rs731236) polymorphisms in the POAG group than in the control group. Participants who had undertaken vitamin D3 or analogs were excluded from this study. Based on their findings, the authors suggested that the presence of these alleles and the vitamin D deficiency represent risk factors of primary open-angle glaucoma. However, it should be noted that this suggestion was made despite the small number of participants in each group, and the fact that serum $1\alpha,25(OH)_2D_3$ levels were within the normal range, and vitamin D deficiency was not measured. Indeed, circulating $1\alpha,25(OH)_2D_3$ levels are normal in vitamin D deficient individuals unless there is a persistent severe vitamin D deficiency, and the measurements of 1,25D levels in circulation are of poor, if any, value, since its half-life is of a few hours. Authors do not report serum 25(OH)D levels which are relevant for vitamin D deficiency. In addition, the VDR polymorphisms presented in this study may indicate only nonfunctional associations as they do not affect VDR expression or actions.

Our knowledge of the effect of vitamin D deficiency on corneal thickness is limited. Cankaya et al. [29] have observed a lower corneal endothelial cell density and other endothelial indices in a case-control study of 58 patients with vitamin D level below 15 ng/ml compared to 40 normal controls. However, central corneal thickness was not statistically different between the two groups, indicating a preserved function of the endothelium. One can hypothesize that the endothelial function and thus the central corneal thickness could be altered according to the level of vitamin D deficiency. Of note is that the intraocular level of vitamin D metabolites is unknown and certainly influences the consequence on the endothelium. This study has major limitations, the main one being its small size, and its results need to be reproduced and confirmed by other larger studies. If central corneal thickness is increased as a result of vitamin D deficiency, this could constitute a major bias that may have misled the authors, as they have attributed higher IOP to vitamin D instead of simply increased corneal thickness. Nevertheless, Kocaturk et al. [30] reported no statistically significant difference in corneal compensated IOP measured by ocular response analyzer between three groups with, respectively, 41 subjects with serum vitamin $D < 20$ ng/ml, 39 subjects with serum vitamin $D > 20$ and < 30 ng/ml, and 40 healthy controls with serum vitamin $D > 30$ ng/ml. The method of measurement and the measured vitamin D were not specified.

2.2. Experimental Studies. An experimental study on seven monkeys has shown significant reduction of IOP after $1\alpha,25(OH)_2D$ or its analog 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D3 (2MD) was topically applied [31] in one eye compared to placebo in the other eye, with lacrimal duct occlusion. The effect of $1,25(OH)_2D$ (5 mg) lasted more than 12 hours and was more effective than 2MD in lowering IOP (20% versus 15%). No control group other than the contralateral eyes was used in this study. The reduction in IOP was not attributed to reduced aqueous humor formation as measured by fluorometry nor to increased uveoscleral outflow, which was reduced by topical use of pilocarpine, suggesting an effect of the administered products on aqueous humor drainage. The authors also found, based on *in vivo* and *in vitro* microarray analysis, that $1,25(OH)_2D$ regulates genes that are known to be involved in the determination of IOP. $1,25(OH)_2D$ markedly suppressed the expression of the angiotensin I converting enzyme (ACE), the carbonic anhydrase (CAI), and the Ras homolog gene family, member A (RHOA) and significantly increased the expression of the chemokine (C-C motif) ligand 20 (CCL20). $1\alpha,25(OH)_2D$ strongly downregulated the expression of the cytoskeleton genes (alpha and gamma actins), the cell adhesion genes (CEACAM and CD44), and the major extracellular matrix (ECM) genes (RHOA and fibronectin I. $1,25(OH)_2D$ also increased the expression of several other ECM genes, viz., the matrix metalloproteinases 3, 11, 13, and 14, while decreasing the expression of their inhibitor TIMP3. It was found that ECM remodeling in the trabecular meshwork can decrease outflow resistance and

thus increase aqueous humor outflow. Other genes affected by 1,25(OH) $_2$D that could be involved in regulating IOP are the purinergic receptors P2Y, the G-protein coupled, 2 (P2RY2), and the aquaporin 1 channel (AQP1). Of note is a clinical case-control study of Caucasian participants with 382 POAG and 363 healthy controls in which there was no association between common polymorphisms in the AQP1 gene and POAG [32]. This suggests that if vitamin D plays a causative role in POAG, its effect probably does not involve a single gene down- or upregulation, but rather follows a more complex pathway. Undoubtedly, 1,25(OH)$_2$D modulates the expression of genes with multiple benefits on the eye. Nevertheless, these correlations do not demonstrate that correction of vitamin D deficiency should help delay the onset of the defects that 1,25(OH) $_2$D controls, because serum 1,25D levels remain normal despite vitamin D deficiency, except in cases of prolonged severe vitamin D deficiency with 25(OH)D levels below 4 ng/ml. Indeed, it is impossible to measure the amount of local 1,25D production within the eye required to mimic the benefits of topical 1,25D. Consequently, it would be very difficult also to know what would be the levels of circulating 25(OH)D levels that ensure the required local 1,25D production within the eye.

In an experimental study on human tissue, Lv et al. [33] reported a protective effect of 1,25(OH)$_2$D on human trabecular meshwork cells which underwent damage from an oxidative stress induced by inhibition of the TGFbeta-SMAD3-VDR pathway. The latter is the primary pathway regulating extracellular matrix deposition in human trabecular meshwork. Again, reshaping the extracellular matrix of the trabecular meshwork can decrease outflow resistance and thus increase aqueous humor outflow through the conventional pathway.

These two experimental studies seem to strongly relate the effect of vitamin D on IOP to both the conventional outflow pathway, and thus to the trabecular meshwork, and the aqueous humor formation, based on the vitamin D modulation of multiple genes expression. Aqueous humor drains mainly through the trabecular meshwork; any deposition within it, increasing outflow resistance, could cause higher IOP, which in turn is a major risk factor for glaucoma development and progression.

3. Discussion

Out of the five clinical studies described above which treated the subject, only two showed an inverse association between serum level of 25(OH)D and IOP [5, 10], and only one showed an association between 25(OH)D and both IOP and glaucoma [10]. The experimental study on monkeys dealt with topical application of 1,25(OH)$_2$D; it found a 20% reduction of IOP after treatment, which seems to confirm the relation between vitamin D and IOP [31]. The study of human trabecular meshwork suggested a higher aqueous humor outflow through trabecular meshwork [33].

A total of four clinical studies out of five showed a direct association between serum levels of 25(OH)D and the presence of glaucoma [6, 7, 9, 10] (see Table 2). Only two studies reported on vitamin D and both glaucoma presence and severity [5, 6]. Only one of these two reported an association between vitamin D and glaucoma severity [5]. The latter studies suggest that vitamin D has an independent effect on the occurrence of glaucoma, unrelated to the IOP. No study could assess vitamin D level as an independent risk factor for glaucoma incidence or progression. All studies harbored significant differences in the sample size, study design, vitamin D measurements (enzyme-linked immunosorbent assay vs. radioimmunoassay), measured forms of vitamin D (25(OH)D versus 1,25(OH)$_2$D), and study population, which influenced the outcome. Conclusions one draws should thus be very careful.

However, published studies suggest vitamin D could have an effect on IOP through gene expression of aqueous humor production or outflow modulation, or through its neuroprotective effect. Vitamin D has been linked to inflammation modulation through different factors [34–37]. Inflammation factors are known to influence neurodegeneration and its severity, and vitamin D has been suggested to be an influencing factor for inflammation and degeneration of neuronal tissue [38, 39]. In addition, vitamin D deficiency was reported in various studies to be associated with major neurodegenerative diseases, such as Alzheimer's disease [40, 41], multiple sclerosis [42], and clinically isolated syndrome [43]. Supplementation of vitamin D on Alzheimer's and multiple sclerosis is reported to be beneficial [40, 42], although larger studies are needed to confirm this conclusion. Through its role in inflammatory, immune, and antioxidant properties, vitamin D is thus expected to play a key role in neuroprotection. Indeed, vitamin D is believed to exert a protective role against several ocular diseases or conditions by modulating the immune system and inhibiting inflammation and angiogenesis [44]. Similar to its effect on other neurodegenerative diseases, vitamin D deficiency could thus be a significant factor in glaucoma pathogenesis, including its development, progression, and severity. The fact that GCC thinning has been reported in vitamin D deficient patients [27] supports this hypothesis. It is noteworthy that, in Alzheimer's disease, retinal ganglion cell loss and RNFL thinning have been described with OCT [45, 46]. A cumulative contribution of multiple factors is not excluded, as is the possibility of different effect in different ethnicities according to the genetic background. This could explain the different findings on the effect of vitamin D levels on either IOP or glaucoma occurrence.

Only one published study, a case-control study [9], deals with serum 1,25(OH)$_2$D levels as measured in humans. In fact, little is known about the presence of the converting enzyme from 25(OH)D to 1,25(OH)D in the human eye. In rabbit, mouse, and human corneal cell lines, Yin et al. [47] showed that both 25(OH)D_3 and 1,25(OH)$_2$D were present in the eye, as well as vitamin D receptor and 1-alpha-hydroxylase, the enzyme required to convert 25(OH)D to 1,25(OH)$_2$ D. Based on a human corneal cell line, Lin et al. [48] showed that human corneal epithelial cells were capable of synthesizing vitamin D3 metabolites (25(OH)D_3 and 24,25(OH)D_3) following UV-B exposure, in the presence of 7-dehydrocholesterol.

Indeed, the intraocular conversion of circulating 25(OH)D to $1,25(OH)_2D$ could have important local benefits with minimal risk of hypercalcemia if there is sufficient production to mimic the effects of topical $1,25(OH)_2D$ application. The systemic conversion of supplemented vitamin D to 25(OH)D provides the eye with the amount of substrate required for its local conversion to 1,25D for VDR transactivation of the vitamin D responsive genes that are critical for normal eye function. If correction of systemic vitamin D deficiency is not adequate, the local levels of 25(OH)D may not be sufficient for the eye to generate the local 1,25D needed. This would explain the observed lack of effect of 25(OH)D systemic administration on IOP [8] and inversely the effect observed on monkey by applying topical $1,25(OH)_2D$ [31]. Meanwhile, in rabbits fed with a vitamin D-supplemented diet, 25(OH)-vitamin D3 and $1,25(OH)_2$-vitamin D3 increased in aqueous humor [48].

This question calls for future prospective observational studies including 25(OH)D dosage in both normal populations and glaucoma patients. Since $1,25(OH)_2D$ has already been described as attenuating stress-induced damage in human trabecular meshwork cells [33], experimental therapy in glaucoma animal models with systematic, topical, and/or intraocular supplementation of $1,25(OH)_2D$ would be of interest in order to characterize the effect of $1,25(OH)_2D$ on IOP and eventual glaucoma progression. It could also pave the way to potential new therapeutic vitamin D related pathways. Larger prospective population-based studies of different ethnicities are needed in order to confirm and further determine the strength of the presumed association in normal and glaucoma patients.

In summary, despite contradicting clinical reports, it seems that vitamin D metabolites may play a role in glaucoma, through either a lowering effect of IOP or a neuroprotection pathway. However, all the clinical studies discussed in this review had a rather limited number of participants and significant bias determined by the chosen study design, precluding the conclusions regarding the involvement of vitamin D in primary angle glaucoma or in IOP. Experimental studies confirm an association between vitamin D and IOP and open the way to pathophysiological explanations of its occurrence. However, the exact molecular mechanism underlying this potential association is still unknown. In this context, population-based studies are needed to give us information on IOP and glaucoma and their eventual association with vitamin D metabolites. Targeting vitamin D could represent a new therapeutic pathway for glaucoma which, given the burden of glaucoma disease, deserves to be thoroughly investigated.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors are grateful to the Mémorial de Recherche Clinique en Ophtalmologie, Mémorial Adolphe de Rothschild, Geneva, Switzerland.

References

[1] Y.-C. Tham, X. Li, T. Y. Wong, H. A. Quigley, T. Aung, and C.-Y. Cheng, "Global prevalence of glaucoma and projections of glaucoma burden through 2040," *Ophthalmology*, vol. 121, no. 11, pp. 2081–2090, 2014.

[2] A. L. Coleman and S. Miglior, "Risk factors for glaucoma onset and progression," *Surv Ophthalmol*, vol. 53, no. Suppl 1, pp. S3–10, 2008.

[3] M. C. Leske, A. Heijl, M. Hussein et al., "Factors for glaucoma progression and the effect of treatment," *Archives of Ophthalmology*, vol. 121, no. 1, pp. 48–56, 2003.

[4] R. Hoehn, A. Mirshahi, E. M. Hoffmann et al., "Distribution of intraocular pressure and its association with ocular features and cardiovascular risk factors," *Ophthalmology*, vol. 120, no. 5, pp. 961–968, 2013.

[5] R. Ayyagari, Y. I. Chen, L. M. Zangwill et al., "Association of severity of primary open-angle glaucoma with serum vitamin D levels in patients of African descent," *Molecular Vision*, vol. 25, pp. 438–445, 2019.

[6] A. Goncalves, D. Milea, P. Gohier et al., "Serum vitamin D status is associated with the presence but not the severity of primary open angle glaucoma," *Maturitas*, vol. 81, no. 4, pp. 470–474, 2015.

[7] H. T. Kim, J. M. Kim, J. H. Kim et al., "The relationship between vitamin D and glaucoma: a kangbuk samsung health study," *Korean Journal of Ophthalmology*, vol. 30, no. 6, pp. 426–433, 2016.

[8] E. A. Krefting, R. Jorde, T. Christoffersen, and G. Grimnes, "Vitamin D and intraocular pressure—results from a case -control and an intervention study," *Acta Ophthalmologica*, vol. 92, no. 4, pp. 345–349, 2014.

[9] Y. Lv, Q. Yao, W. Ma, H. Liu, J. Ji, and X. Li, "Associations of vitamin D deficiency and vitamin D receptor (Cdx-2, Fok I, Bsm I and Taq I) polymorphisms with the risk of primary open-angle glaucoma," *BMC Ophthalmology*, vol. 16, p. 116, 2016.

[10] T. K. Yoo, E. Oh, and S. Hong, "Is vitamin D status associated with open-angle glaucoma? A cross-sectional study from South Korea," *Public Health Nutrition*, vol. 17, no. 4, pp. 833–843, 2014.

[11] R. Scragg, M. Sowers, and C. Bell, "Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the third national health and nutrition examination survey," *Diabetes Care*, vol. 27, no. 12, pp. 2813–2818, 2004.

[12] M. C. Leske, A. M. Connell, A. P. Schachat, and L. Hyman, "The Barbados eye study," *Archives of Ophthalmology*, vol. 112, no. 6, pp. 821–829, 1994.

[13] M. C. Leske, A. M. S. Connell, S.-Y. Wu, L. Hyman, and A. P. Schachat, "Distribution of intraocular pressure," *Archives of Ophthalmology*, vol. 115, no. 8, pp. 1051–1057, 1997.

[14] K. Makris, C. Sempos, and E. Cavalier, "The measurement of vitamin D metabolites: part I-metabolism of vitamin D and the measurement of 25-hydroxyvitamin D," *Hormones (Athens)*, vol. 19, no. 2, pp. 81–96, 2020.

[15] G. Jones, "Extrarenal vitamin D activation and interactions between vitamin D2, vitamin D3, and vitamin D analogs," *Annual Review of Nutrition*, vol. 33, no. 1, pp. 23–44, 2013.

[16] A. Dusso, A. Brown, and E. Slatopolsky, "Extrarenal production of calcitriol," *Seminars in Nephrology*, vol. 14, no. 2, pp. 144–155, 1994.

[17] S. Christakos, P. Dhawan, A. Verstuyf, L. Verlinden, and G. Carmeliet, "Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects," *Physiological Reviews*, vol. 96, no. 1, pp. 365–408, 2016.

[18] S. Sirajudeen, I. Shah, and A. Al Menhali, "A narrative role of vitamin D and its receptor: with current evidence on the gastric tissues," *International Journal of Molecular Sciences*, vol. 20, no. 15, p. 3822, 2019.

[19] M. A. Maestro, F. Molnár, A. Mouriño, and C. Carlberg, "Vitamin D receptor 2016: novel ligands and structural insights," *Expert Opinion on Therapeutic Patents*, vol. 26, no. 11, pp. 1291–1306, 2016.

[20] J. W. CraigLanders and M. B. Meyer, "Fundamentals of vitamin D hormone-regulated gene expression," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 144, pp. 5–11, 2014.

[21] M. Abouzid, M. Karazniewicz-Lada, and F. Glowka, "Genetic determinants of vitamin D-related disorders; focus on vitamin D receptor," *Current Drug Metabolism*, vol. 19, no. 12, pp. 1042–1052, 2018.

[22] S. Christakos, M. Hewison, D. G. Gardner et al., "Vitamin D: beyond bone," *Annals of the New York Academy of Sciences*, vol. 1287, no. 1, pp. 45–58, 2013.

[23] S.-M. Jeon and E.-A. Shin, "Exploring vitamin D metabolism and function in cancer," *Experimental & Molecular Medicine*, vol. 50, no. 4, p. 20, 2018.

[24] M. Miraglia Del Giudice, C. Indolfi, and C. Strisciuglio, "Vitamin D: immunomodulatory aspects," *Journal of Clinical Gastroenterology*, vol. 52, no. Suppl 1, pp. S86–S88, 2018, Proceedings from the 9th Probiotics, Prebiotics and New Foods, Nutraceuticals and Botanicals for Nutrition & Human and Microbiota Health Meeting, held in Rome, Italy from September 10 to 12, 2017.

[25] D. D. Bikle, "Vitamin D metabolism, mechanism of action, and clinical applications," *Chemistry & Biology*, vol. 21, no. 3, pp. 319–329, 2014.

[26] S. Mazzaferro, D. Goldsmith, T. E. Larsson, Z. A. Massy, and M. Cozzolino, "Vitamin D metabolites and/or analogs: which D for which patient?" *Current Vascular Pharmacology*, vol. 12, no. 2, pp. 339–349, 2014.

[27] M. Uro, O. Beauchet, M. Cherif, A. Graffe, D. Milea, and C. Annweiler, "Age-related vitamin D deficiency is associated with reduced macular ganglion cell complex: a cross-sectional high-definition optical coherence tomography study," *PLoS One*, vol. 10, no. 6, Article ID e0130879, 2015.

[28] A. Graffe, O. Beauchet, B. Fantino, D. Milea, and C. Annweiler, "Vitamin D and macular thickness in the elderly: an optical coherence tomography study," *Investigative Opthalmology & Visual Science*, vol. 55, no. 8, pp. 5298–5303, 2014.

[29] C. Cankaya, T. Cumurcu, and A. Gunduz, "Corneal endothelial changes in patients with vitamin D deficiency," *Indian Journal of Ophthalmology*, vol. 66, no. 9, pp. 1256–1261, 2018.

[30] T. Kocaturk, S. Bekmez, and M. Unubol, "Effects of vitamin D deficiency on intraocular pressure values obtained by ocular response analyzer," *International Ophthalmology*, vol. 40, no. 3, pp. 697–701, 2019.

[31] G. D. Kutuzova, B. A. T. Gabelt, J. A. Kiland, E. A. Hennes-Beann, P. L. Kaufman, and H. F. DeLuca, "1α,25-Dihydroxyvitamin D3 and its analog, 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D3 (2MD), suppress intraocular pressure in non-human primates," *Archives of Biochemistry and Biophysics*, vol. 518, no. 1, pp. 53–60, 2012.

[32] W. Liu, Y. Liu, X. J. Qin, S. Schmidt, M. A. Hauser, and R. R. Allingham, "AQP1 and SLC4A10 as candidate genes for primary open-angle glaucoma," *Molecular Vision*, vol. 20, no. 16, pp. 93–97, 2010.

[33] Y. Lv, X. Han, Q. Yao et al., "1α,25-dihydroxyvitamin D3 attenuates oxidative stress-induced damage in human trabecular meshwork cells by inhibiting TGFβ-SMAD3-VDR pathway," *Biochemical and Biophysical Research Communications*, vol. 516, no. 1, pp. 75–81, 2019.

[34] F. Colotta, B. Jansson, and F. Bonelli, "Modulation of inflammatory and immune responses by vitamin D," *Journal of Autoimmunity*, vol. 85, pp. 78–97, 2017.

[35] W. Liu, L. Zhang, H. J. Xu et al., "The anti-inflammatory effects of vitamin D in tumorigenesis," *International Journal of Molecular Sciences*, vol. 19, no. 9, p. 2736, 2018.

[36] M. Mangin, R. Sinha, and K. Fincher, "Inflammation and vitamin D: the infection connection," *Inflammation Research*, vol. 63, no. 10, pp. 803–819, 2014.

[37] T. K. Wöbke, B. L. Sorg, and D. Steinhilber, "Vitamin D in inflammatory diseases," *Frontiers in Physiology*, vol. 5, p. 244, 2014.

[38] T. L. Briones and H. Darwish, "Vitamin D mitigates age-related cognitive decline through the modulation of pro-inflammatory state and decrease in amyloid burden," *Journal of Neuroinflammation*, vol. 9, p. 244, 2012.

[39] K.-P. Jiao, S.-M. Li, W.-Y. Lv, M.-L. Jv, and H.-Y. He, "Vitamin D3 repressed astrocyte activation following lipopolysaccharide stimulation in vitro and in neonatal rats," *Neuroreport*, vol. 28, no. 9, pp. 492–497, 2017.

[40] M. O. W. Grimm, A. Thiel, A. A. Lauer et al., "Vitamin D and its analogues decrease amyloid-beta (abeta) formation and increase abeta-degradation," *International Journal of Molecular Sciences*, vol. 18, no. 12, p. 2764, 2017.

[41] T. J. Littlejohns, W. E. Henley, I. A. Lang et al., "Vitamin D and the risk of dementia and Alzheimer disease," *Neurology*, vol. 83, no. 10, pp. 920–928, 2014.

[42] B. S. Martina, M. Rametta, and A. T. Anthony, "Reder vitamin D and multiple sclerosis: a comprehensive review," *Neurology and Therapy*, vol. 7, no. 1, pp. 59–85, 2018.

[43] K. O'Connell, J. Sulaimani, S. A. Basdeo et al., "Effects of vitamin D(3) in clinically isolated syndrome and healthy control participants: a double-blind randomised controlled trial," *Multiple Sclerosis Journal—Experimental, Translational and Clinical*, vol. 3, no. 3, 2017.

[44] M. Nebbioso, G. Buomprisco, A. Pascarella, and N. Pescosolido, "Modulatory effects of 1,25-dihydroxyvitamin D3 on eye disorders: a critical review," *Critical Reviews in Food Science and Nutrition*, vol. 57, no. 3, pp. 559–565, 2017.

[45] V. T. T. Chan, Z. Sun, S. Tang et al., "Spectral-domain OCT measurements in alzheimer's disease," *Ophthalmology*, vol. 126, no. 4, pp. 497–510, 2019.

[46] A. Kesler, V. Vakhapova, A. D. Korczyn, E. Naftaliev, and M. Neudorfer, "Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease," *Clinical Neurology and Neurosurgery*, vol. 113, no. 7, pp. 523–526, 2011.

[47] Z. Yin, V. Pintea, Y. Lin, B. D. Hammock, and M. A. Watsky, "Vitamin D enhances corneal epithelial barrier function," *Investigative Opthalmology & Visual Science*, vol. 52, no. 10, pp. 7359–7364, 2011.

[48] Y. Lin, J. L. Ubels, M. P. Schotanus et al., "Enhancement of vitamin D metabolites in the eye following vitamin D3 supplementation and UV-B irradiation," *Current Eye Research*, vol. 37, no. 10, pp. 871–878, 2012.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 3595610, 8 pages
https://doi.org/10.1155/2020/3595610

Research Article

Digital Image Analysis of the Angle and Optic Nerve: A Simple, Fast, and Low-Cost Method for Glaucoma Assessment

Greg Russell,[1,2] Silvia N. W. Hertzberg,[3] Natalia Anisimova,[4,5] Natalia Gavrilova,[5] Beáta É. Petrovski,[6] and Goran Petrovski[2,3]

[1]*Eyenuk Inc., Clinical Development, Woodland Hills, CA, USA*
[2]*University of Szeged, Department of Ophthalmology, Szeged, Hungary*
[3]*Center for Eye Research, Department of Ophthalmology, Oslo University Hospital, Institute for Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway*
[4]*The A. I. Evdokimov* Moscow *State University of Medicine and Dentistry of the Ministry of Healthcare the Russian Federation, Moscow, Russia*
[5]*Eye Center "Vostok-Prozrenie", Moscow, Russia*
[6]*Faculty of Dentistry, University of Oslo, Oslo, Norway*

Correspondence should be addressed to Goran Petrovski; goran.petrovski@medisin.uio.no

Received 31 January 2020; Accepted 17 February 2020; Published 28 October 2020

Guest Editor: Barbara Cvenkel

Purpose. To devise a simple, fast, and low-cost method for glaucoma assessment using digital image analysis of the angle and optic nerve in human subjects. *Methods*. Images from glaucoma and fundus assessment were used in this study, including color fundus photographs, standard optic nerve optical coherence tomography (OCT), and digital slit-lamp images of the angle/gonioscopy. Digital image conversion and analysis of the angle using ImageJ (NIH, USA) and adaptive histogram equalization contrast-limited AHE (CLAHE) to prevent noise amplification were implemented. Angle and optic nerve images were analyzed separately in the red, green, and blue (RGB) channels followed by 3D volumetric analysis of the degrees of angle depth and cup volume of the optic nerve. Horizontal tomogram reconstitution and nerve fiber detection methods were developed and compared to standard OCT images. *Results*. Digital slit-lamp angle images showed similar accuracy as standard anterior OCT measurements. Comparative analysis of RGB channels produced volumetric cup and horizontal tomogram, which closely resembled the 3D OCT appearance and *B*-scan of the cup, respectively. RGB channel splitting and image subtraction produced a map closely resembling that of the retinal nerve fiber layer (RNFL) thickness map on OCT. *Conclusions*. While OCT imaging is rapidly progressing in the area of optic disc and chamber angle assessment, rising healthcare costs and lack of availability of the technology open a demand for alternative and cost-minimizing forms of image analysis in glaucoma. Volumetric, geometric, and segmentational data obtained through digital image analysis correspond well to those obtained by OCT imaging.

1. Introduction

The need for improved management of glaucoma seems obvious with the increasing prevalence worldwide. A recent study correctly predicted that a total of 79.6 million people will be affected by glaucoma in 2020, which is indeed the case today. Out of these, bilateral blindness was estimated to occur in 11.2 million [1]. The following 20 years might bring rise in the number of glaucoma patients up to 111.8 million [2]. While the prevalence of glaucoma has a societal burden, there is an associated economic burden, which affects both developing and developed countries with at least the United States spending approximately $6 billion in 2013; the latter accounted for productivity losses and individual patient care per year [3]. In the United Kingdom, medical costs were approximately over 40% of the direct costs [4]. In sub-Saharan African countries, the lowest income patients diagnosed with glaucoma can spend

almost 100% of their salary on treatment, while middle-income earners spent at least 50% of their salary on the disease treatment [5, 6].

Glaucoma is classified as a group of diseases with progressive damage to the optic nerve. It can be divided into primary open angle glaucoma (POAG), primary angle-closure glaucoma (PACG), and secondary glaucoma. The most recognized risk factor for glaucomatous progression is increased intraocular pressure (IOP), which occurs in eyes with an imbalance between aqueous liquor production and drainage through the angle. In this matter, the angle drainage becomes essential, and angle anatomy is doubtlessly an important factor for assessment in glaucoma management. Worldwide, the prevalence of POAG shows variation, which can be attributed to multiple factors including ethnicity. Meta-analysis reveals a prevalence of POAG of 1.4% in Asian, 2.0% in Caucasian, and 4.2% in the African population [7]. In contrast, PACG occurs more frequently in Asia than in Europe with estimates of 1.5% and 0.04%, respectively [8, 9].

The burden of blindness from glaucoma is challenged not only by the increasing number of cases but also by the difficulties to recognize the affected population. Glaucoma is an asymptomatic disease, which only raises suspicion when it becomes advanced. Studies have estimated the prevalence of undetected glaucoma to be 50% in developed countries and up to a striking 98.5% in developing countries [10–12]. On the other hand, approximately half of the patients diagnosed and treated with glaucoma are found to be ocular hypertensives that may not be benefiting from treatment at all [11, 13, 14].

The notable number of undetected glaucoma cases and overtreated patients has given rise to multiple evaluations of screening tools to trace unrecognized glaucoma. Yet, the United States Preventive Services Task Force (USPSTF) has indicated a lack of convincing evidence to facilitate regular screening, whereas no screening tool has been shown cost-beneficial to date [15, 16]. The current effort focuses on how to manage and follow the increasing number of patients with glaucoma [15]. Nevertheless, there is an increasing demand for organized screening programs that are cost-effective to prevent the load of future visual impairment in glaucoma patients, similar to diabetic retinopathy screening [10, 12, 15].

In addition to the increasing burden of glaucoma, the imminent shortage of healthcare professionals worldwide strengthens the need for the development of cost-beneficial screening methods [17, 18]. In this matter, emphasis on office visits may shift to alternative methods with reading centers or even automated image analysis. While noninvasive optical coherence tomography (OCT) is rapidly progressing in means of optic disc and chamber angle assessment and is considered a standard in angle-closure detection compared to gonioscopy [19], with rising healthcare costs, demand and availability for the technology can split, leaving lower-financed practices short of aid. Other simple, fast, and low-cost forms of image analysis need to be incorporated to help in the management of glaucoma or to preassess those attending for care. The sharp rise in the number of smartphones with high resolution cameras worldwide poses a potential source of documentation, storage, sharing, and eventually image analysis of large numbers of photographs taken to be used in ophthalmic decision-making [20–23]. Though, at present, image acquisition and interpretation are still having many limitations, it seems reasonable to develop methods and algorithms that can assess a mass of people at an affordable price to allocate further attention to those who are in need of medical care. In this pilot study, we present the information that is to be gained from color photographs of the fundus and the chamber angle, regarding glaucoma. Volumetric, geometric, and segmentational data are gathered and compared with corresponding high-definition OCT images to test for comparison and possible validity, while the cost-benefits of such processing are shown as well.

2. Methods

Images from glaucoma and fundus assessment were used in this study, including freely available/online color fundus photographs, standard optic nerve optical coherence tomography (OCT), and additional digital slit-lamp images of the angle obtained by gonioscopy (no patient contact was needed for the image analysis, and the whole process was carried out without knowing any personal data for the images included in the processing). Applying the diagnostic criteria for glaucoma, a novel method is hereby aimed at showing similarities and possible data correlation between findings, data presented by *B*-scans, and the provided standard grade for glaucoma.

2.1. Contrast Enhancement. Standardization of the fundus images was achieved through known methods of image processing by ImageJ (NIH, USA). Normalization of the histographic information across each image was achieved as volumetric representation and was derived from image intensity. As image intensity varied according to camera exposures, histogram equalization (HE) was needed to standardize the images. In addition, comparative to HE, contrast-limited adaptive histogram equalization (CLAHE), a tool designed to prevent the over amplification of noise that adaptive HE can give rise to, was used in the study. Splitting of the color fundus images into red, green, and blue (RGB) channels was performed to achieve optimal comparison and dynamic range of the images acquired.

2.2. 3D Volumetric Measurement of Image Intensity and Analysis of the Profile of the Cup. After application of CLAHE in the regions of interest (ROI), the images were converted into a 3D representation in ImageJ by adjusting volumetric measurements of the image intensity through a 3D rendering on the screen and an interactive 3D-surface plot function. Splitting of the RGB was carried out consequently, and the optimal channel was selected for further analysis. Images taken from the fundus were imported into ImageJ for further analysis.

2.3. Retinal Nerve Fiber Layer (RNFL) Detection Method. Images were split into RGB, and then, the differences between the channels were observed, and a spectrum lookup table was applied to allow image intensities to be compared to the gold standard images and data produced by the RNFL analysis given by the spectral domain Topcon 3D-2000 OCT. RGB fundus imaging separates a fundus image into three wavelength components to help visualize the retinal pigment epithelium/choroid (red filter), neural retina (green filter), and nerve fiber layer (blue filter). This allows for better visualization of the retinal tissues [24].

3. Results

3.1. Contrast Enhancement. RGB color splitting allowed for a more averaged distribution of the intensity range across the ROI selected by the user (Figure 1). The color fundus and gonioscopy images were split into RGB channels to allow for expert observation and comparison of findings. The green channel was found to show the highest dynamic range for the anterior images and was used to further select the ROI to be processed with the CLAHE filter.

3.2. 3D Volumetric Measurement of Image Intensity in the Angle. Parameters in the screen were adjusted to the range as shown in Figure 2 for the gonioscopy images before any adjustments, to avoid clipping of the intensity range, and then, the angle was calculated as shown. The detected angle was then compared to the angle shown from the anterior OCT scan.

3.3. 3D Volumetric Measurement of Image Intensity of the Fundus and Analysis of the Cup Profile. The review of split RGB channels concluded that the green channel harvested better information (Figure 1). However, when applied to the fundus images and the optic nerve (Figure 3), the red channels offered a less-obstructed view of the cup region of the images compared to the blue or green channel. It was considered important here for the dynamic range to support analysis of the cup volume through the profile shown.

The images, both by profile and when rendered in 3D, appeared to show a close resemblance to the tomographical images shown in Figure 4.

3.4. RNFL Detection. Splitting of the RGB channels in the fundus/optic nerve images (Figure 5) and obtaining differential images between the color channels resulted in the blue channel showing similar intensity when analyzed by ImageJ as the Topcon 3D OCT channel shown in the same figure.

4. Discussion

4.1. Image Analysis Technique. Normalization of histographic information across angle images obtained from gonioscopy is important to standardize the image intensity across a dynamic range and across the image ROIs. The CLAHE filter can be applied initially to RGB split images to look for the best possible profile in the intensity images. Figure 5 illustrates the strength of the green channel, showing more dynamic range and resolving more information than the other color profiles do.

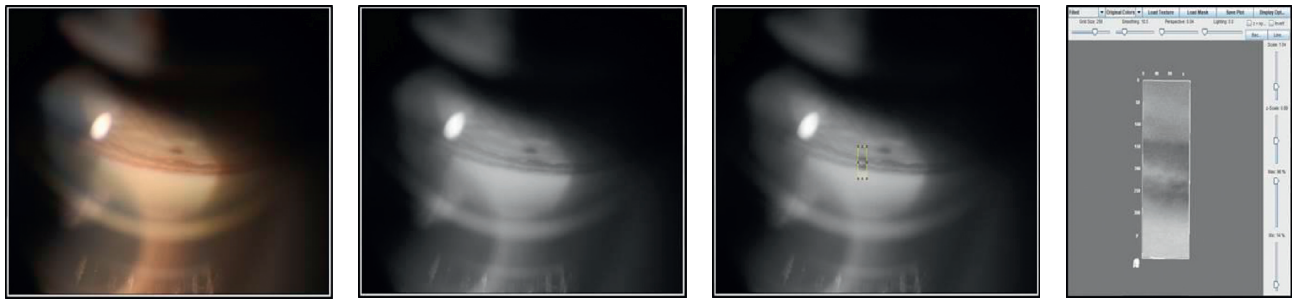
ImageJ splitting of fundus images into red, green, and blue color channels allows a simple way to obtain monochromatic renderings from any full color acquisitions; a disadvantage of doing so is that some loss in resolution can occur as a result of viewing just a single channel. Nevertheless, as exposure often needs to be increased to compensate for light loss when a physical filter is introduced into the light path of the fundus camera, this splitting technique allows for a more comfortable exposure for the patient when taking the image.

Green light is typically focused deeper into the retina, while red light is particularly good for visualizing the retinal pigment epithelium and the choroid, and blue light is good for determining the nerve fiber layer. Digital retinal photography is unique in comparison to film-based techniques; it allows for the immediate adjustment of exposure settings and offers easy contrast enhancement. The disadvantage to digital imaging is the linear response and narrow exposure latitude of currently available digital sensors. The problem of dynamic range has been acknowledged by a number of digital single lens reflex (DSLR) manufacturers; so, for example, some cameras have an automatic exposure bracketing mode that is used in conjunction with the high dynamic range imaging software. Some sensors such as the Fujifilm Super CCD combines sensors of different sizes to give increased dynamic range, while other manufacturers use in-camera software to prevent highlight overexposure such as the D-Lighting feature from Nikon.

Unlike the traditional single-lens reflect camera, screeners take images on a fundus camera where the exposure settings have been typically predetermined by the software for standardization. The only variable the screener has to mitigate is the limited dynamic range of digital sensors due to small pupil settings or the flash level of the fundus camera. This is especially important to consider when imaging the optic nerve because of the stark contrast between the nerve and the surrounding retina. Images can also suffer from degradation caused by media opacity, and assessability has to be considered before reading such images [25].

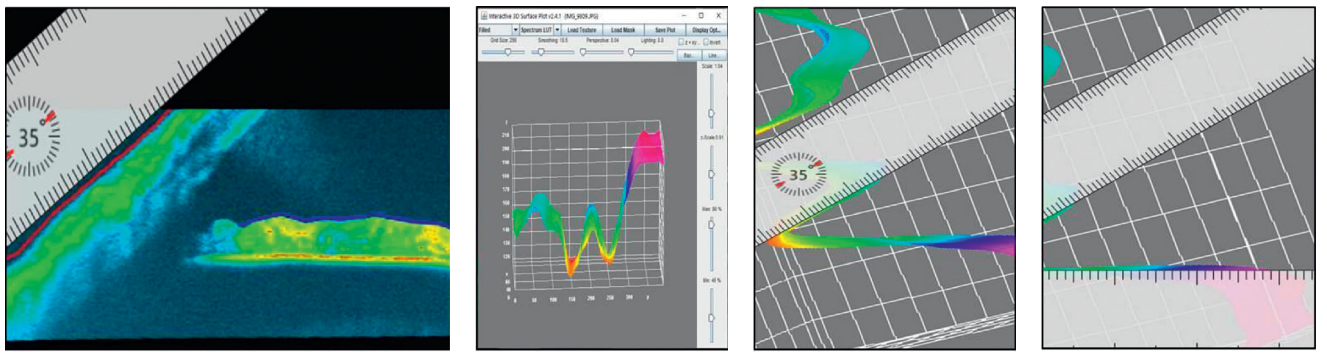
It is important to have proper exposure as well; over or under exposure can be detrimental to digital image quality, while the exposure control requires a delicate balance between flash output, sensor gain, and gamma settings. The International Standards Organization (ISO) has a standardized scale for measuring the sensitivity of the film to light. These standards have traditionally been used with film-based fundus cameras [26].

The 3D volumetric measurement of image intensity and analysis of the profile of the cup was made possible by applying CLAHE in the ROI and by conversion of the images into 3D representative images in ImageJ and adjusting the volumetric measurements of the image intensity through a 3D rendering on the screen and an interactive 3D-surface plot function. Splitting of the RGB allowed for selection of optimal channel for further analysis. Using multiple filters in



(a) (b) (c) (d)

Figure 1: Green channel of a gonioscopy image used for ROI selection and consequent CLAHE filtering. (a) Gonioscopy image. (b) 8-bit converted image. (c) Selection of area of interest. (d) Interactive 3D-surface plot.



OCT-based angle measurement (a)

Gonioscopy-based image processing (b)

Gonioscopy-based angle measurement (c)

Gonioscopy-based angle measurement (d)

Figure 2: 3D measurement of the image intensity and angle from gonioscopy images in comparison to angle measurements obtained by anterior OCT. A gonioscopy-based image processing includes intensity adjustment and clipping of the intensity range, followed by angle measurement; for comparison, the corresponding angle obtained by anterior OCT measurement is shown. (a) OCT-based angle measurement. (b) Gonioscopy-based image processing. (c) Gonioscopy-based angle measurement. (d) Gonioscopy-based angle measurement.

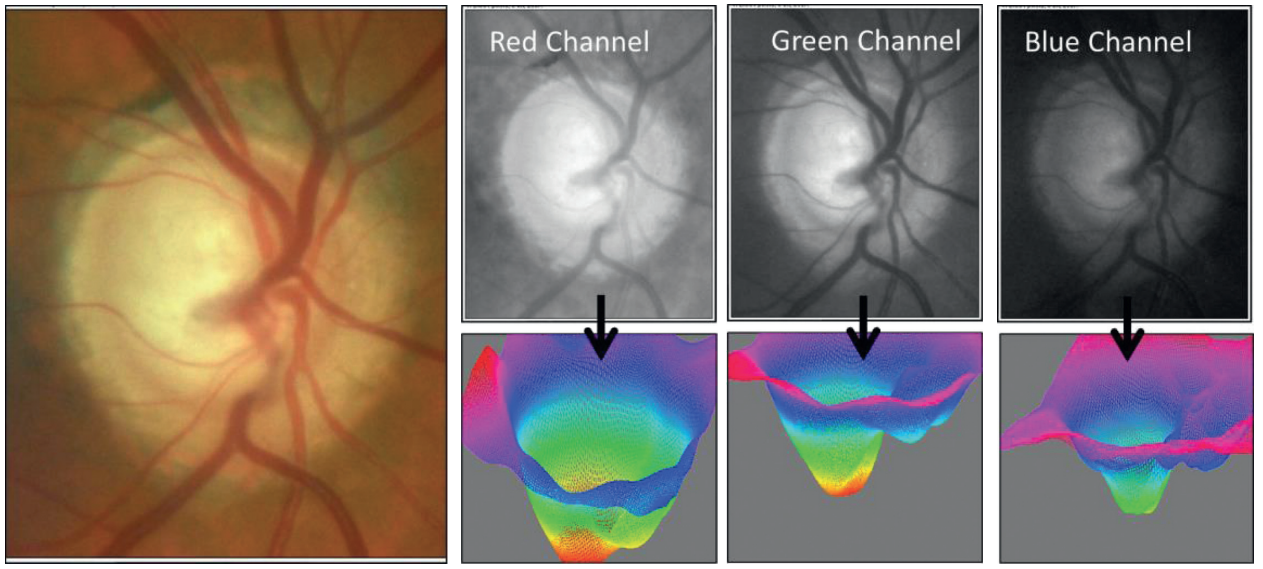


Figure 3: Splitting of the RGB channels in fundus images of the optic nerve and 3D volumetric conversion.

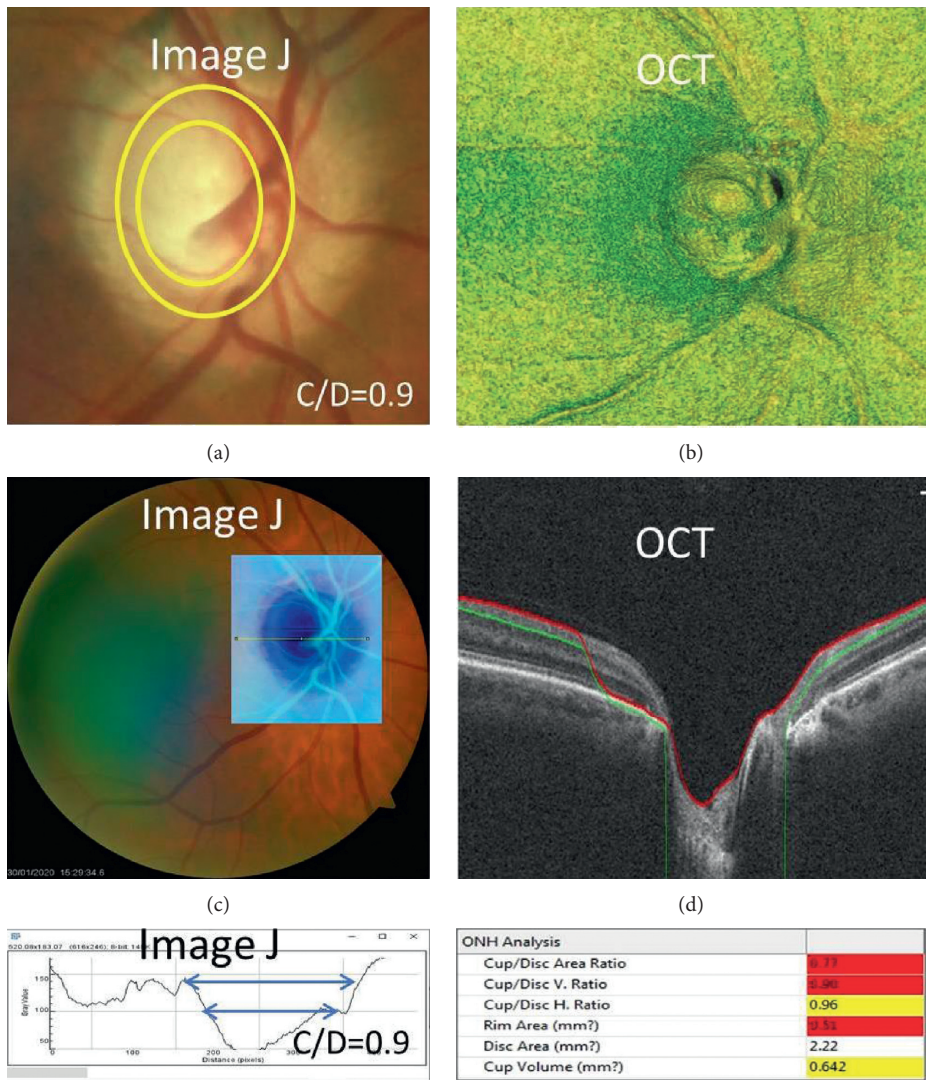


Figure 4: Resemblance of the ImageJ histogram and cup/disc (C/D) ratio based on the 3D fundus images compared to the analogous images obtained by OCT.

a photo assay style approach, one can provide the examiner with more than one monochromatic interpretation of the fundus, as is the case of epiretinal membranes and macular pigmentary changes detected at 490 nm, 540 nm, and 615 nm wavelengths.

Using a modern retinal fundus camera, retinal ganglion axons can also be directly observed. The fine nerve fibers reflect when imaged [27] and allow implementation of a scoring system; furthermore, texture analysis for severe RNFL defects can be performed from such images [28]. The microtexture analysis of the RNFL in the grey level digitized photographs has been described before [29]; depending on the aperture of the pupil, the flash intensity settings, and presence of, if any, polarization or cross polarizing filters in the optical pathway, appropriate images for assessment of RNFL can be obtained. One would also need to ensure some degree of normalization to average out the color range, hence our use of the CLAHE contrast enhancement filter. In the present study, we made an attempt to 'color' the nerve fiber layer with a lookup table to show spectral analysis and compare it to the OCT structural texture analysis. Under such conditions, the dynamic and tonal range is important to be able to see the finest differences within the visible color gamut. The justification for using the blue channel comes naturally from the optical properties of the blue filter, which corresponds to the absorption spectrum of rhodopsin, which is around 500 nm.

4.2. Possible Applications in Ophthalmology. Multiple examinations are used in conjunction for diagnosis of glaucoma. The lack of a definitive reference standard limits both their specificity and sensitivity. Furthermore, there is no evidence that any combination of tests is superior in terms of patient outcome or cost-effectiveness [30]. Glaucoma is most frequently diagnosed via opportunistic case findings.

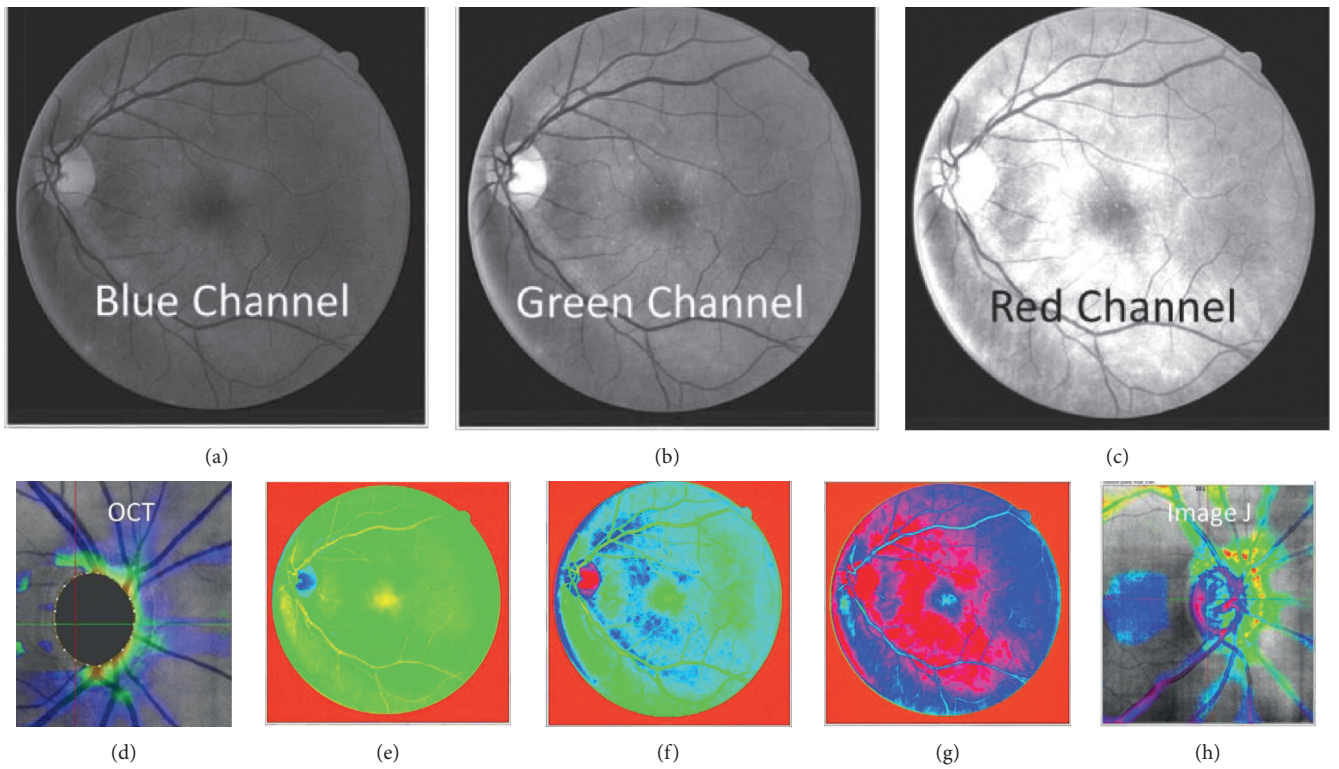


Figure 5: Splitting of the RGB channels in fundus images/optic nerve and resemblance of the blue channel ImageJ to the OCT thickness map of the RNFL.

Of all parameters that are taken into consideration, many practitioners are still relying heavily on IOP measurements, while disc assessment alone with or without visual field damage is underrepresented [31]. Findings suggest that variation amongst observers is large, and the rate of improper diagnosis is high, with more than half of glaucoma patients being missed on former ophthalmic visits [32]. With glaucoma being an optic neuropathy with functional loss occurring at later stages [33], it would seem reasonable to give emphasis to structural changes first, if one were to detect those at risk of a debilitating future visual disturbance and allocate further appointments to specialist care accordingly. Because the quality of life can be severely impaired if the disease is advanced bilaterally [34], selected cases require further attention. Current experience in glaucoma screening is not promising. The range of sensitivity and specificity for tests is large, while the study design, execution, and examined populations differ greatly. Though some tests may outperform others, no combination suitable for implementation to the general population has been established [35]. Identifying the population at risk may pose some challenges. Performing a community-based mass screening in an office setting has high financial, temporal, and professional requirements, thus not suitable for health systems already lacking funding. Narrowing of the target population is highly desired. Prepublicizing the risk factors for glaucoma does not seem to increase the number of patients successfully screened, for in self-recruited screening, overall health anxiety may surpass the actual rate of risk factors present [36]. Several studies suggest that there is a possible relationship between diabetes and POAG, the condition occurring almost twice as often in diabetics than in non-diabetics [37, 38]. It would seem plausible to use the large amount of fundus photos acquired during diabetic screening to devise and test an algorithm that can aid in the detection of optic disc pathologies, notably glaucoma. Additional costs for screening this way would be minimal, and a higher-than-normal prevalence of POAG might be anticipated. Furthermore, as individuals screened for any reason might have a false sense of security that they underwent a comprehensive eye test [39], screening for more than one condition could improve the hit ratio and lower the number of missed follow-up visits.

When making the diagnosis of glaucoma, physicians fail to properly complete gonioscopy about 50% of the time [40]. The true prevalence of PACG can easily be underestimated. Since all cases of glaucoma should be considered closed until confirmed otherwise [41], gonioscopy is an essential and compulsory tool for decision-making. PACG, although less frequent than POAG, leads to blindness more often; therefore, screening for narrow or closed angles would be worthwhile. Currently, only a few reports exist discussing the topic, mainly in well-defined Asian subgroups [8]. A popular method for detecting narrow angles, the Van Herick method for assessing the peripheral limbal depth, has been found to have a sensitivity and specificity of 61.9% and 89.3%, respectively [42]. Even with emerging OCT technology [19], 360° visualization of the drainage structures is

the best achieved by gonioscopy, which is still a gold standard in angle grading. To our current knowledge, total internal reflection from the chamber angle cannot be surpassed; otherwise, the need of a contact gonio lens appears to be a major weakness in designing a fast, infection-proof, noncontact, and reliable method suitable for screening. Nevertheless, image analysis of angle anatomy can be useful for future implications. Indeed, by applying the same contrast enhancement and volumetric measurement techniques described above on the temporal corneal periphery in photographs of the anterior segment, we can simulate the examination without the need of an additional light source for the slit beam.

Screening strategies for glaucoma with early diagnostic and prognostic value may decrease the societal cost in all regions of the world, especially among low-income earners. With the rising availability of less costly retina digital cameras, which cost 80% less to acquire than an OCT equipment itself [43, 44], a cost-minimizing image assessment system may lead to decreased costs and reduced individual burden associated to glaucoma such as fear of blindness, psychological health, and physical functioning due to eye sight loss. In addition, given the needed training in an optometry setting, such assessment can reduce the number of referrals for double screening, which takes on patient time, and which may also lead to disease progression. This will also provide a form of standardization for the rising concern about optometrists using gonioscopy in the primary care setting for glaucoma detection and the need for training nonophthalmic personnel in screening techniques, which can increase the sensitivity and specificity to a level of accurate positive prediction (62%) [45–48].

Altogether, we hereby present an alternative low-cost way to detect and manage glaucoma prospectively by applying a glaucoma assessment method using volumetric, geometric, and segmentational data obtained through digital image analysis, which correspond well to those obtained by high-definition OCT imaging. While the OCT technology is rapidly progressing in the area of optic disc and chamber angle assessment, rising healthcare costs and lack of availability of the technology open a demand for alternative forms of image analysis in glaucoma.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] H. A. Quigley and A. T. Broman, "The number of people with glaucoma worldwide in 2010 and 2020," *British Journal of Ophthalmology*, vol. 90, no. 3, pp. 262–267, 2006.

[2] Y.-C. Tham, X. Li, T. Y. Wong, H. A. Quigley, T. Aung, and C.-Y. Cheng, "Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis," *Ophthalmology*, vol. 121, no. 11, pp. 2081–2090, 2014.

[3] J. S. Wittenborn and D. B. Rein, "Cost of vision problems: the economic burden of vision loss and eye disorders in the United States," *Ophthalmology*, vol. 60603, 2013.

[4] C. E. Traverso, J. G. Walt, S. P. Kelly et al., "Direct costs of glaucoma and severity of the disease: a multinational long term study of resource utilisation in Europe," *British Journal of Ophthalmology*, vol. 89, no. 10, pp. 1245–1249, 2005.

[5] A. O. Adio and A. A. Onua, "Economic burden of glaucoma in rivers state, Nigeria," *Clinical Ophthalmology*, vol. 6, no. 1, pp. 2023–2031, 2012.

[6] G. Lazcano-Gomez, D. L. A. Ramos-Cadena, M. Torres-Tamayo, A. H. De Oteyza, M. Turati-Acosta, and J. Jimenez-Román, "Cost of glaucoma treatment in a developing country over a 5-year period," *Medicine*, vol. 95, no. 47, Article ID e5341, 2016.

[7] A. R. Rudnicka, S. Mt-Isa, C. G. Owen, D. G. Cook, and D. Ashby, "Variations in primary open-angle glaucoma prevalence by age, gender, and race: a Bayesian meta-analysis," *Investigative Opthalmology & Visual Science*, vol. 47, no. 10, pp. 4254–4261, 2006.

[8] M. He, P. J. Foster, J. Ge et al., "Prevalence and clinical characteristics of glaucoma in adult Chinese: a population-based study in Liwan District, Guangzhou," *Investigative Opthalmology Visual Science*, vol. 47, no. 7, pp. 2782–2788, 2006.

[9] B. E. Klein, R. Klein, W. E. Sponsel et al., "Prevalence of glaucoma. The beaver dam eye study," *Ophthalmology*, vol. 99, no. 10, pp. 1499–1504, 1992.

[10] I. Dielemans, J. R. Vingerling, R. C. W. Wolfs, A. Hofman, D. E. Grobbee, and P. T. V. M. De Jong, "The prevalence of primary open-angle glaucoma in a population-based study in The Netherlands. The Rotterdam study," *Ophthalmology*, vol. 101, no. 11, pp. 1851–1855, 1994.

[11] S. M. Kymes, M. A. Kass, D. R. Anderson, J. P. Miller, and M. O. Gordon, "Management of ocular hypertension: a cost-effectiveness approach from the ocular hypertension treatment study," *American Journal of Ophthalmology*, vol. 141, no. 6, pp. 997–1008.e3, 2006.

[12] L. Vijaya, R. George, P. G. Paul et al., "Prevalence of open-angle glaucoma in a rural south Indian population," *Investigative Opthalmology & Visual Science*, vol. 46, no. 12, pp. 4461–4467, 2005.

[13] W. C. Stewart, J. A. Stewart, Q. J. Nassar, and M. A. Mychaskiw, "Cost-effectiveness of treating ocular hypertension," *Ophthalmology*, vol. 115, no. 1, pp. 94–98, 2008.

[14] H. Vaahtoranta-Lehtonen, A. Tuulonen, P. Aronen et al., "Cost effectiveness and cost utility of an organized screening programme for glaucoma," *Acta Ophthalmologica Scandinavica*, vol. 85, no. 5, pp. 508–518, 2007.

[15] C. Fleming, E. P. Whitlock, T. Beil, B. Smit, and R. P. Harris, "Screening for primary open-angle glaucoma in the primary care setting: an update for the US preventive services task force," *The Annals of Family Medicine*, vol. 3, no. 2, pp. 167–170, 2005.

[16] V. A. Moyer, "Screening for glaucoma: U.S. Preventive services task force recommendation statement," *Annals of Internal Medicine*, vol. 159, no. 7, pp. 484–489, 2013.

[17] S. Naicker, J. Plange-Rhule, R. C. Tutt, and J. B. Eastwood, "Shortage of healthcare workers in developing countries—Africa," *Ethnicity Disease*, vol. 19, no. S1, pp. 60–64, 2009.

[18] NHS, *Annual Report English National Screening Programme for Diabetic Retinopathy*, National Health Service, London, UK, 2011.

[19] W. P. Nolan, J. L. See, P. T. K. Chew et al., "Detection of primary angle closure using anterior segment optical coherence tomography in Asian eyes," *Ophthalmology*, vol. 114, no. 1, pp. 33–39, 2007.

[20] J. Chhablani, S. Kaja, and V. A. Shah, "Smartphones in ophthalmology," *Indian Journal of Ophthalmology*, vol. 60, no. 2, pp. 127–131, 2012.

[21] J. H. Luis, D. Y. Kim, and S. Mukai, "Simple, inexpensive technique for high-quality smartphone fundus photography in human and animal eyes," *Journal of Ophthalmology*, vol. 2013, Article ID 518479, 5 pages, 2013.

[22] T. N. Kim, F. Myers, C. Reber et al., "A smartphone-based tool for rapid, portable, and automated wide-field retinal imaging," *Translational Vision Science and Technology*, vol. 7, no. 5, 2018.

[23] D. Myung, A. Jais, L. He, and R. T. Chang, "Simple, low-cost smartphone adapter for rapid, high quality ocular anterior segment imaging: a photo diary," *Journal of Mobile Technology in Medicine*, vol. 3, no. 1, pp. 2–8, 2014.

[24] A. Shah, B. Szirth, I. Sheng, T. Xia, and A. S. Khouri, "Optic disc drusen in a child: diagnosis using noninvasive imaging tools," *Optometry and Vision Science*, vol. 90, no. 10, pp. e269–e273, 2013.

[25] G. Russell, N. McLoughlin, V. Nourrit, and J. P. Oakley, "Enhancement of color retinal images in poor imaging conditions," in *Proceedings of the IEEE International Conference on Imaging Systems and Techniques*, Manchester, UK, July 2012.

[26] N. C. Roger, *Film versus Digital Summary*, Clarkvision, Cambridge, UK, 2014.

[27] P. J. Airaksinen, S. M. Drance, G. R. Douglas, D. K. Mawson, and H. Nieminen, "Diffuse and localized nerve fiber loss in glaucoma," *American Journal of Ophthalmology*, vol. 98, no. 5, pp. 566–571, 1984.

[28] K. Yogesan, R. H. Eikelboom, and C. J. Barry, "Texture analysis of retinal images to determine nerve fibre loss," in *Proceedings of the 14th International Conference on Pattern Recognition*, vol. 2, pp. 1665–1667, Queensland, Australia, August 1998.

[29] A. Tuulonen, H. Alanko, P. Hyytinen, J. Veijola, T. Seppänen, and P. J. Airaksinen, "Digital imaging and microtexture analysis of the nerve fiber layer," *Journal of Glaucoma*, vol. 9, no. 1, pp. 5–9, 2000.

[30] EGS, *Terminology and Guidelines for Glaucoma*, European Glaucoma Society, Brussels, Belgium, 4th edition, 2014.

[31] H. A. Quigley and H. D. Jampel, "How are glaucoma patients identified?" *Journal of Glaucoma*, vol. 12, no. 6, pp. 451–455, 2003.

[32] K. Grødum, A. Heijl, and B. Bengtsson, "A comparison of glaucoma patients identified through mass screening and in routine clinical practice," *Acta Ophthalmologica Scandinavica*, vol. 80, no. 6, pp. 627–631, 2002.

[33] L. A. Kerrigan-Baumrind, H. A. Quigley, M. E. Pease, D. F. Kerrigan, and R. S. Mitchell, "Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons," *Invest Ophthalmology Visual Science*, vol. 41, no. 3, pp. 741–748, 2000.

[34] I. Goldberg, C. I. Clement, T. H. Chiang et al., "Assessing quality of life in patients with glaucoma using the Glaucoma Quality of Life-15 (GQL-15) questionnaire," *Journal of Glaucoma*, vol. 18, no. 1, pp. 6–12, 2009.

[35] A. M. Ervin, M. V. Boland, E. H. Myrowitz et al., *Screening for Glaucoma: Comparative Effectiveness*, Agency for Healthcare Research and Quality, Rockville, MD, USA, 2012.

[36] G. Holló, P. Kóthy, A. Géczy, and P. Vargha, "Health anxiety in a non-population-based, pre-publicised glaucoma screening exercise," *Eye*, vol. 24, no. 4, pp. 699–705, 2009.

[37] E. K. K. Barbara, R. Klein, and S. C. Jensen, "Open-angle glaucoma and older-onset diabetes," *Ophthalmology*, vol. 101, no. 7, pp. 1173–1177, 1994.

[38] P. Mitchell, W. Smith, T. Chey, and P. R. Healey, "Open-angle glaucoma and diabetes," *Ophthalmology*, vol. 104, no. 4, pp. 712–718, 1997.

[39] S. Salim, P. A. Netland, K. H. Fung, M. E. Smith, and A. Aldridge, "Assessment of the student sight savers program methods for glaucoma screening," *Ophthalmic Epidemiology*, vol. 16, no. 4, pp. 238–242, 2009.

[40] H. A. Quigley, D. S. Friedman, and S. R. Hahn, "Evaluation of practice patterns for the care of open-angle glaucoma compared with claims data: the Glaucoma Adherence and Persistency study," *Ophthalmology*, vol. 114, no. 9, pp. 1599–1606, 2007.

[41] A. C. Day, G. Baio, G. Gazzard et al., "The prevalence of primary angle closure glaucoma in European derived populations: a systematic review," *British Journal of Ophthalmology*, vol. 96, no. 9, pp. 1162–1167, 2012.

[42] R. Thomas, T. George, A. Braganza, and J. Muliyil, "The flashlight test and van Herick's test are poor predictors for occludable angles," *Australian and New Zealand Journal of Ophthalmology*, vol. 24, no. 3, pp. 251–256, 1996.

[43] Grafton Optical, "D-EYE—smartphone ophthalmoscop," 2019, https://www.graftonoptical.com/graftonshop?gclid=CjwKCAiA1L_xBRA2EiwAgcLKAzNRzsweGfXu8aaCYJRHbk6mSCVe5dm4nJHQXW_IiXZhdfs0NnrnxoCJ0UQAvD_BwEHaddock.

[44] J. Olson, P. Sharp, K. Goatman et al., "Improving the economic value of photographic screening for optical coherence tomography-detectable macular oedema: a prospective, multicentre, UK study," *Health Technology Assessment*, vol. 17, no. 51, pp. 141-142, 2013.

[45] R. Annoh, C. Y. Loo, B. Hogan, H. L. Tan, L. S. Tang, and A. J. Tatham, "Accuracy of detection of patients with narrow angles by community optometrists in Scotland," *Ophthalmic and Physiological Optics*, vol. 39, no. 2, pp. 104–112, 2019.

[46] K. F. Jamous, M. Kalloniatis, A. Hayen, P. Mitchell, F. J. Stapleton, and B. Zangerl, "Application of clinical techniques relevant for glaucoma assessment by optometrists: concordance with guidelines," *Ophthalmic and Physiological Optics*, vol. 34, no. 5, pp. 580–591, 2014.

[47] A. U. Kumar, G. B. Jonnadula, C. Garudadri et al., "Agreement of glaucoma specialists and experienced optometrists in gonioscopy and optic disc evaluation," *Journal of Optometry*, vol. 6, no. 4, pp. 212–218, 2013.

[48] Primary Care Optometry, "Gonioscopy: how its results affect your glaucoma management decisions," 2000, https://www.healio.com/optometry/glaucoma/news/print/primary-care-optometry-news/%7Bb82ed12d-e45c-49a4-9ed9-47569ed68211%7D/gonioscopy-how-its-results-affect-your-glaucoma-management-decisions.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 6138132, 14 pages
https://doi.org/10.1155/2020/6138132

Review Article

Current Medical Therapy and Future Trends in the Management of Glaucoma Treatment

Barbara Cvenkel[1,2] **and Miriam Kolko**[3,4]

[1]*Department of Ophthalmology, University Medical Centre Ljubljana, Ljubljana, Slovenia*
[2]*Medical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[3]*Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark*
[4]*Department of Ophthalmology, Copenhagen University Hospital, Rigshospitalet, Glostrup, Copenhagen, Denmark*

Correspondence should be addressed to Barbara Cvenkel; barbara.cvenkel@gmail.com

Received 27 January 2020; Accepted 29 June 2020; Published 21 July 2020

Academic Editor: Tomasz Zarnowski

Glaucoma is a neurodegenerative disease characterized by progressive loss of retinal ganglion cells and their axons. Lowering of intraocular pressure (IOP) is currently the only proven treatment strategy for glaucoma. However, some patients show progressive loss of visual field and quality of life despite controlled IOP which indicates that other factors are implicated in glaucoma. Therefore, approaches that could prevent or decrease the rate of progression and do not rely on IOP lowering have gained much attention. Effective neuroprotection has been reported in animal models of glaucoma, but till now, no neuroprotective agents have been clinically approved. The present update provides an overview of currently available IOP-lowering medications. Moreover, potential new treatment targets for IOP-lowering and neuroprotective therapy are discussed. Finally, future trends in glaucoma therapy are addressed, including sustained drug delivery systems and progress toward personalized medicine.

1. Introduction

Glaucoma encompasses a group of eye conditions, which cause progressive optic nerve damage, retinal ganglion cell death, and corresponding visual field defects. It is the third leading cause of global blindness after uncorrected refractive error and cataract. Glaucoma contributed 8.49% to world blindness among adults aged 50 years and older in 2015 [1]. In the future, the number of glaucoma patients is expected to increase due to growing and ageing populations [2]. More importantly with ageing, time with glaucoma diagnosis will be longer and the lifetime risk of blindness will increase correspondingly. In Sweden, one out of six patients with 12-year median time of diagnosis was bilaterally blind from glaucoma at the last visit [3]. The classification of glaucoma relies on the appearance and obstruction of the drainage pathway and whether it is primary or associated with detectable comorbidity, i.e., secondary glaucoma. The most common type of glaucoma is primary open-angle glaucoma (POAG) with normal, open anterior chamber angle and restricted aqueous outflow associated with increased intraocular pressure (IOP), i.e. high-pressure glaucoma. There is no evidence of a threshold IOP for the onset of glaucoma, but the relative risk for the disease rises with the level of IOP. Nevertheless, most subjects with IOP outside the "normal" range (ocular hypertension) in a population will not develop POAG [4]. In a subtype of POAG, i.e., normal-pressure glaucoma, there is glaucomatous optic neuropathy at the statistically "normal" IOP. It is presumed that risk factors other than IOP have a relatively greater importance and/or sensitivity to IOP may be increased [5, 6]. Even though IOP-lowering therapy delays the onset and progression of glaucoma, the pathogenesis is debatable and not completely understood.

This review aims to (1) summarize current and recently launched IOP-lowering medications, (2) provide a brief overview of new targets for IOP lowering and targets for IOP-independent therapy, and (3) address future trends in therapy.

2. Current and Recently Launched IOP-Lowering Medications

IOP is the principle known and modifiable risk factor for development and progression of glaucoma. Hence, reducing IOP has been the mainstay of glaucoma treatment and its lowering by 20–40% has been shown to delay or halt the progression of glaucoma [7–9].

IOP-lowering medications reduce IOP by increasing aqueous outflow and/or reducing aqueous production. There are several types of IOP-lowering eye drops used to treat glaucoma with different mechanisms of action and efficacy (Table 1). The eye drops available in Europe include prostaglandin analogues, β-blockers, carbonic anhydrase inhibitors, α-2 adrenergic agonists, and parasympathomimetic drugs. In addition, systemic carbonic anhydrase inhibitor drugs are available and can be considered for short time use when eye drops are not effective. Combining agents of different classes with different mechanism of action is associated with superior IOP-lowering efficacy compared to each of the components used alone. Fixed combinations eyedrops in Europe include prostaglandin analogues/β-blockers, carbonic anhydrase inhibitors/β-blockers, α-2 adrenergic agonists/β-blockers, carbonic anhydrase inhibitor/α-2 adrenergic agonists, and β-blockers/parasympathomimetics.

New medications have been approved by the Food and Drug Administration (FDA) in 2017 and not yet by the European Medicines Agency (EMA). These include latanoprostene bunod and netarsudil, and in 2019, fixed combination netarsudil/latanoprost was launched.

2.1. Prostaglandin Analogues. Prostaglandin analogues (PGAs) are recommended as first choice treatment for POAG, because of their efficacy, limited systemic side effects, and once daily dosing [10, 11]. Differences among drugs within this class in the IOP reduction did not exceed 1 mmHg [12]. They lower IOP by increasing uveoscleral outflow. The most common side effects are conjunctival hyperaemia, increased pigmentation of periocular skin, longer and thicker eyelashes, and change in iris colour in some eyes (mostly in green-brown or grey-brown eyes) [13, 14]. A few cases of recurrence of herpetic keratitis have been reported with the use of prostaglandins [15].

2.2. β-Blockers (Adrenergic Antagonists). These drugs decrease aqueous humour production by blocking β-adrenergic receptors in the ciliary epithelium. β-Blockers are less effective during night time, because of naturally reduced aqueous humour production at night [16]. Nonselective β1 and β2 receptor antagonists may have higher efficacy (Table 1) compared to the β1-selective antagonist, betaxolol. Ocular adverse effects include conjunctival hyperaemia, epithelial keratopathy, and slight decrease in corneal sensitivity. Systemic adverse reactions include decreased heart rate and cardiac contractility, bronchospasm, depression, impotence, and anxiety [17]. β-Blockers should not be used in patients with bradycardia, heart block, manifest cardiac failure, and asthma. Respiratory adverse reactions are mediated via β2 receptor blockage. Hence, betaxolol can be considered in cases with respiratory issues [18].

2.3. Carbonic Anhydrase Inhibitors (CAIs). Carbonic anhydrase inhibitors reduce production of aqueous humour by inhibiting carbonic anhydrase in the ciliary epithelium [19]. Systemic CAIs effectively lower IOP, but the adverse effects limit their use for long-term therapy. Common adverse effects include paraesthesia, nausea, vomiting, depression, kidney stones, and metabolic acidosis. Topical CAIs are systemically safe; ocular adverse effects include stinging, burning, foreign body sensation, and, with brinzolamide, transient blurring of vision. Carbonic anhydrase is naturally present in the endothelial cells, and topical CAIs were reported to cause irreversible corneal decompensation in patients with corneal endothelial disorders [20].

2.4. Adrenergic Agonists. Adrenergic agonists decrease aqueous humour production and increase uveoscleral outflow. Nonselective adrenergic agonists have been in clinical practice replaced by α-2 selective agents, of which brimonidine only is used for chronic therapy. Apraclonidine has been associated with a high rate of allergic blepharoconjunctivitis and is used only for short-term prophylaxis to prevent IOP increase after laser procedures. Ocular adverse effects include allergic reactions and periocular contact dermatitis occurring in 12–15% of patients [21, 22]. Systemic side effects include dry mouth, fatigue, and headache [21]. These agents should not be used in small children, because they cross the blood-brain barrier and can cause respiratory and central nervous system depression. A randomized controlled trial comparing brimonidine versus β-blocker timolol in patients with low-tension glaucoma found that patients treated with brimonidine were less likely to have visual field progression than patients treated with timolol [23]. This non-IOP-dependent mechanism suggests a potential neuroprotective role of α-2 agonists.

2.5. Parasympathomimetics (Cholinergic Drugs). Parasympathomimetics increase aqueous outflow through trabecular meshwork. Pilocarpine is a direct agonist of parasympathetic receptors, whereas echothiophate is an indirect acting agonist and inhibits acetylcholinesterase. The ocular side effects include miosis, pseudomyopia, brow ache, red eyes, miosis-induced visual field constriction, and decreased vision at night. Miotics may cause increased salivation, sweating, diarrhoea, vomiting, and tachycardia [19].

3. Novel IOP-Lowering Medications for Treatment of Glaucoma

3.1. Rho-Kinase Inhibitor Netarsudil. Netarsudil is the only available rho-kinase inhibitor, which represents the first new class glaucoma drug in more than 20 years. It has received approval by the FDA in 2017 (Rhopressa™, Aerie Pharmaceuticals, Inc., USA), and recently the European

Table 1: IOP-lowering medications, efficacy, and mechanism of action.

Medication Class	Compound	IOP reduction (%)	Mechanism of action			
			Increases uveoscleral outflow	Increases trabecular outflow	Decreases aqueous production	Decreases episcleral venous pressure
Prostaglandin analogues	Bimatoprost, latanoprost, tafluprost, travoprost	25–35	Yes	No	No	No
β-Blockers (i) Nonselective	Timolol, levobonolol, carteolol, metipranolol	20–25	No	No	Yes	No
(ii) *β*1-Selective	Betaxolol	20	No	No	Yes	No
Carbonic anhydrase inhibitors (i) *Topical*	Dorzolamide, brinzolamide	20	No	No	Yes	No
(ii) *Systemic*	Acetazolamide, methazolamide, dichlorphenamide	30–40	No	No	Yes	No
Adrenergic agonists (i) *α*-2 Selective	Brimonidine, apraclonidine	20–25	Yes	No	Yes	No
(ii) Nonselective	Dipivefrin, epinephrine	15–20	Yes	No	Yes	No
Parasympathomimetics	Pilocarpine, echothiophate	20–25	No	Yes	No	No
Novel IOP-lowering medications						
Rho-kinase inhibitors	Netarsudil	16–21	No	Yes	Yes	Yes
Nitric oxide-donating prostaglandin analogue	Latanoprostene bunod	32–34	Yes	Yes	No	No
FC rho-kinase inhibitor/ latanoprost	Netarsudil/latanoprost	30–36	Yes	Yes	Yes	Yes

Medicines Agency's Committee for Medicinal Product for Human Use approved the use of netarsudil 0.02% (Rhokiinsa™, Aerie Pharmaceuticals Ireland Ltd.) for treatment of open-angle glaucoma and ocular hypertension [24]. Rho-kinase is a serine/threonine protein kinase that regulates cytoskeletal activities and calcium-dependent smooth muscle contraction. Its functions include modulation of cell adhesion, increasing cell stiffness and contraction of actomyosin, and influencing aqueous humour outflow [25]. Netarsudil lowers resistance to outflow through trabecular meshwork, decreases aqueous production, and decreases episcleral venous pressure. It is supplied as a buffered aqueous solution with a pH~5 and dosed once daily. In a 28-day clinical trial comparing IOP-lowering efficacy of netarsudil 0.02% versus latanoprost, netarsudil was found to be less effective in patients with open-angle glaucoma and ocular hypertension by approximately 1 mmHg [26]. Other clinical trials compared IOP-lowering efficacy of netarsudil versus timolol, with two studies of three months' and one trial of 12 months' duration [27–29]. Netarsudil was found to be effective, consistently lowering IOP through 12 months, and noninferior to timolol, but with higher incidence of conjunctival hyperaemia and subconjunctival haemorrhages versus both latanoprost and timolol. The ocular adverse effects included conjunctival hyperaemia noted in 48–60%, small microhaemorrhages in or around the limbus in 6–20%, and cornea verticillata in 5–24% of patients. Other ocular side effects include blurred vision, eyelid erythema, instillation-site pain, increased lacrimation, and reduced visual acuity. Conjunctival hyperaemia is due to rho-kinase inhibition of calcium sensitization and leads to blood vessel smooth muscle relaxation and consequently to vessel dilation [27]. The conjunctival hyperaemia is usually mild and was a reason for discontinuation of treatment in 4% of patients [29]. Subconjunctival haemorrhages resolved with continued use of netarsudil. Cornea verticillata was reported to be mild, without impact on vision, and resolved within few months after stopping therapy. Drugs that are both cationic and amphiphilic can induce cornea verticillate, which is due to lysosomal accumulation of phospholipids within corneal epithelial cells, a process called phospholipidosis. Netarsudil is a cationic amphiphilic drug and can induce phospholipidosis [28]. Systemic side effects were not observed with netarsudil.

3.2. Nitric Oxide-Donating Prostaglandin Analogue: Latanoprostene Bunod. Latanoprostene bunod is a novel nitric oxide-donating prostaglandin F2*α* receptor agonist that is metabolised to latanoprost acid and butanediol mononitrate, which releases the second active component, nitric oxide. Latanoprostene bunod ophthalmic solution 0.024% (Vyzulta, Bausch & Lomb Incorporated, Rochester, New York, USA) was approved by the FDA in 2017 for the reduction of IOP in patients with open-angle glaucoma or ocular hypertension and is not available in Europe. It has dual mechanism of IOP lowering: latanoprost acid increases uveoscleral aqueous humour outflow and nitric oxide increases trabecular meshwork and Schlemm's canal outflow. Nitric oxide activates the nitric oxide-guanylate cyclase-1-

cGMP cascade, resulting in trabecular meshwork relaxation and consequently increased aqueous humour outflow [30]. Nitric oxide is a regulator of blood flow through relaxation of the vascular smooth muscle and has been shown to have either neuroprotective or neurodegenerative effect on retinal ganglion cells in animal models [30–32]. Very high concentrations of nitric oxide caused oxidative damage to the retinal ganglion cells in some animal models [33]. Because of very short half-life of nitric oxide (less than three seconds in extravascular tissues), it is highly unlikely that nitric oxide released from latanoprostene bunod would reach the retina at toxic levels [34]. In two clinical trials with a three months' duration comparing latanoprostene bunod once daily versus timolol twice daily at three time points, latanoprostene bunod achieved significantly lower IOP at all time points [35, 36]. In the pooled analysis of both studies, the percentage of subjects with mean IOP ≤18 mmHg and the percentage with IOP reduction ≥25% were significantly higher in the latanoprostene bunod group versus the timolol group (mean IOP ≤ 18 mmHg: 20.2% vs. 11.2%, $P = 0.001$; IOP reduction ≥ 25%: 32.9% vs. 19.0%, $P < 0.001$). Both trials extended as open-label studies showed that patients treated with latanoprostene bunod maintained consistently lowered IOP at 6 and 12 months. Patients switched from timolol to latanoprostene bunod had an additional and sustained decrease in mean diurnal IOP [37]. The adverse effects of latanoprostene bunod are similar to those of prostaglandin analogues and were more frequent than in the timolol group [38]. The most common ocular adverse effects through 1 year of treatment were conjunctival hyperaemia (17.7%), growth of eyelashes (16.2%), eye irritation (11.5%), eye pain (10.0%), and increase in iris pigmentation (9%) [39]. There were no systemic adverse effects related to this drug.

3.3. Fixed Combination: Rho-Kinase Inhibitor/Latanoprost. Fixed combination netarsudil 0.02%/latanoprost 0.005% ophthalmic solution (Rocklatan™, Aerie Pharmaceuticals, Inc., USA) is the first fixed combination of a prostaglandin analogue and the rho-kinase inhibitor. It received FDA approval for the treatment of open-angle glaucoma and ocular hypertension in 2019 [40]. Netarsudil lowers IOP by increasing aqueous outflow through trabecular meshwork, decreasing aqueous production, and decreasing episcleral venous pressure, and its mechanism of action complements that of latanoprost which lowers IOP by increasing uveoscleral outflow. Fixed combination is prepared as a buffered aqueous solution with a pH~5 and dosed once daily. Two clinical trials of 3 months' duration compared fixed combination of netarsudil/latanoprost versus monotherapy with netarsudil or latanoprost [41, 42]. Both studies found that fixed combination showed statistically and clinically significant superior IOP-lowering efficacy compared to its individual components. Fixed combination netarsudil/latanoprost lowered IOP by an additional 1.8–3.3 mmHg compared with netarsudil and 1.3–2.5 mmHg compared with latanoprost. From both clinical trials, mean diurnal IOP reduction of ≥30% was achieved in 58.8–64.5% of patients treated with fixed combination netarsudil/latanoprost, 20.6–28.8% of netarsudil, and 29.4–37.2% of latanoprost groups. Ocular side effects include those related to individual component. Currently there is one ongoing clinical trial in Europe comparing fixed combination netarsudil/latanoprost to fixed combination bimatoprost/timolol (NCT03284853). The most frequent ocular side effects were mild conjunctival hyperaemia (44%), conjunctival haemorrhage (10%), and cornea verticillate (5–13%). Conjunctival hyperaemia was a reason to discontinue therapy in 7% of patients [41, 42]. Other ocular side effects include pain at the site of instillation, increased lacrimation, eye pruritus, and asymptomatic corneal changes. Corneal changes refer to changes in the appearance of endothelial cells with specular microscopy that were found in 5.7% of patients treated with fixed combination netarsudil/latanoprost, 4.7% with netarsudil, and in none with latanoprost [42]. No systemic adverse effects were reported.

The novel drugs netarsudil, a rho-kinase inhibitor, latanoprostene bunod, and fixed combination of netarsudil/latanoprost represent an extension of treatment options with different mechanisms of action. Interestingly, in clinical trial, the new compounds were compared to timolol and not to prostaglandin analogues (except for netarsudil that showed lower IOP lowering compared to latanoprost in a 28-day trial; NCT01731002), and the new fixed combination was compared to its separate individual components and not concomitant treatment. All three drugs are preserved with benzalkonium chloride (BAK). Netarsudil contains 0.015% BAK, and latanoprostene bunod and fixed combination netarsudil/latanoprost contain 0.02% BAK. Because of the well-known toxic-inflammatory effects of BAK, these drugs are not suitable for patients with signs or symptoms of dry eye. Another issue is the cost of medication, which if paid out of the pocket would be an important obstacle for long-term treatment.

4. New Targets for IOP-Lowering and for IOP-Independent Therapy

4.1. IOP-Lowering Treatment Strategies. Currently, lowering of IOP is the only clinically proven strategy for successful neuroprotection. Depending on the severity of glaucoma at diagnosis and years with the diagnosis, IOP lowering to the individual target delays progression of disease and preserves adequate visual function in most, but not all glaucoma patients. Reduction of IOP removes stress causing glaucomatous optic nerve damage, but it does not stimulate cell survival or cell resilience to withstand pathological insults or prevent cells' death. Ideally, the new targets for glaucoma treatment should include both IOP-lowering and non-IOP-related effects (neuroprotective). Some of the new targets have shown to achieve either one or both effects in animal models of glaucoma, but translation of preclinical results into clinical glaucoma practice is challenging and has so far not been successful.

4.1.1. Cannabinoids. Cannabinoids have been investigated for their IOP-lowering effect for the past few decades. The

exact mechanism is incompletely understood. They lower IOP by inhibiting calcium influx through presynaptic channels and in this way reduce the noradrenaline release in the ciliary body, leading to a decrease in the production of aqueous humour [43]. The main active component is Δ^9-tetrahydrocannabinol (THC) which acts with G-protein-coupled type-1 and type-2 cannabinoid (CB1 and CB2) receptors that are the most important endogenous cannabinoid-binding targets within the so-called "endocannabinoid system" [44]. CB1 and CB2 receptors are present in the central nervous system and within the eye in the retina. CB1 receptors in the trabecular meshwork, Schlemm's canal, and ciliary body are involved in the regulation of IOP [45]. THC's action on CB1 receptors in ciliary body leads to vasodilation of blood vessels in the anterior uvea, favouring aqueous humour efflux [46]. In addition, THC increased retinal ganglion cell survival in an animal model of glaucoma and inhibited glutamate release by increasing K^+ and decreasing Ca^{2+} permeability [47, 48]. Oral administration of cannabinoids is not a suitable treatment modality, because of side effects, variable absorption, and poor predictability of timing and peak effect. Topical administration could potentially be ideal for glaucoma patients, but at present there is no solid evidence supporting use of cannabinoids for glaucoma [49]. Recently, a hydrophilic prodrug of THC, Δ^9-tetrahydrocannabinol-valine-hemisuccinate, has been synthesized with the aim to improve the ocular bioavailability of THC [50]. The prodrug formulated in a lipid-based nanoparticle carrier was evaluated in an animal model. It lowered IOP by 30% from baseline at peak and the IOP decrease lasted for six hours.

4.1.2. Melatonin. Melatonin is synthesized by several ocular structures. Melatonin and its analogues decrease IOP by activation of membrane receptors MT1 and MT2, located in ocular tissues, including ciliary processes. Melatonin receptors belong to the G-protein-coupled receptors [51]. In healthy, normotensive eyes, melatonin receptors form complexes with α1-adrenoceptors. These functional units couple to Gs which leads to an increase in cAMP levels and protein kinase A activity. In the hypertensive eyes, these functional adrenergic/melatonin receptor units are not formed. The individually expressed α1-adrenoceptors allow adrenergic agonists to increase cytosolic Ca^{2+} levels and the expression of individual melatonin receptors, which couple to Gi leading to decrease in cAMP levels and protein kinase A activity. Several analogues were studied for their IOP-lowering effect, which depended on the status of the eye (normotensive or hypertensive) [52, 53]. The most promising melatonin analogue is agomelatine which is used to treat depressive disorders. In glaucoma patients on topical IOP-lowering medication, agomelatine further lowered IOP by 30% [54]. Melatonin has also antioxidant function acting as effective free radical scavenger, and its analogues may have promising application in glaucoma therapy [55].

4.1.3. Connective Tissue Growth Factor. Connective Tissue Growth Factor (CTGF) is a downstream molecule in the Transforming Growth Factor (TGF) β-2 signalling cascade. CTGF is a matricellular protein which is expressed by the cells of trabecular meshwork, ciliary body, and retina [56]. Increased levels of CTGF were found in the aqueous humour of patients with the secondary glaucoma subtype, pseudoexfoliation glaucoma [57]. CTGF increases the expression of fibrotic extracellular matrix, fibronectin, and cells' stiffness [58]. Consequently, the trabecular meshwork outflow facility decreases. In a transgenic mice model with overexpression of CTGF, IOP was higher in mice overexpressing CTGF compared to control mice [59]. Inhibiting CTGF-induced extracellular matrix production does not interfere with TGFβ-2 pleiotropic effects, therefore targeting CTGF may prove beneficial and safer in the treatment of glaucoma. Recently, the intracameral delivery of anti-CTGF small interfering RNA (siRNA) by using nanoparticles coated by hyaluronan succeeded to penetrate deeply in the outflow region and showed binding of hyaluronan to the CD44 receptors, which were overexpressed in glaucomatous eyes [60]. Hyaluronan-coated nanoparticles combined with RNA interference may provide a potential strategy for glaucoma therapy.

4.1.4. Adenosine. Adenosine and several adenosine derivatives increase or decrease IOP via modulation of G-protein-coupled receptors. There are four adenosine receptors subtypes known as A1, A2a, A2B, and A3 [61]. The activation of A1 receptors in the trabecular meshwork and ciliary body reduces the outflow resistance and aqueous production and lowers IOP. Activation of A2a receptors in Schlemm's canal cells can decrease or increase IOP, whereas activation of A3 receptors increases IOP [62, 63]. Trabodenoson is an A1 receptor-selective adenosine derivative. It lowers IOP by increasing aqueous outflow through trabecular meshwork. Trabodenoson topically was well tolerated without clinically important ocular and systemic side effects. Its IOP-lowering effect was dose-dependent with a mean change of 4 mmHg from baseline at the highest dose tested [64, 65].

4.2. Non-IOP-Dependent Treatment Strategies: Neuroprotection. Neuroprotection strategies use signalling pathways to improve cell survival and/or prevent cell death after a pathological insult. Some of the cellular processes that result in retinal ganglion cell death and are targets of neuroprotective agents include production of external nerve-derived risk factors such as glutamate and nitric oxide (NO), deprivation of internal trophic (nutritional) factors in the nerve cells, loss of intracellular self-repair processes, or generation of intracellular destructive processes [66]. Treatment strategies can be grouped into targets that interfere with excitotoxicity, oxidative stress and mitochondrial dysfunction, inflammation-abnormal immune response, glial cell modulation, and stem cell therapy. However, any division is arbitrary as most targets are involved in several pathways and/or mechanism of action is incompletely understood. A brief overview of some promising treatment strategies is summarized.

4.2.1. Excitotoxicity. Excitotoxicity refers to cell death resulting from the toxic actions of excitatory amino acids. Glutamate is the major excitatory neurotransmitter in the central nervous system. In glaucoma, pathological insult leads to elevated levels of extracellular glutamate. Sustained activation of ionotropic N-methyl-D-aspartate (NMDA) receptors by glutamate impairs cellular calcium homeostasis and activates nitric oxide synthesis, formation of free radicals, and apoptosis. In physiological conditions, MÜller cells remove the extracellular glutamate and are neuroprotective. When the homeostasis is impaired, MÜller cells contribute to excitotoxicity and neuronal degeneration [67]. Therapy targeting excitotoxicity has been studied for application in glaucoma.

Memantine is a noncompetitive NMDA receptor antagonist and is approved for treatment of Alzheimer's and Parkinson's disease. In animal glaucoma models, memantine protected against retinal ganglion cell loss [68, 69]. Unfortunately, in two large clinical trials, daily treatment with memantine for 4 years did not prevent or delay progression in patients with open-angle glaucoma and was no different from placebo [70]. This indicates the need for better trial design, such as selecting patients with rapid progression and more sensitive endpoint for detecting progression [70, 71].

Brimonidine, an α-2 adrenergic agonist, is used to lower IOP. It has been shown to reduce optic nerve damage in animal glaucoma model unrelated to IOP. Brimonidine modulates glutamate-induced toxicity through several pathways. In a clinical trial, patients with low-tension glaucoma receiving brimonidine monotherapy had lower rate of progression compared with those treated with timolol over 30 months despite similar IOP (9% vs. 30%) [23]. There was a considerable dropout rate in the brimonidine group and too short follow-up, both of which limit the conclusion [72].

4.2.2. Oxidative Stress and Mitochondrial Dysfunction. Mitochondria are the main source of intracellular reactive oxygen species (ROS) formed as by-product of oxidative phosphorylation. ROS are highly reactive molecules and tightly regulated under physiological conditions. In dysfunctional mitochondria, the impaired homeostasis leads to increased production of ROS with chronic oxidative damage which contributes to cellular dysfunction and neurotoxicity. Oxidative stress refers to an imbalance between generation of ROS and the cells' ability to detoxify the reactive intermediates or repair the resulting damage. Oxidative stress has been shown to play a role in retinal ganglion cell death in glaucoma. Decreased antioxidant defence status and increased oxidative stress were found in serum of patients with POAG and pseudoexfoliation glaucoma compared to controls [73]. Tanito et al. have reported that lower systemic antioxidant capacity was associated with more severe visual field loss [74]. Thus, interventions that target elevated oxidative stress and potential mitochondrial dysfunction may prove beneficial neuroprotective treatment [75]. Several compounds with antioxidant function have been studied, especially vitamin E (α-tocopherol), vitamin C, and *Ginkgo biloba* as oral supplementation. A systematic review did not find evidence to support the use of nutritional substances in glaucoma; the randomized clinical trials were small and biased [76]. *Ginkgo biloba* extract, mainly composed of flavonoids, is widely used nutritional supplement for treatment of cognitive impairment. *Ginkgo biloba* increases blood flow, reduces free radical damage, and interferes with glutamate signalling [77–79]. In patients with normal-tension glaucoma, *Gingko biloba* slowed progression of visual field damage [80].

Mitochondrial dysfunction has its role in the glaucoma pathogenesis. Coenzyme Q10 (CoQ10) is a mitochondrial targeted antioxidant that plays an essential role in the normal function of the electron transport chain. CoQ10 has been reported to have neuroprotective activity in neurodegenerative diseases and cerebral ischemia [81]. In addition to its antioxidant function, CoQ10 is also reported to protect against glutamate excitotoxicity [82]. In an animal model of acute IOP rise, CoQ10 was able to reduce significantly the pathological increase of glutamate observed during reperfusion and this may contribute to the neuroprotection [83]. CoQ10 associated with vitamin E topical administration in open-angle glaucoma has shown a beneficial effect on the inner retinal function with consequent enhancement of the visual cortical responses [84]. Topical CoQ10 prevented retinal ganglion cell apoptosis and loss as assessed in vivo by Detecting Apoptotic Retinal Cells (DARC) in an animal glaucoma model [84]. Two ongoing randomized clinical trials in patients with primary open-angle glaucoma treated with IOP-lowering medications are comparing addition of CoQ10 versus placebo. One trial (Phase IV, NCT04038034) is evaluating oral supplementation of CoQ10 on the functional (electrophysiological test, visual field, and contrast sensitivity) and structural (OCT) changes. The second trial (NCT03611530) is looking at the time to progression in a larger number of open-angle glaucoma patients treated with eye drops containing both CoQ10 and vitamin E versus placebo [85].

Citicoline or cytidine 5′-diphosphocholine functions as an intermediate in the membrane phospholipids. Citicoline has shown neuroprotective effects in neurodegenerative diseases, after stroke, and in cognitive impairment, brain trauma, amblyopia, and glaucoma [86]. Its mechanism of action is not clarified. Neuroprotection of retinal ganglion cells may include mimicking neurotrophic factors, reducing oxidative stress, improving axonal transport, and inhibiting excitotoxicity in retinal tissues [87–90]. Oral and intramuscular citicoline treatment used as an adjunct to IOP-lowering therapy in glaucoma patients improved pattern electroretinogram (PERG) and visually evoked potentials (VEP) and better preserved visual field compared to the placebo treated group [91–94]. Similar effects, i.e., enhanced PERG and VEP responses, in patients with glaucoma were achieved with topical citicoline therapy [95]. Recently, a clinical trial looking at the difference in glaucoma progression between the citicoline eye drop group versus the placebo group has been completed, but the results have not been published. The ongoing trial (NCT04046809) has the

aim to test whether the intake of citicoline oral solution (Neurotidine®, Omikron Italia S.r.l.) can improve quality of life in patients with glaucoma.

4.2.3. Inflammation-Abnormal Immune Response (Autoimmunity). Degeneration of retinal ganglion cells and axons following pathological insult is associated with activation of microglial cells which release proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [96]. Higher levels of TNF-α were detected in aqueous humour of patients with glaucoma compared to controls [97]. Its binding to the TNF-receptor-1 (TNF-R1) mediates retinal ganglion cell death in glaucoma [98]. Production and release of TNF-α occur very early following exposure to stress. In an animal model, intravitreal injection of TNF-α was found to induce axonal degeneration from two weeks to two months after injection, whereas significant retinal ganglion cell loss was noted at two months after injection. This effect of TNF-α is mediated through nuclear factor (NF)-kappa B p65 [99]. TNF-α can also act as a downstream mediator of proapoptotic factors such as pro-nerve growth factor (pro NGF) [100]. The finding that retinal ganglion cell apoptosis was attenuated by a neutralizing antibody against TNF-α supports TNF-α as an attractive therapeutic target [101]. The usefulness of anti TNF-α therapy in glaucoma will depend upon its ability to block selectively excessive TNF-α and TNF-R1 expression without significantly affecting its physiological functions such as local immunity.

Several studies have shown difference in the concentrations of autoantibodies in serum and aqueous humour of patients with glaucoma compared to controls. Autoantibodies changes detected include elevated levels of antibodies against α-fodrin, glutathione-S-transferase, spectrin, and Heat Shock Protein (HSP) 70 and decreased levels of antibodies against αB crystalline, vimentin, Glial Fibrillary Acidic Protein (GFAP), and Υ-Synuclein [102–105]. *In vitro* studies have shown that antibodies against Υ-Synuclein and GFAP possess direct and indirect (through Müller cells) protective effect on the retinal ganglion cells [106]. Results from clinical studies revealed altered immunoreactivities against retina and optic nerve in sera and aqueous humour of glaucoma patients which indicates a role of autoimmunity in glaucomatous neurodegeneration and retinal ganglion cells death. Targeting immune changes in the retina of glaucoma patients, such as the antibody against Υ-Synuclein, may be a promising therapeutic strategy [107].

4.2.4. Glial Cell Modulation. Glial cells regulate tightly retinal ganglion cells and their response to injury is important for maintaining the health of retina or its degeneration. The glial cells include microglial cells, which are immunocompetent cells involved in the process of apoptosis and removal of dead cells, and macroglial cells. In the nonmyelinated region in the retina, the major macroglia cells are astrocytes and Müller cells which form blood-retina barrier, connect the neurons to the blood-vessels, and maintain homeostasis by removing ions and neurotransmitters [108]. Müller cells were shown to increase uptake of excitatory glutamate in limited energy supply condition thus protecting retinal ganglion cells [109]. Macroglial cells produce cytokines such as TGF-α, ciliary neurotrophic factor (CNTF), and platelet-derived growth factor [110–112]. CNTF is one of the most extensively studied neurotrophic factors, which was able to induce neuronal cell differentiation and neurite outgrowth and protect cells from neurodegeneration in an animal model following axotomy [113]. The CNTF concentration was reduced in aqueous humour and lacrimal fluid of patients with primary open-angle glaucoma, especially in those with severe visual loss [114]. CNTF is a promising target and its neuroprotective effect was evaluated in phase I study (NCT01408472) in patients with POAG who received intraocular implant NT-501 CNTF (made by Neurotech) into one eye. The NT-501 implant contains encapsulated retinal pigment epithelial cells that have been modified to release CNTF across a semipermeable membrane in a selective and sustained way. The results of the study have not been published. Another ongoing phase 2 trial (NCT02862938) is evaluating safety and efficacy of NT-501 CNTF intravitreal implant versus sham surgery.

Intravitreal injection of platelet-derived growth factor (PDGF) inhibited retinal ganglion cell death. The neuroprotective effect of PDGF has been shown to be mediated by astrocytes and amacrine cells which are in the presence of PDGF stimulated to secrete factors protecting ganglion cells [115, 116]. Therefore, modulation of macroglial cell activity has a potential in neuroprotection.

4.2.5. Stem Cell Therapy. Regarding the origin, stem cells can be divided into embryonic, adult, and induced pluripotent stem cells. The latter are artificially produced from any somatic cell by reprogramming its properties into a pluripotent stem cell. Among the adult stem cells, mesenchymal stromal cells (MSC) have been shown to be neuroprotective and promote regeneration in an animal glaucoma model and after optic nerve injury [117–119]. Therapeutic effects of MSC are mediated by their immunomodulatory and secretory properties, production of numerous cytokines, and growth factors. MSC can also differentiate into different cell types [120]. It has been shown in ex vivo human retinal explants that PDGF plays an important role in MSC-mediated retinal ganglion cell protection and may represent a new target in retinal ganglion cell neuroprotection [121, 122]. The spectrum and concentration of immunoregulatory molecules produced by the MSC depend on the environment [123]. The side effects of MSC following intravitreal administration have been reported and may be influenced by the difference of diseased environment, indications, and inconsistencies in isolation and preparation of MSC [124–126]. Two ongoing trials (NCT01920867; NCT03011541) aim to evaluate autologous bone marrow-derived MSC for treatment of multiple retinal diseases including glaucoma. A completed phase 1 study evaluating intravitreal application of autologous bone marrow-derived MSC in advanced glaucoma (NCT02330978) enrolled 2 patients. One patient developed retinal detachment with

proliferative vitreoretinopathy and lost light perception. Study using autologous adipose tissue-derived stem cells delivered sub-Tenon's in glaucoma patients is going on in Russia (NCT02144103). These trials are mainly focused on safety, followed by efficacy, and are designed to determine the best method of delivery and the required level of immunosuppression [127]. Stem cell therapy has a potential for glaucoma treatment and needs further evaluation in well-designed clinical studies.

4.2.6. Gene Therapy. Gene therapy aims to correct a specific, well defined genetic defect or deliver protective factors using different pathways to stimulate survival and regeneration of retinal ganglion cells. The most promising vector systems for successful gene delivery in the eye are recombinant adenoassociated viral vectors (AAVs), which lead to long and sustained levels of gene expression within a select target cell [128]. Genetic approach is still in preclinical phase for glaucoma. Correcting a specific genetic defect is feasible in primary congenital or primary juvenile open-angle glaucoma, both of which have a clear genetic basis. Between 10 and 30% of patients with primary juvenile open-angle glaucoma have mutations in the gene encoding myocilin that affects trabecular meshwork function with an increase in IOP [129, 130]. Recently, Jain et al. have disrupted the effects of the mutant myocilin gene using AAV-CRISP/Cas9 in a mouse model of myocilin-associated glaucoma and were able to lower IOP and prevent further glaucomatous damage [131]. The etiopathogenesis of adult-onset glaucoma is not clear and includes various genetic, environmental, and individual risk factors. For these reasons, gene therapy strategies are based on enhancing retinal ganglion cell survival or inhibiting cell death pathways [128]. Supplementation of brain-derived neurotrophic factors showed transient neuroprotective effect due to BDNF receptor (TrKB) downregulation [132]. A novel AAV gene therapy (AAV2 TrKB-2A-mBDNF) increased production of BDNF and the expression of its receptor. The neuroprotective efficacy was confirmed in an experimental animal model of glaucoma and optic nerve injury and was present over 6 months without vector-related adverse effects [133]. Although there are major advances in gene therapy such as in Leber's hereditary optic neuropathy, in adult-onset glaucoma there are many unresolved issues such as which molecular pathways to be targeted, long-term modification of gene expression, and immunogenic and mutagenic effects [129]. Gene therapy is a promising treatment strategy for neuroprotection, but further research and studies are needed.

5. Future Trends in Glaucoma Therapy

5.1. Sustained Drug Delivery Systems: Sustained Release Drug Formulations. Poor adherence is an important issue in the long-term glaucoma therapy. To avoid active instillation of eye drops, several sustained drug delivery systems have been developed.

Bimatoprost SR (Allergan, Dublin, Ireland) is a biodegradable implant which is injected in the anterior chamber and enables a slow, extended release of medication. In phase I/II, bimatoprost SR was safe and showed comparable efficacy to topical bimatoprost through 6 months [134]. The side effects of bimatoprost SR included conjunctival hyperaemia, foreign body sensation, punctate keratitis, increased lacrimation, conjunctival haemorrhage, eye pain, transient iritis, and progression of cataracts [134]. Bimatoprost SR is currently in six phase III studies. Two studies (NCT 02636946; NCT02507687) are comparing efficacy and safety of bimatoprost SR to selective laser trabeculoplasty, 3 studies aim to assess long-term efficacy and safety of bimatoprost SR (NCT03850782; NCT 03891446; NCT02250651), and one completed study (NCT02247804) compared safety and efficacy of bimatoprost SR to topical timolol bid. The results have not been published yet.

The topical bimatoprost ocular insert (Allergan, Dublin, Ireland) is an ocular ring which contains 13 mg of bimatoprost incorporated within a silicone matrix with an inner polypropylene structure. The ring is inserted between the upper and lower fornix. It releases drug into the tear film in a decreasing concentration over six months. Bimatoprost ring lowered IOP by 3.2–6.4 mmHg from baseline IOP and was noninferior to topical timolol [135]. The ring was safe and well tolerated and stayed in place in 95% of subjects [136]. There is no data about potential availability of this delivery system on the market.

Currently there are 3 ongoing trials evaluating a titanium intraocular implant filled with travoprost (Glaukos, Inc.) that releases travoprost with two different elution rates (NCT02754596; NCT03868124; NCT03519386) and comparing it to topical timolol treatment.

Travoprost extended release as a biodegradable intracameral implant (Envisia Therapeutics) has been evaluated in a phase II study for up to 24 months. Ocular side effects included ocular hyperaemia, photophobia, anterior chamber inflammation (iritis), cataract, and corneal endothelial cell loss (NCT02371746).

Intracanalicular insert of sustained release travoprost OTX-TP (Ocular Therapeutix, Inc.) is a hydrogel punctum plug eluting drug into the tear film. Completed phase III study (NCT02914509) has not published results yet. Currently an ongoing open-label phase III study is evaluating long-term safety of repeat dose punctum plug delivery over 12 months.

Recently, the micelles-laden contact lenses have been developed and were able to achieve sustained release of timolol and latanoprost simultaneously [137].

The development in sustained drug release is promising, but there are still unsolved issues, such as long-term safety with intraocular implants compared to eye drops, variation in the length of time of IOP-lowering effect, and costs. Also, approximately half of patients require more than one drug for IOP control and development of delivery systems with simultaneous release of more than one drug with different properties to avoid instillation of eye drops is still a challenge.

At present, there is no solid evidence that topical or systemic neuroprotective agents and nutritional supplements may be beneficial for individuals with open-angle glaucoma and IOP-lowering eye drops remain the only proven and available treatment for glaucoma [72, 76].

Adherence represents a treatment burden and it has been reported that 60% of patients had one or more problems with taking their medication [138]. One-third up to 75% of glaucoma patients do not use their eye drops as prescribed [139, 140]. Lower adherence has been reported with younger age, male gender, forgetfulness, lower social status and education, medication cost, side effects, greater number of daily instillations, and situational obstacles (travel and change of daily routine) [139, 141–143]. In the longitudinal assessment of patients in the Collaborative Initial Glaucoma Treatment Study lower adherence was associated with faster visual field loss [144]. A systematic review assessing different interventions to improve patients' adherence to topical glaucoma therapy found that there was insufficient evidence to recommend any interventions to improve adherence, but simplified drug regimens could be of benefit [145].

In the near future, the sustained drug-release implants and nanotechnology based-drugs for glaucoma using nano delivery systems have a potential to overcome the limitations of topical IOP-lowering drops by improving bioavailability, providing sustained release, targeted delivery, dose accuracy, and reducing side effects [146, 147].

5.2. Personalised Medicine and Biomarkers of Disease. Personalised medicine refers to tailoring glaucoma prevention and treatment individually based on genetic and other characteristics of the individual patient. Most of the open-angle glaucoma forms are complex and have polygenic basis resulting from a combined effect of several common gene variants, each of which has a small effect size on the disease [148]. Genome-wide association analyses have identified several loci associated with glaucoma risk factors such as IOP, vertical cup-disc ratio, and central corneal thickness [149–151]. The genetic findings need to be integrated with risk factors to identify patients at high risk of progression to visual impairment [152]. Using the large data set machine learning can cluster patients based on their genomic similarity and detect relevant pathways that are disrupted in glaucoma [153]. Investigation of these pathways can detect new biomarkers for glaucoma diagnosis, prognosis, and new therapeutic targets, which after validation will help ophthalmologists to identify patients with high risk of progression and treat them more aggressively and avoid unnecessary treatment to many subjects [154].

Many different measurable indicators can serve as potential biomarkers for glaucoma such as IOP or OCT measurements of retinal nerve fibre layer thickness [155]. With the advances in technology including imaging, genomic, metabolomic, and proteomic techniques, potential new biomarkers are generated and need to be validated in large patients' populations with different ethnicity and stage of glaucoma [156, 157]. These biomarkers may serve as diagnostic, predictive, prognostic biomarkers or indicate patients' response to drug or surgery [158, 159]. Recently, aqueous veins were found to be a potential structural biomarker predicting the outcome of Schlemm's canal-based glaucoma surgery [160].

6. Conclusions

The present review summarizes current treatment strategies in glaucoma therapy and addresses potential future targets and ways to protect and improve survival and regeneration of retinal ganglion cells. Targeting several pathways has been shown to improve survival of retinal ganglion cells in animal glaucoma models. However, translation to the clinic is hampered due to the limitations of glaucoma models and the fact that glaucoma pathogenesis is multifactorial and incompletely understood. Further research is required to identify molecules and pathways to be able to improve clinical translation of neuroprotection in glaucoma. Currently lowering of IOP remains the only treatment strategy and adherence to treatment is essential. Sustained drug delivery systems aim to overcome adherence problem, but there are still unresolved issues with safety, duration of IOP-lowering effect, treatment with several compounds simultaneously, quality of life, and costs.

For future personalized medicine that considers individual variability in genes, environmental and lifestyle factors for each person hold a promise to predict optimal treatment and prevention strategies for the individual glaucoma patient.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this work.

References

[1] S. R. Flaxman, R. R. A. Bourne, S. Resnikoff et al., "Global causes of blindness and distance vision impairment 1990–2020: a systematic review and meta-analysis," *The Lancet Global Health*, vol. 5, no. 12, pp. e1221–e1234, 2017.

[2] Y.-C. Tham, X. Li, T. Y. Wong, H. A. Quigley, T. Aung, and C.-Y. Cheng, "Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis," *Ophthalmology*, vol. 121, no. 11, pp. 2081–2090, 2014.

[3] D. Peters, B. Bengtsson, and A. Heijl, "Lifetime risk of blindness in open-angle glaucoma," *American Journal of Ophthalmology*, vol. 156, no. 4, pp. 724–730, 2013.

[4] K. S. Yadav, R. Rajpurohit, and S. Sharma, "Glaucoma: current treatment and impact of advanced drug delivery systems," *Life Sciences*, vol. 221, pp. 362–376, 2019.

[5] M. Mozaffarieh and J. Flammer, "New insights in the pathogenesis and treatment of normal tension glaucoma," *Current Opinion in Pharmacology*, vol. 13, no. 1, pp. 43–49, 2013.

[6] J. Adeghate, K. Rahmatnejad, M. Waisbourd, and L. J. Katz, "Intraocular pressure-independent management of normal tension glaucoma," *Survey of Ophthalmology*, vol. 64, no. 1, pp. 101–110, 2019.

[7] M. A. Kass, D. K. Heuer, E. J. Higginbotham et al., "The ocular hypertension treatment study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma," *Archives of Ophthalmology*, vol. 120, no. 6, pp. 701–713, 2002.

[8] A. Heijl, M. C. Leske, B. Bengtsson, L. Hyman, B. Bengtsson, and M. Hussein, "Reduction of intraocular pressure and

glaucoma progression: results from the early manifest glaucoma trial," *Archives of Ophthalmology*, vol. 120, no. 10, pp. 1268–1279, 2002.

[9] The Advanced Glaucoma Intervention Study (AGIS): 7, "The relationship between control of intraocular pressure and visual field deterioration. The AGIS investigators," *American Journal of Ophthalmology*, vol. 130, no. 4, pp. 429–440, 2000.

[10] T. Li, K. Lindsley, B. Rouse et al., "Comparative effectiveness of first-line medications for primary open-angle glaucoma: a systematic review and network meta-analysis," *Ophthalmology*, vol. 123, no. 1, pp. 129–140, 2016.

[11] F. Li, W. Huang, and X. Zhang, "Efficacy and safety of different regimens for primary open-angle glaucoma or ocular hypertension: a systematic review and network meta-analysis," *Acta Ophthalmologica*, vol. 96, no. 3, pp. e277–e284, 2018.

[12] F. Aptel, M. Cucherat, and P. Denis, "Efficacy and tolerability of prostaglandin analogs: a meta-analysis of randomized controlled clinical trials," *Journal of Glaucoma*, vol. 17, no. 8, pp. 667–673, 2008.

[13] C. B. Camras, A. Alm, P. Watson, and J. Stjernschantz, "Latanoprost, a prostaglandin analog, for glaucoma therapy. Efficacy and safety after 1 year of treatment in 198 patients. Latanoprost study groups," *Ophthalmology*, vol. 103, no. 11, pp. 1916–1924, 1996.

[14] K. Inoue, M. Shiokawa, R. Higa et al., "Adverse periocular reactions to five types of prostaglandin analogs," *Eye*, vol. 26, no. 11, pp. 1465–1472, 2012.

[15] M. Wand, C. M. Gilbert, and T. J. Liesegang, "Latanoprost and herpes simplex keratitis," *American Journal of Ophthalmology*, vol. 127, no. 5, pp. 602–604, 1999.

[16] R. L. Coakes and R. F. Brubaker, "The mechanism of timolol in lowering intraocular pressure. In the normal eye," *Archives of Ophthalmology*, vol. 96, no. 11, pp. 2045–2048, 1978.

[17] T. Taniguchi and Y. Kitazawa, "The potential systemic effect of topically applied β-blockers in glaucoma therapy," *Current Opinion in Ophthalmology*, vol. 8, no. 2, pp. 55–58, 1997.

[18] D. R. Caldwell, C. R. Salisbury, and J. P. Guzek, "Effects of topical betaxolol in ocular hypertensive patients," *Archives of Ophthalmology*, vol. 102, no. 4, pp. 539-540, 1984.

[19] R. E. Marquis and J. T. Whitson, "Management of glaucoma: focus on pharmacological therapy," *Drugs & Aging*, vol. 22, no. 1, pp. 1–21, 2005.

[20] A. Konowal, J. C. Morrison, S. V. L. Brown et al., "Irreversible corneal decompensation in patients treated with topical dorzolamide," *American Journal of Ophthalmology*, vol. 127, no. 4, pp. 403–406, 1999.

[21] J. S. Schuman, B. Horwitz, N. T. Choplin, R. David, D. Albracht, and K. Chen, "A 1-year study of brimonidine twice daily in glaucoma and ocular hypertension. A controlled, randomized, multicenter clinical trial. Chronic brimonidine study group," *Archives of Ophthalmology*, vol. 115, no. 7, pp. 847–852, 1997.

[22] L. J. Katz, "Brimonidine tartrate 0.2% twice daily vs timolol 0.5% twice daily: 1-year results in glaucoma patients. Brimonidine study group," *American Journal of Ophthalmology*, vol. 127, no. 1, pp. 20–26, 1999.

[23] T. Krupin, J. M. Liebmann, D. S. Greenfield, R. Ritch, S. Gardiner, and G. Low-Pressure Glaucoma Study, "A randomized trial of brimonidine versus timolol in preserving visual function: results from the low-pressure glaucoma treatment study," *American Journal of Ophthalmology*, vol. 151, no. 4, pp. 671–681, 2011.

[24] Aerie Pharmaceuticals, "Aerie pharmaceuticals receives positive CHMP opinion for Rhokiinsa in the European Union," 2019, https://eyewire.news/articles/aerie-pharmaceuticals-receives-positive-chmp-opinion-for-rhokiinsa-in-the-european-union/.

[25] A. P. Tanna and M. Johnson, "Rho kinase inhibitors as a novel treatment for glaucoma and ocular hypertension," *Ophthalmology*, vol. 125, no. 11, pp. 1741–1756, 2018.

[26] J. Bacharach, H. B. Dubiner, B. Levy, C. C. Kopczynski, G. D. Novack, and A.-C. S. Group, "Double-masked, randomized, dose-response study of AR-13324 versus latanoprost in patients with elevated intraocular pressure," *Ophthalmology*, vol. 122, no. 2, pp. 302–307, 2015.

[27] J. B. Serle, L. J. Katz, E. McLaurin et al., "Two phase 3 clinical trials comparing the safety and efficacy of netarsudil to timolol in patients with elevated intraocular pressure: rho kinase elevated IOP treatment trial 1 and 2 (ROCKET-1 and ROCKET-2)," *American Journal of Ophthalmology*, vol. 186, pp. 116–127, 2018.

[28] M. Y. Kahook, J. B. Serle, F. S. Mah et al., "Long-term safety and ocular hypotensive efficacy evaluation of netarsudil ophthalmic solution: rho kinase elevated IOP treatment trial (ROCKET-2)," *American Journal of Ophthalmology*, vol. 200, pp. 130–137, 2019.

[29] A. S. Khouri, J. B. Serle, J. Bacharach et al., "Once-daily netarsudil versus twice-daily timolol in patients with elevated intraocular pressure: the randomized phase 3 ROCKET-4 study," *American Journal of Ophthalmology*, vol. 204, pp. 97–104, 2019.

[30] M. E. Cavet, J. L. Vittitow, F. Impagnatiello, E. Ongini, and E. Bastia, "Nitric oxide (NO): an emerging target for the treatment of glaucoma," *Investigative Opthalmology & Visual Science*, vol. 55, no. 8, pp. 5005–5015, 2014.

[31] I. M. Goldstein, P. Ostwald, and S. Roth, "Nitric oxide: a review of its role in retinal function and disease," *Vision Research*, vol. 36, no. 18, pp. 2979–2994, 1996.

[32] L. Schmetterer and K. Polak, "Role of nitric oxide in the control of ocular blood flow," *Progress in Retinal and Eye Research*, vol. 20, no. 6, pp. 823–847, 2001.

[33] G. C. Brown and A. Bal-Price, "Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria," *Molecular Neurobiology*, vol. 27, no. 3, pp. 325–355, 2003.

[34] D. D. Thomas, X. Liu, S. P. Kantrow, and J. R. Lancaster, "The biological lifetime of nitric oxide: implications for the perivascular dynamics of NO and O_2," *Proceedings of the National Academy of Sciences*, vol. 98, no. 1, pp. 355–360, 2001.

[35] R. N. Weinreb, B. Scassellati Sforzolini, J. Vittitow, and J. Liebmann, "Latanoprostene bunod 0.024% versus timolol maleate 0.5% in subjects with open-angle glaucoma or ocular hypertension: the APOLLO study," *Ophthalmology*, vol. 123, no. 5, pp. 965–973, 2016.

[36] F. A. Medeiros, K. R. Martin, J. Peace, B. Scassellati Sforzolini, J. L. Vittitow, and R. N. Weinreb, "Comparison of latanoprostene bunod 0.024% and timolol maleate 0.5% in open-angle glaucoma or ocular hypertension: the LUNAR study," *American Journal of Ophthalmology*, vol. 168, pp. 250–259, 2016.

[37] R. N. Weinreb, J. M. Liebmann, K. R. Martin, P. L. Kaufman, and J. L. Vittitow, "Latanoprostene bunod 0.024% in subjects with open-angle glaucoma or ocular hypertension: pooled phase 3 study findings," *Journal of Glaucoma*, vol. 27, no. 1, pp. 7–15, 2018.

[38] P. L. Kaufman, "Latanoprostene bunod ophthalmic solution 0.024% for IOP lowering in glaucoma and ocular

hypertension," *Expert Opinion on Pharmacotherapy*, vol. 18, no. 4, pp. 433–444, 2017.

[39] K. Kawase, J. L. Vittitow, R. N. Weinreb, M. Araie, and J. S. Group, "Long-term safety and efficacy of latanoprostene bunod 0.024% in Japanese subjects with open-angle glaucoma or ocular hypertension: the JUPITER study," *Advances in Therapy*, vol. 33, no. 9, pp. 1612–1627, 2016.

[40] Aerie Pharmaceuticals, "Aerie pharmaceuticals receives FDA approval of rocklatan for reduction of IOP," 2019, https://eyewire.news/articles/aerie-pharmaceuticals-receives-fda-approval-of-rocklatan-for-reduction-of-iop/.

[41] S. Asrani, A. L. Robin, J. B. Serle et al., "Netarsudil/latanoprost fixed-dose combination for elevated intraocular pressure: three-month data from a randomized phase 3 trial," *American Journal of Ophthalmology*, vol. 207, pp. 248–257, 2019.

[42] T. R. Walters, I. I. K. Ahmed, R. A. Lewis et al., "Once-daily netarsudil/Latanoprost fixed-dose combination for elevated intraocular pressure in the randomized phase 3 MERCURY-2 study," *Ophthalmology Glaucoma*, vol. 2, no. 5, pp. 280–289, 2019.

[43] M. F. Sugrue, "New approaches to antiglaucoma therapy," *Journal of Medicinal Chemistry*, vol. 40, no. 18, pp. 2793–2809, 1997.

[44] E. A. Cairns, W. H. Baldridge, and M. E. Kelly, "The endocannabinoid system as a therapeutic target in glaucoma," *Neural Plasticity*, vol. 2016, Article ID 9364091, 10 pages, 2016.

[45] C. Rapino, D. Tortolani, L. Scipioni, and M. Maccarrone, "Neuroprotection by (endo)cannabinoids in glaucoma and retinal neurodegenerative diseases," *Current Neuropharmacology*, vol. 16, no. 7, pp. 959–970, 2018.

[46] A. Porcella, C. Maxia, G. L. Gessa, and L. Pani, "The synthetic cannabinoid WIN55212-2 decreases the intraocular pressure in human glaucoma resistant to conventional therapies," *European Journal of Neuroscience*, vol. 13, no. 2, pp. 409–412, 2001.

[47] J. Crandall, S. Matragoon, Y. M. Khalifa et al., "Neuroprotective and intraocular pressure-lowering effects of (−)Δ^9-tetrahydrocannabinol in a rat model of glaucoma," *Ophthalmic Research*, vol. 39, no. 2, pp. 69–75, 2007.

[48] A. B. El-Remessy, I. E. Khalil, S. Matragoon et al., "Neuroprotective effect of (−)Δ^9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite," *The American Journal of Pathology*, vol. 163, no. 5, pp. 1997–2008, 2003.

[49] P. F. Whiting, R. F. Wolff, S. Deshpande et al., "Cannabinoids for medical use: a systematic review and meta-analysis," *JAMA*, vol. 313, no. 24, pp. 2456–2473, 2015.

[50] P. S. Taskar, A. Patil, P. Lakhani et al., "Delta(9)-tetrahydrocannabinol derivative-loaded nanoformulation lowers intraocular pressure in normotensive rabbits," *Translational Vision Science & Technology*, vol. 8, no. 5, p. 15, 2019.

[51] H. A. Alkozi, G. Navarro, R. Franco, and J. Pintor, "Melatonin and the control of intraocular pressure," *Progress in Retinal and Eye Research*, vol. 75, Article ID 100798, 2019.

[52] P. Alarma-Estrany, A. Guzman-Aranguez, F. Huete et al., "Design of novel melatonin analogs for the reduction of intraocular pressure in normotensive rabbits," *Journal of Pharmacology and Experimental Therapeutics*, vol. 337, no. 3, pp. 703–709, 2011.

[53] A. Martinez-Aguila, B. Fonseca, M. J. Perez de Lara, and J. Pintor, "Effect of melatonin and 5-methoxycarbonylamino-N-acetyltryptamine on the intraocular pressure of normal and glaucomatous mice," *Journal of Pharmacology and Experimental Therapeutics*, vol. 357, no. 2, pp. 293–299, 2016.

[54] N. Pescosolido, V. Gatto, A. Stefanucci, and D. Rusciano, "Oral treatment with the melatonin agonist agomelatine lowers the intraocular pressure of glaucoma patients," *Ophthalmic and Physiological Optics*, vol. 35, no. 2, pp. 201–205, 2015.

[55] P. O. Lundmark, S. R. Pandi-Perumal, V. Srinivasan, D. P. Cardinali, and R. E. Rosenstein, "Melatonin in the eye: implications for glaucoma," *Experimental Eye Research*, vol. 84, no. 6, pp. 1021–1030, 2007.

[56] R. Fuchshofer and E. R. Tamm, "Modulation of extracellular matrix turnover in the trabecular meshwork," *Experimental Eye Research*, vol. 88, no. 4, pp. 683–688, 2009.

[57] J. G. Browne, S. L. Ho, R. Kane et al., "Connective tissue growth factor is increased in pseudoexfoliation glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 52, no. 6, pp. 3660–3666, 2011.

[58] B. Junglas, A. H. Yu, U. Welge-Lussen, E. R. Tamm, and R. Fuchshofer, "Connective tissue growth factor induces extracellular matrix deposition in human trabecular meshwork cells," *Experimental Eye Research*, vol. 88, no. 6, pp. 1065–1075, 2009.

[59] B. Junglas, S. Kuespert, A. A. Seleem et al., "Connective tissue growth factor causes glaucoma by modifying the actin cytoskeleton of the trabecular meshwork," *American Journal of Pathology*, vol. 180, no. 6, pp. 2386–2403, 2012.

[60] A. E. Dillinger, M. Guter, F. Froemel et al., "Intracameral delivery of layer-by-layer coated siRNA nanoparticles for glaucoma therapy," *Small*, vol. 14, no. 50, Article ID e1803239, 2018.

[61] P. Guglielmi, S. Carradori, C. Campestre, and G. Poce, "Novel therapies for glaucoma: a patent review (2013–2019)," *Expert Opinion on Therapeutic Patents*, vol. 29, no. 10, pp. 769–780, 2019.

[62] Y. Zhong, Z. Yang, W. C. Huang, and X. Luo, "Adenosine, adenosine receptors and glaucoma: an updated overview," *Biochimica et Biophysica Acta*, vol. 1830, no. 4, pp. 2882–2890, 2013.

[63] T. W. Shearer and C. E. Crosson, "Adenosine A1 receptor modulation of MMP-2 secretion by trabecular meshwork cells," *Investigative Ophthalmology & Visual Science*, vol. 43, no. 9, pp. 3016–3020, 2002.

[64] A. Laties, C. C. Rich, R. Stoltz et al., "A randomized phase 1 dose escalation study to evaluate safety, tolerability, and pharmacokinetics of trabodenoson in healthy adult volunteers," *Journal of Ocular Pharmacology and Therapeutics: The Official Journal of the Association for Ocular Pharmacology and Therapeutics*, vol. 32, no. 8, pp. 548–554, 2016.

[65] J. S. Myers, K. N. Sall, H. DuBiner et al., "A dose-escalation study to evaluate the safety, tolerability, pharmacokinetics, and efficacy of 2 and 4 weeks of twice-daily ocular trabodenoson in adults with ocular hypertension or primary open-angle glaucoma," *Journal of Ocular Pharmacology and Therapeutics: The Official Journal of the Association for Ocular Pharmacology and Therapeutics*, vol. 32, no. 8, pp. 555–562, 2016.

[66] M. Schwartz and E. Yoles, "Neuroprotection: a new treatment modality for glaucoma?" *Current Opinion in Ophthalmology*, vol. 11, no. 2, pp. 107–111, 2000.

[67] T. Furuya, Z. Pan, and K. Kashiwagi, "Role of retinal glial cell glutamate transporters in retinal ganglion cell survival following stimulation of NMDA receptor," *Current Eye Research*, vol. 37, no. 3, pp. 170–178, 2012.

[68] W. A. Hare, E. WoldeMussie, R. K. Lai et al., "Efficacy and safety of memantine treatment for reduction of changes

associated with experimental glaucoma in monkey, I: functional measures," *Investigative Ophthalmology & Visual Science*, vol. 45, no. 8, pp. 2625–2639, 2004.

[69] Y. H. Yucel, N. Gupta, Q. Zhang, A. P. Mizisin, M. W. Kalichman, and R. N. Weinreb, "Memantine protects neurons from shrinkage in the lateral geniculate nucleus in experimental glaucoma," *Archives of Ophthalmology*, vol. 124, no. 2, pp. 217–225, 2006.

[70] R. N. Weinreb, J. M. Liebmann, G. A. Cioffi et al., "Oral memantine for the treatment of glaucoma: design and results of 2 randomized, placebo-controlled, phase 3 studies," *Ophthalmology*, vol. 125, no. 12, pp. 1874–1885, 2018.

[71] N. N. Osborne, "Recent clinical findings with memantine should not mean that the idea of neuroprotection in glaucoma is abandoned," *Acta Ophthalmologica*, vol. 87, no. 4, pp. 450–454, 2009.

[72] D. F. Sena and K. Lindsley, "Neuroprotection for treatment of glaucoma in adults," *Cochrane Database Systematic Review*, vol. 1, Article ID CD006539, 2017.

[73] M. Erdurmus, R. Yagci, O. Atis, R. Karadag, A. Akbas, and I. F. Hepsen, "Antioxidant status and oxidative stress in primary open angle glaucoma and pseudoexfoliative glaucoma," *Current Eye Research*, vol. 36, no. 8, pp. 713–718, 2011.

[74] M. Tanito, S. Kaidzu, Y. Takai, and A. Ohira, "Association between systemic oxidative stress and visual field damage in open-angle glaucoma," *Scientific Reports*, vol. 6, p. 25792, 2016.

[75] M. I. Lopez Sanchez, J. G. Crowston, D. A. Mackey, and I. A. Trounce, "Emerging mitochondrial therapeutic targets in optic neuropathies," *Pharmacology & Therapeutics*, vol. 165, pp. 132–152, 2016.

[76] E. Loskutova, C. O'Brien, I. Loskutov, and J. Loughman, "Nutritional supplementation in the treatment of glaucoma: a systematic review," *Survey of Ophthalmology*, vol. 64, no. 2, pp. 195–216, 2019.

[77] D. Janssens, C. Michiels, E. Delaive, F. Eliaers, K. Drieu, and J. Remacle, "Protection of hypoxia-induced ATP decrease in endothelial cells by Ginkgo biloba extract and bilobalide," *Biochemical Pharmacology*, vol. 50, no. 7, pp. 991–999, 1995.

[78] R. Abdel-Kader, S. Hauptmann, U. Keil et al., "Stabilization of mitochondrial function by Ginkgo biloba extract (EGb 761)," *Pharmacological Research*, vol. 56, no. 6, pp. 493–502, 2007.

[79] A. Eckert, U. Keil, I. Scherping, S. Hauptmann, and W. E. Muller, "Stabilization of mitochondrial membrane potential and improvement of neuronal energy metabolism by Ginkgo biloba extract EGb 761," *Annals of the New York Academy of Sciences*, vol. 1056, pp. 474–485, 2005.

[80] J. Lee, S. W. Sohn, and C. Kee, "Effect of Ginkgo biloba extract on visual field progression in normal tension glaucoma," *Journal of Glaucoma*, vol. 22, no. 9, pp. 780–784, 2013.

[81] E. Ahmed, T. Donovan, L. Yujiao, and Q. Zhang, "Mitochondrial targeted antioxidant in cerebral ischemia," *Journal of Neurology and Neuroscience*, vol. 6, no. 2, 2015.

[82] L. Papucci, N. Schiavone, E. Witort et al., "Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property," *Journal of Biological Chemistry*, vol. 278, no. 30, pp. 28220–28228, 2003.

[83] R. Russo, F. Cavaliere, L. Rombola et al., "Rational basis for the development of coenzyme Q10 as a neurotherapeutic agent for retinal protection," *Progress in Brain Research*, vol. 173, pp. 575–582, 2008.

[84] V. Parisi, M. Centofanti, S. Gandolfi et al., "Effects of coenzyme Q10 in conjunction with vitamin E on retinal-evoked and cortical-evoked responses in patients with open-angle glaucoma," *Journal of Glaucoma*, vol. 23, no. 6, pp. 391–404, 2014.

[85] L. Quaranta, I. Riva, E. Biagioli et al., "Evaluating the effects of an ophthalmic solution of coenzyme Q10 and vitamin E in open-angle glaucoma patients: a study protocol," *Advances in Therapy*, vol. 36, no. 9, pp. 2506–2514, 2019.

[86] Citicoline. Monograph," *Alternative Medicine Review*, vol. 13, no. 1, pp. 50–57, 2008.

[87] T. Oshitari, N. Yoshida-Hata, and S. Yamamoto, "Effect of neurotrophic factors on neuronal apoptosis and neurite regeneration in cultured rat retinas exposed to high glucose," *Brain Research*, vol. 1346, pp. 43–51, 2010.

[88] A. Matteucci, M. Varano, L. Gaddini et al., "Neuroprotective effects of citicoline in in vitro models of retinal neurodegeneration," *International Journal of Molecular Sciences*, vol. 15, no. 4, pp. 6286–6297, 2014.

[89] P. Grieb, A. Junemann, M. Rekas, and R. Rejdak, "Citicoline: a food beneficial for patients suffering from or threated with glaucoma," *Frontiers in Aging Neuroscience*, vol. 8, p. 73, 2016.

[90] K. Qian, Y. Gu, Y. Zhao, Z. Li, and M. Sun, "Citicoline protects brain against closed head injury in rats through suppressing oxidative stress and calpain over-activation," *Neurochemical Research*, vol. 39, no. 7, pp. 1206–1218, 2014.

[91] M. Virno, J. Pecori-Giraldi, A. Liguori, and F. Gregorio, "The protective effect of citicoline on the progression of the perimetric defects in glaucomatous patients (perimetric study with a 10-year follow-up)," *Acta Ophthalmologica Scandinavica*, vol. 78, no. 232, pp. 56-57, 2000.

[92] V. Parisi, "Electrophysiological assessment of glaucomatous visual dysfunction during treatment with cytidine-5′-diphosphocholine (citicoline): a study of 8 years of follow-up," *Documenta Ophthalmologica*, vol. 110, no. 1, pp. 91–102, 2005.

[93] L. Ottobelli, G. L. Manni, M. Centofanti, M. Iester, F. Allevena, and L. Rossetti, "Citicoline oral solution in glaucoma: is there a role in slowing disease progression?" *Ophthalmologica*, vol. 229, no. 4, pp. 219–226, 2013.

[94] M. Lanza, U. A. Gironi Carnevale, L. Mele, M. Bifani Sconocchia, S. Bartollino, and C. Costagliola, "Morphological and functional evaluation of oral citicoline therapy in chronic open-angle glaucoma patients: a pilot study with a 2-year follow-up," *Frontiers in Pharmacology*, vol. 10, p. 1117, 2019.

[95] V. Parisi, M. Centofanti, L. Ziccardi et al., "Treatment with citicoline eye drops enhances retinal function and neural conduction along the visual pathways in open angle glaucoma," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 253, no. 8, pp. 1327–1340, 2015.

[96] N. N. Osborne, "Mitochondria: their role in ganglion cell death and survival in primary open angle glaucoma," *Experimental Eye Research*, vol. 90, no. 6, pp. 750–757, 2010.

[97] H. Sawada, T. Fukuchi, T. Tanaka, and H. Abe, "Tumor necrosis factor-alpha concentrations in the aqueous humor of patients with glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 51, no. 2, pp. 903–906, 2010.

[98] G. Tezel, L. Y. Li, R. V. Patil, and M. B. Wax, "TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes," *Investigative Ophthalmology & Visual Science*, vol. 42, no. 8, pp. 1787–1794, 2001.

[99] Y. Kitaoka, Y. Kitaoka, J. M. Kwong et al., "TNF-alpha-induced optic nerve degeneration and nuclear factor-kappaB p65," *Investigative Ophthalmology & Visual Science*, vol. 47, no. 4, pp. 1448–1457, 2006.

[100] M. M. Al-Gayyar, S. Matragoon, B. A. Pillai, T. K. Ali, M. A. Abdelsaid, and A. B. El-Remessy, "Epicatechin blocks pro-nerve growth factor (proNGF)-mediated retinal neurodegeneration via inhibition of p75 neurotrophin receptor expression in a rat model of diabetes [corrected]," *Diabetologia*, vol. 54, no. 3, pp. 669–680, 2011.

[101] G. Tezel and M. B. Wax, "Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells," *The Journal of Neuroscience*, vol. 20, no. 23, pp. 8693–8700, 2000.

[102] G. Tezel, G. M. Seigel, and M. B. Wax, "Autoantibodies to small heat shock proteins in glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 39, no. 12, pp. 2277–2287, 1998.

[103] J. Yang, G. Tezel, R. V. Patil, C. Romano, and M. B. Wax, "Serum autoantibody against glutathione S-transferase in patients with glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 42, no. 6, pp. 1273–1276, 2001.

[104] S. C. Joachim, J. Reichelt, S. Berneiser, N. Pfeiffer, and F. H. Grus, "Sera of glaucoma patients show autoantibodies against myelin basic protein and complex autoantibody profiles against human optic nerve antigens," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 246, no. 4, pp. 573–580, 2008.

[105] S. C. Joachim, K. Bruns, K. J. Lackner, N. Pfeiffer, and F. H. Grus, "Antibodies to alpha B-crystallin, vimentin, and heat shock protein 70 in aqueous humor of patients with normal tension glaucoma and IgG antibody patterns against retinal antigen in aqueous humor," *Current Eye Research*, vol. 32, no. 6, pp. 501–509, 2007.

[106] K. Bell, C. Wilding, S. Funke et al., "Neuroprotective effects of antibodies on retinal ganglion cells in an adolescent retina organ culture," *Journal of Neurochemistry*, vol. 139, no. 2, pp. 256–269, 2016.

[107] K. Bell, S. Funke, and F. H. Grus, "Autoimmunity and glaucoma," *Ophthalmologe*, vol. 116, no. 1, pp. 18–27, 2019.

[108] J. W. Wang, S. D. Chen, X. L. Zhang, and J. B. Jonas, "Retinal microglia in glaucoma," *Journal of Glaucoma*, vol. 25, no. 5, pp. 459–465, 2016.

[109] A. K. Toft-Kehler, D. M. Skytt, K. A. Poulsen et al., "Limited energy supply in Muller cells alters glutamate uptake," *Neurochemical Research*, vol. 39, no. 5, pp. 941–949, 2014.

[110] M. P. Junier, "What role(s) for TGFalpha in the central nervous system?" *Progress in Neurobiology*, vol. 62, no. 5, pp. 443–473, 2000.

[111] X. Liu, A. F. Clark, and R. J. Wordinger, "Expression of ciliary neurotrophic factor (CNTF) and its tripartite receptor complex by cells of the human optic nerve head," *Molecular Vision*, vol. 13, pp. 758–763, 2007.

[112] P. Gris, A. Tighe, D. Levin, R. Sharma, and A. Brown, "Transcriptional regulation of scar gene expression in primary astrocytes," *Glia*, vol. 55, no. 11, pp. 1145–1155, 2007.

[113] S. Pasquin, M. Sharma, and J. F. Gauchat, "Ciliary neurotrophic factor (CNTF): new facets of an old molecule for treating neurodegenerative and metabolic syndrome pathologies," *Cytokine & Growth Factor Reviews*, vol. 26, no. 5, pp. 507–515, 2015.

[114] A. A. Shpak, A. B. Guekht, T. A. Druzhkova, K. I. Kozlova, and N. V. Gulyaeva, "Ciliary neurotrophic factor in patients with primary open-angle glaucoma and age-related cataract," *Molecular Vision*, vol. 23, pp. 799–809, 2017.

[115] R. S. Chong, A. Osborne, R. Conceicao, and K. R. Martin, "Platelet-derived growth factor preserves retinal synapses in a rat model of ocular hypertension," *Investigative Ophthalmology & Visual Science*, vol. 57, no. 3, pp. 842–852, 2016.

[116] S. Takahama, M. O. Adetunji, T. Zhao, S. Chen, W. Li, and S. I. Tomarev, "Retinal astrocytes and GABAergic wide-field amacrine cells express PDGFRalpha: connection to retinal ganglion cell neuroprotection by PDGF-AA," *Investigative Ophthalmology & Visual Science*, vol. 58, no. 11, pp. 4703–4711, 2017.

[117] T. V. Johnson, N. D. Bull, D. P. Hunt, N. Marina, S. I. Tomarev, and K. R. Martin, "Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 51, no. 4, pp. 2051–2059, 2010.

[118] E. Emre, N. Yuksel, G. Duruksu et al., "Neuroprotective effects of intravitreally transplanted adipose tissue and bone marrow-derived mesenchymal stem cells in an experimental ocular hypertension model," *Cytotherapy*, vol. 17, no. 5, pp. 543–559, 2015.

[119] B. Mead, A. Logan, M. Berry, W. Leadbeater, and B. A. Scheven, "Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury," *Investigative Ophthalmology & Visual Science*, vol. 54, no. 12, pp. 7544–7556, 2013.

[120] V. Holan, B. Hermankova, M. Krulova, and A. Zajicova, "Cytokine interplay among the diseased retina, inflammatory cells and mesenchymal stem cells—a clue to stem cell-based therapy," *World Journal of Stem Cells*, vol. 11, no. 11, pp. 957–967, 2019.

[121] T. V. Johnson, N. W. DeKorver, V. A. Levasseur et al., "Identification of retinal ganglion cell neuroprotection conferred by platelet-derived growth factor through analysis of the mesenchymal stem cell secretome," *Brain*, vol. 137, no. Pt 2, pp. 503–519, 2014.

[122] A. Osborne, J. Sanderson, and K. R. Martin, "Neuroprotective effects of human mesenchymal stem cells and platelet-derived growth factor on human retinal ganglion cells," *Stem Cells*, vol. 36, no. 1, pp. 65–78, 2018.

[123] V. Holan, B. Hermankova, P. Bohacova et al., "Distinct immunoregulatory mechanisms in mesenchymal stem cells: role of the cytokine environment," *Stem Cell Reviews and Reports*, vol. 12, no. 6, pp. 654–663, 2016.

[124] J. Y. Kim, Y. S. You, S. H. Kim, and O. W. Kwon, "Epiretinal membrane formation after intravitreal autologous stem cell implantation in a retinitis pigmentosa patient," *Retinal Cases and Brief Reports*, vol. 11, no. 3, pp. 227–231, 2017.

[125] A. E. Kuriyan, T. A. Albini, J. H. Townsend et al., "Vision loss after intravitreal injection of autologous "stem cells" for AMD," *New England Journal of Medicine*, vol. 376, no. 11, pp. 1047–1053, 2017.

[126] M. Dominici, K. Le Blanc, I. Mueller et al., "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement," *Cytotherapy*, vol. 8, no. 4, pp. 315–317, 2006.

[127] N. Cuenca, L. Fernandez-Sanchez, L. Campello et al., "Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases," *Progress in Retinal and Eye Research*, vol. 43, pp. 17–75, 2014.

[128] S. E. Ratican, A. Osborne, and K. R. Martin, "Progress in gene therapy to prevent retinal ganglion cell loss in glaucoma and Leber's hereditary optic neuropathy," *Neural Plasticity*, vol. 2018, Article ID 7108948, 11 pages, 2018.

[129] A. M. Wilson and A. Di Polo, "Gene therapy for retinal ganglion cell neuroprotection in glaucoma," *Gene Therapy*, vol. 19, no. 2, pp. 127–136, 2012.

[130] S. Shimizu, P. R. Lichter, A. T. Johnson et al., "Age-dependent prevalence of mutations at the GLC1A locus in primary open-angle glaucoma," *American Journal of Ophthalmology*, vol. 130, no. 2, pp. 165–177, 2000.

[131] A. Jain, G. Zode, R. B. Kasetti et al., "CRISPR-Cas9-based treatment of myocilin-associated glaucoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 42, pp. 11199–11204, 2017.

[132] M. T. Sommerfeld, R. Schweigreiter, Y. A. Barde, and E. Hoppe, "Down-regulation of the neurotrophin receptor TrkB following ligand binding. Evidence for an involvement of the proteasome and differential regulation of TrkA and TrkB," *Journal of Biological Chemistry*, vol. 275, no. 12, pp. 8982–8990, 2000.

[133] A. Osborne, T. Z. Khatib, L. Songra et al., "Neuroprotection of retinal ganglion cells by a novel gene therapy construct that achieves sustained enhancement of brain-derived neurotrophic factor/tropomyosin-related kinase receptor-B signaling," *Cell Death & Disease*, vol. 9, no. 10, p. 1007, 2018.

[134] R. A. Lewis, W. C. Christie, D. G. Day et al., "Bimatoprost sustained-release implants for glaucoma therapy: 6-month results from a phase I/II clinical trial," *American Journal of Ophthalmology*, vol. 175, pp. 137–147, 2017.

[135] J. D. Brandt, K. Sall, H. DuBiner et al., "Six-month intraocular pressure reduction with a topical bimatoprost ocular insert: results of a phase II randomized controlled study," *Ophthalmology*, vol. 123, no. 8, pp. 1685–1694, 2016.

[136] J. D. Brandt, H. B. DuBiner, R. Benza et al., "Long-term safety and efficacy of a sustained-release bimatoprost ocular ring," *Ophthalmology*, vol. 124, no. 10, pp. 1565-1566, 2017.

[137] J. Xu, Y. Ge, R. Bu et al., "Co-delivery of latanoprost and timolol from micelles-laden contact lenses for the treatment of glaucoma," *Journal of Controlled Release*, vol. 305, pp. 18–28, 2019.

[138] B. Sleath, A. L. Robin, D. Covert, J. E. Byrd, G. Tudor, and B. Svarstad, "Patient-reported behavior and problems in using glaucoma medications," *Ophthalmology*, vol. 113, no. 3, pp. 431–436, 2006.

[139] C. Y. Kim, K. H. Park, J. Ahn et al., "Treatment patterns and medication adherence of patients with glaucoma in South Korea," *The British Journal of Ophthalmology*, vol. 101, no. 6, pp. 801–807, 2017.

[140] C. O. Okeke, H. A. Quigley, H. D. Jampel et al., "Adherence with topical glaucoma medication monitored electronically the Travatan dosing aid study," *Ophthalmology*, vol. 116, no. 2, pp. 191–199, 2009.

[141] J. C. Tsai, C. A. McClure, S. E. Ramos, D. G. Schlundt, and J. W. Pichert, "Compliance barriers in glaucoma: a systematic classification," *Journal of Glaucoma*, vol. 12, no. 5, pp. 393–398, 2003.

[142] C. M. Olthoff, J. S. Schouten, B. W. van de Borne, and C. A. Webers, "Noncompliance with ocular hypotensive treatment in patients with glaucoma or ocular hypertension an evidence-based review," *Ophthalmology*, vol. 112, no. 6, pp. 953–961, 2005.

[143] A. P. Tse, M. Shah, N. Jamal, and A. Shaikh, "Glaucoma treatment adherence at a United Kingdom general practice," *Eye*, vol. 30, no. 8, pp. 1118–1122, 2016.

[144] P. A. Newman-Casey, L. M. Niziol, B. W. Gillespie, N. K. Janz, P. R. Lichter, and D. C. Musch, "The association between medication adherence and visual field progression in the collaborative initial glaucoma treatment study," *Ophthalmology*, vol. 127, no. 4, pp. 477–483, 2020.

[145] H. Waterman, J. R. Evans, T. A. Gray, D. Henson, and R. Harper, "Interventions for improving adherence to ocular hypotensive therapy," *Cochrane Database Systematic Reviews*, vol. 4, Article ID CD006132, 2013.

[146] F. R. Juliana, S. Kesse, K. O. Boakye-Yiadom, H. Veroniaina, H. Wang, and M. Sun, "Promising approach in the treatment of glaucoma using nanotechnology and nanomedicine-based systems," *Molecules*, vol. 24, no. 20, 2019.

[147] M. L. Occhiutto, R. C. Maranhao, V. P. Costa, and A. G. Konstas, "Nanotechnology for medical and surgical glaucoma therapy—a review," *Advances in Therapy*, vol. 37, no. 1, pp. 155–199, 2020.

[148] M. I. McCarthy, G. R. Abecasis, L. R. Cardon et al., "Genome-wide association studies for complex traits: consensus, uncertainty and challenges," *Nature Reviews Genetics*, vol. 9, no. 5, pp. 356–369, 2008.

[149] A. P. Khawaja, J. N. Cooke Bailey, N. J. Wareham et al., "Genome-wide analyses identify 68 new loci associated with intraocular pressure and improve risk prediction for primary open-angle glaucoma," *Nature Genetics*, vol. 50, no. 6, pp. 778–782, 2018.

[150] W. D. Ramdas, L. M. van Koolwijk, M. K. Ikram et al., "A genome-wide association study of optic disc parameters," *PLoS Genetics*, vol. 6, no. 6, Article ID e1000978, 2010.

[151] M. D. Benson, C. C. Khor, P. J. Gage, and O. J. Lehmann, "A targeted approach to genome-wide studies reveals new genetic associations with central corneal thickness," *Molecular Vision*, vol. 23, pp. 952–962, 2017.

[152] S. E. Moroi, D. A. Raoof, D. M. Reed, S. Zollner, Z. Qin, and J. E. Richards, "Progress toward personalized medicine for glaucoma," *Expert Review of Ophthalmology*, vol. 4, no. 2, pp. 145–161, 2009.

[153] C. Lopez, S. Tucker, T. Salameh, and C. Tucker, "An unsupervised machine learning method for discovering patient clusters based on genetic signatures," *Journal of Biomedical Informatics*, vol. 85, pp. 30–39, 2018.

[154] S. E. Moroi, D. M. Reed, D. S. Sanders et al., "Precision medicine to prevent glaucoma-related blindness," *Current Opinion in Ophthalmology*, vol. 30, no. 3, pp. 187–198, 2019.

[155] A. Wu, A. P. Khawaja, L. R. Pasquale, and J. D. Stein, "A review of systemic medications that may modulate the risk of glaucoma," *Eye*, vol. 34, no. 1, pp. 12–28, 2019.

[156] T. E. Yap, E. Shamsher, L. Guo, and M. F. Cordeiro, "Ophthalmic research lecture 2018: DARC as a potential surrogate marker," *Ophthalmic Research*, vol. 63, no. 1, pp. 1–7, 2019.

[157] L. Agnifili, D. Pieragostino, A. Mastropasqua et al., "Molecular biomarkers in primary open-angle glaucoma: from noninvasive to invasive," *Progress in Brain Research*, vol. 221, pp. 1–32, 2015.

[158] A. G. Hindle, R. Thoonen, J. V. Jasien et al., "Identification of candidate miRNA biomarkers for glaucoma," *Investigative Ophthalmology & Visual Science*, vol. 60, no. 1, pp. 134–146, 2019.

[159] F. S. Ong, J. Z. Kuo, W. C. Wu et al., "Personalized medicine in ophthalmology: from pharmacogenetic biomarkers to therapeutic and dosage optimization," *Journal of Personalized Medicine*, vol. 3, no. 1, pp. 40–69, 2013.

[160] A. S. Huang, R. C. Penteado, S. K. Saha et al., "Fluorescein aqueous angiography in live normal human eyes," *Journal of Glaucoma*, vol. 27, no. 11, pp. 957–964, 2018.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 8324319, 10 pages
https://doi.org/10.1155/2020/8324319

Research Article

Nailfold Capillary Hemorrhages: Microvascular Risk Factors for Primary Open-Angle Glaucoma

Nicholas M. Pfahler,[1] Jordan L. Barry,[1] Indre E. Bielskus,[1] Agni Kakouri,[1,2] Michael C. Giovingo,[2] Nicholas J. Volpe,[3] and Paul A. Knepper[1,3]

[1]*Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago College of Medicine, Chicago, IL, USA*
[2]*Division of Ophthalmology, John H. Stroger Jr. Hospital of Cook County, Chicago, IL, USA*
[3]*Department of Ophthalmology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*

Correspondence should be addressed to Paul A. Knepper; pknepper@northwestern.edu

Received 2 January 2020; Revised 12 May 2020; Accepted 20 May 2020; Published 9 June 2020

Guest Editor: Miriam Kolko

Background. Primary open-angle glaucoma (POAG) is associated with systemic microvascular dysfunction including hemorrhages and other abnormalities of the nailfold capillary bed. This study aimed to verify the specificity of nailfold capillary hemorrhages and other abnormalities as risk factors for POAG. *Methods*. Nailfold video capillaroscopy was performed using a JH-1004 capillaroscope on the fourth and fifth digits of the nondominant hand in control ($n = 277$), POAG ($n = 206$), OHT ($n = 57$), and SG ($n = 29$) subjects. The number of hemorrhages, dilated capillaries >50 μm, and avascular zones ≥200 μm were counted and adjusted to counts per 100 capillaries. Descriptive analyses as well as univariate- and multivariable-adjusted logistic regression were performed comparing all groups with controls and POAG with OHT and SG. Subanalyses were conducted in POAG patients examining the association between nailfold capillary outcomes and previous glaucoma surgery, successful IOP control, or disease severity. *Results*. All nailfold capillary outcomes were significantly increased in POAG, no outcomes were increased in SG, and only hemorrhages were mildly increased in OHT. Hemorrhages were significantly more frequent in POAG compared with both OHT ($P < 0.0001$) and SG ($P = 0.001$). There were significant trends between higher numbers of hemorrhages and POAG compared with controls, OHT, and SG, with odds ratios of 18.3 (8.5–39.4), 9.1 (1.9–13.4), and 11.8 (1.7–7.3), respectively, for the presence of two or more hemorrhages per 100 capillaries. Hemorrhages were not significantly associated with previous glaucoma surgery, successful postoperative IOP control, or disease severity in POAG. *Conclusions*. These findings suggest that systemic microvascular dysfunction is frequent in POAG and occurs early in the disease process. The high specificity of nailfold hemorrhages makes them viable clinical risk factors for POAG.

1. Introduction

Primary open-angle glaucoma (POAG) is a leading cause of irreversible vision loss worldwide. The disease is characterized by progressive excavation of the optic disc with corresponding visual field defects due to loss of retinal ganglion cells [1]. POAG has no known cause, is frequently asymptomatic, and may not be discovered until late in the disease process. Elevated intraocular pressure (IOP) is the most important risk factor for POAG and can be well controlled in most patients with topical medications or surgical intervention. Some POAG patients, however, do not exhibit increased IOP, i.e., normal tension glaucoma (NTG), and others progress despite all current treatment modalities. Non-IOP risk factors for POAG are primarily vascular in nature and include altered systemic blood pressure and reduced ocular blood flow. Systemic manifestations associated with POAG include diabetes [2], systemic hypertension [3], vasospasm [4], vascular dysfunction [5], and microvascular disease [6]. Numerous biomarkers relating to the extracellular matrix, cell signaling, oxidative stress, and innate and adaptive immunity have also been associated with POAG [7].

Two important research goals for POAG are the identification of persons at high risk for the disease and the

advancement of neuroprotection for the optic nerve. Towards these goals, one key research aim is to identify systemic risk factors that can help predict POAG before vision loss occurs and potentially provide insight into the disease pathogenesis. One such marker may be microvascular hemorrhages identified using nailfold capillaroscopy [8, 9]. Nailfold capillaroscopy is a safe, noninvasive, and inexpensive clinical procedure that serves as a diagnostic tool in rheumatology and has been used to detect systemic microvascular abnormalities in POAG [9, 10]. Previous nailfold capillaroscopy studies have documented that hemorrhages, dilated capillaries, and avascular zones are increased in POAG compared with healthy control subjects [8, 9]. It remains unclear, however, whether this trend extends to high-risk ocular hypertension (OHT) patients or those with secondary forms of glaucoma.

The purpose of this study was to verify the specificity of nailfold capillary hemorrhages and other microvascular abnormalities as risk factors or predictors of POAG. Nailfold abnormalities were measured and compared between normal control subjects, POAG patients, OHT patients, and secondary angle-closure or angle-recession glaucoma (SG) patients. To examine the possibility that systemic microvascular abnormalities may be associated with IOP or the extent of visual field loss, subanalyses were conducted in POAG patients examining the association between nailfold capillary outcomes and previous glaucoma surgery, successful IOP control, or disease severity.

2. Materials and Methods

2.1. Study Population. All subjects were recruited from the Department of Ophthalmology, Northwestern University Feinberg School of Medicine, Chicago IL; the Division of Ophthalmology, John H. Stroger, Jr. Hospital of Cook County, Chicago IL; or the private practice of Zaparackas and Knepper Ltd. in Chicago, IL. In total, 569 participants were recruited from January 2014 to September 2019 including 277 control, 206 POAG, 29 SG, and 57 OHT subjects. This clinic-based, cross-sectional prospective study included a comprehensive ocular examination and nailfold capillaroscopy. All procedures were approved by the Institutional Review Boards of each study site. The study conformed to the tenets of Declaration of Helsinki, and all participants provided written informed consent.

All subjects were at least 35 years of age at the time of recruitment. Eyes with glaucoma had clinical findings consistent with glaucomatous optic neuropathy, including cup-disc ratio ≥0.6 and abnormal visual fields. Reliable visual field tests were acquired closest to the time of nailfold capillaroscopy using a Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, California, USA). Glaucoma severity was graded using visual field mean deviation scores from the more severely affected eye according to the Hodapp–Parrish–Anderson scale [11]. Early, intermediate, and late POAGs were defined as MD scores better than −6 dB, between −6 and −12 dB, and worse than −12 dB, respectively. Eyes with NTG had signs of glaucomatous optic neuropathy and no history of IOP >21 mm Hg. Eyes with high-tension POAG (HTG) had signs of glaucomatous optic neuropathy with a highest known IOP >21 mm Hg. OHT was defined as untreated IOP >21 mm Hg without visual field loss or any signs of glaucomatous optic neuropathy. SG patients had a diagnosis of angle-closure ($n = 20$) or angle-recession ($n = 9$) glaucoma.

Exclusion criteria consisted of connective tissue disease including systemic sclerosis, systemic lupus erythematosus, Sjogren's syndrome, antiphospholipid syndrome, dermatomyositis, psoriasis, or rheumatoid arthritis as these conditions have been associated with abnormal nailfold capillary patterns [12]. No subjects had comorbid mental cognitive impairment, dementia, or age-related macular degeneration, which have been associated with nailfold capillary abnormalities [13–15]. Additional exclusion criteria included bleeding diathesis, current treatment with chemotherapy, and history of trauma to the fingers or hands.

2.2. Collection of Covariate Data. Covariate data from the date of capillaroscopy were extracted from the subjects' medical record for use in multivariable logistic regression. Demographic information included age, sex, and race/ethnicity. Ocular information included IOP, cup-disc ratio, visual acuity, refractive error, lens status, and previous glaucoma surgery. Surgical success in patients with previous glaucoma surgery was defined as IOP ≤15 mm Hg at the time of examination. Information regarding preexisting medical conditions included the presence of hypertension, diabetes, arthritis, hyperlipidemia, cardiovascular disease, and cancer as well as any family history of glaucoma. Information about the use of medications included use of antiplatelet or anticoagulant medications (aspirin, warfarin, clopidogrel, etc.) which may cause nailfold capillary hemorrhages.

2.3. Nailfold Capillaroscopy. Nailfold capillaroscopy was performed using a JH-1004 microscope (Jiangsu Jiahua Electronic Instrument Co., Jiangsu, China) set at ×280 magnification as previously described [9]. In brief, subjects were asked to remain in a seated position for 15 minutes at room temperature prior to recording in order to stabilize blood flow. Cedar oil was applied to the nailfold to enhance epidermal translucency and visualization of the nailfold microvasculature. Videos were recorded of the fourth and fifth digits on each subject's nondominant hand to minimize confounding microvascular disruptions and ensure a sufficient sample of capillaries. Each video was 2 to 4 minutes in length and spanned the width of the capillary bed. Videos were analyzed by two masked graders who counted the total number of hemorrhages, dilated capillaries, and avascular zones for each participant. All values were normalized to counts per 100 capillaries based on the number of capillaries sampled to account for variability in capillary density. Hemorrhages were defined as extravascular deposits consisting of fresh, bright-red blood or old blood containing hemosiderin deposits. Dilated capillaries were defined as capillaries with a maximum width >50 μm. Avascular zones were defined as horizontal regions ≥200 μm displaying no capillaries.

2.4. Statistical Analysis. Statistical analysis was performed using Minitab 19 statistical software (State College, PA, US). Continuous demographic and clinical features were compared using unpaired *t*-tests. Frequencies of univariate categorical variables were compared between groups using χ^2 tests. The mean numbers of hemorrhages, dilated capillaries, and avascular zones per 100 capillaries were compared using nonparametric Mann–Whitney *U* tests. Mantel–Haenszel χ^2 tests were used to determine whether ordinal categories of nailfold findings were associated with case status.

To further assess associations between nailfold capillary abnormalities and case status, univariate and multivariable-adjusted logistic regression analyses were conducted with odds ratios (ORs) and 95% confidence intervals (CIs) calculated for each analysis. In the multivariable-adjusted model, adjustments were made for age, sex, race, study site, family history of glaucoma, hypertension, diabetes, non-skin cancer malignancy, and use of antiplatelet or anticoagulant medication. For each nailfold capillaroscopy finding, associations between binary values (e.g., any versus no hemorrhages) as well as between ordinal categories of values ("1" for 0 hemorrhages per 100 capillaries, "2" for >0 and <1, "3" for ≥1 and <2, and "4" for ≥(2) were evaluated between groups. All statistical tests were two sided with significance levels set at $P < 0.05$.

Interrater and intrarater reliability were calculated using weighted Cohen's kappa statistics accounting for ordinal categorical variables ($n = 50$). Agreement was high between graders for both hemorrhages (OR = 0.91, $P < 0.0001$) and all capillary abnormalities (OR = 0.89, $P < 0.0001$). Repeatability within graders was also high for both hemorrhages (OR = 0.96, $P < 0.0001$) and all capillary abnormalities (OR = 0.90, $P < 0.0001$).

3. Results

A total of 277 control, 206 POAG, 57 OHT, and 29 SG patients were included in this study. POAG patients were significantly older, more likely to be female, and more likely to be African-American compared with control subjects (Table 1). POAG patients also had a higher frequency of arthritis, diabetes, cardiovascular disease, and family history of glaucoma. The 206 POAG patients enrolled consisted of 173 (84.0%) with highest known IOP ≤21 mm Hg (HTG) and 33 (16.0%) with highest known IOP <21 mm Hg (NTG). HTG subjects were more likely than controls to have hypertension, diabetes, arthritis, cardiovascular disease, and a family history of glaucoma, whereas none of these variables were associated with NTG (Supplementary Table 1). No other demographic or clinical features differed between any subject type and controls.

The number of capillaries counted was variable within groups but lower in POAG and SG than controls and OHT (Supplementary Table 2). The higher age of POAG and SG patients could account for this difference; however, the correlation between age and capillaries counted was very weak (0.02). Because it is possible that factors such as video quality and natural variability in capillary density may influence the number of capillaries counted, the numbers of hemorrhages, dilated capillaries, and avascular zones were normalized to counts per 100 capillaries for all analyses. The effect of this normalization on hemorrhages is shown in Supplementary Table 2.

In the descriptive analysis of means (Table 2), POAG patients had significantly more hemorrhages ($P < 0.0001$), dilated capillaries ($P = 0.002$), and avascular zones ($P = 0.0005$) compared with controls. POAG patients also had significantly more hemorrhages than both SG ($P = 0.0007$) and OHT ($P < 0.0001$) patients. There were no differences between HTG and NTG patients, POAG patients who had undergone glaucoma surgery and those who had not, or POAG patients with successful versus unsuccessful glaucoma surgery. OHT patients had more dilated capillaries than controls ($P = 0.04$), whereas there was no difference in any measure between SG and controls.

In the descriptive frequency analysis (Table 3), the presence of one or more nailfold microvascular abnormalities was significantly higher in POAG than in controls: 85.9% versus 43.7% had at least one hemorrhage ($P < 0.0001$); 47.6% versus 33.2% had at least one dilated capillary ($P < 0.0001$); and 19.4% versus 8.7% had at least one avascular zone ($P = 0.001$). In diagnostic terms, the presence of any hemorrhage in POAG yielded a sensitivity of 0.86, specificity of 0.54, and accuracy of 0.70. The presence of any of the three nailfold capillary abnormalities yielded a sensitivity of 0.95, specificity of 0.40, and accuracy of 0.67. In OHT, the presence of one or more hemorrhages was moderately more frequent compared with controls (59.6% versus 43.7%, $P = 0.03$), although POAG patients were significantly more likely to have one or more hemorrhages compared with both OHT ($P < 0.0001$) and SG ($P = 0.001$). When we evaluated the relationship between ordinal categories of abnormalities and subject type, there were significant associations between hemorrhages ($P < 0.0001$), dilated capillaries ($P = 0.03$), and avascular zones ($P = 0.0007$) in POAG compared with controls (Table 3). These trends were preserved when POAG was classified as HTG and NTG with the exception of dilated capillaries, which were associated with NTG ($P = 0.01$) but not HTG ($P = 0.10$).

In the univariate analysis in relation to control subjects, the presence of any hemorrhage (OR = 7.9, $P < 0.0001$) and increasing numbers of hemorrhages (OR = 14.2, $P < 0.0001$) were strongly associated with POAG (Table 4). There was a significant association between increasing hemorrhages and OHT compared with controls (OR = 1.9; $P = 0.006$) and no association between any nailfold microvascular abnormalities and SG. In POAG, the association with increasing hemorrhages was significantly greater compared with both SG ($P = 0.006$) and OHT ($P < 0.0001$), with the odds of two or more hemorrhages being 7.5 and 15.7 times greater in POAG compared with SG and OHT, respectively (Table 5).

Compared with the univariate analysis, the multivariable-adjusted model showed stronger associations between all microvascular abnormalities and POAG (Table 6). The presence of any hemorrhage ($P < 0.0001$), dilated capillary ($P < 0.0001$), or avascular zone ($P < 0.0001$) was significantly

Table 1: Demographic and clinical features of the study cohort.

	Control ($n = 277$)	POAG ($n = 206$)	P value vs. control	SG ($n = 29$)	P value vs. control	OHT ($n = 57$)	P value vs. control
Age in years, mean (SD)	63.2 (10.8)	67.5 (10.6)	<0.0001*	65.0 (11.2)	0.40*	62.5 (12.5)	0.68*
Sex, *n* (%)							
Female	157 (56.7)	95 (46.1)	0.02	18 (62.1)	0.42	36 (63.2)	0.36
Male	120 (43.3)	111 (53.9)		11 (37.9)		21 (36.8)	
Race/ethnicity, *n* (%)							
Caucasian	169 (61.0)	88 (42.7)	<0.0001	9 (33.3)	0.002	39 (68.4)	0.29
African-American	59 (21.3)	95 (46.1)		15 (55.6)		12 (21.1)	
Asian/Pacific Islander	7 (2.5)	11 (5.3)		1 (3.7)		3 (5.3)	
Hispanic	42 (15.2)	12 (5.8)		4 (14.8)		3 (5.3)	
Any cancer, *n* (%)	30 (10.8)	20 (9.7)	0.69	3 (10.3)	0.94	3 (5.3)	0.17
Non-skin cancer malignancy, *n* (%)	19 (6.9)	19 (9.2)	0.35	2 (6.9)	0.99	1 (1.8)	0.09
Anticoagulant medication, *n* (%)	69 (24.9)	46 (22.3)	0.51	5 (17.2)	0.36	9 (15.8)	0.13
Cataract, *n* (%)	90 (32.5)	70 (34.0)	0.73	6 (20.7)	0.19	16 (28.1)	0.51
Cataract surgery, *n* (%)	27 (1.0)	26 (12.6)	0.33	1 (3.4)	0.26	6 (10.5)	0.87
Arthritis, *n* (%)	50 (18.1)	19 (9.2)	0.005	1 (3.4)	0.05	6 (10.5)	0.15
Hypertension, *n* (%)	116 (41.9)	105 (51.0)	0.05	9 (31.0)	0.26	23 (40.4)	0.83
Diabetes, *n* (%)	58 (20.9)	65 (31.6)	0.008	5 (17.2)	0.64	12 (22.8)	0.76
Hyperlipidemia, *n* (%)	61 (22.0)	42 (20.4)	0.66	7 (24.1)	0.79	15 (26.3)	0.49
Cardiovascular disease, any, *n* (%)	30 (10.8)	34 (16.5)	0.05	1 (3.4)	0.21	5 (8.8)	0.70
Family history of glaucoma, *n* (%)	57 (20.6)	76 (36.9)	<0.0001	7 (24.1)	0.65	13 (22.8)	0.71
IOP (mm Hg), mean (SD)	14.6 (3.5)	17.2 (6.7)	<0.0001*	20.4 (8.2)	<0.0001*	19.4 (4.2)	<0.0001*
Disease severity, *n* (%)							
Early	———	111 (53.9)		———		———	
Intermediate	———	48 (23.3)		———		———	
Late	———	47 (22.8)		———		———	

*the *t*-test; all other *P* values, the $\chi 2$ test. HTG, high-tension glaucoma; IOP, intraocular pressure; NTG, normal-tension glaucoma; OHT, ocular hypertension; POAG, primary open-angle glaucoma; SG, secondary glaucoma.

Table 2: Mean number of nailfold capillary abnormalities per 100 capillaries.

Subject type	*n*	Hemorrhages/100				Dilated capillaries/100				Avascular zones/100			
		Mean	*SD*	*P* value*	*P* value†	Mean	*SD*	*P* value*	*P* value†	Mean	*SD*	*P* value*	*P* value†
Control	277	0.77	1.39			0.69	1.37			0.09	0.34		
POAG	206	1.88	1.68	<0.0001		1.02	1.55	0.002		0.20	0.47	0.0005	
High-tension	173	1.87	1.71	<0.0001		1.00	1.63	0.02		0.19	0.47	0.003	
Normal-tension	33	1.91	1.60	<0.0001		1.10	1.05	0.0007		0.24	0.44	0.001	
No surgery	151	1.87	1.82	<0.0001		0.98	1.62	0.03		0.53	0.53	0.89	
Surgery	55	1.88	1.44	<0.0001		1.05	1.68	0.005		0.29	0.29	<0.0001	
Successful	19	2.05	1.57	<0.0001		1.24	1.69	0.06		0.13	0.32	0.38	
Unsuccessful	36	2.00	1.70	<0.0001		0.94	1.69	0.21		0.07	0.28	0.60	
SG	29	0.89	0.93	0.08	0.0007	0.47	0.82	0.87	0.12	0.09	0.34	0.78	0.12
OHT	57	0.87	1.19	0.09	<0.0001	0.98	1.36	0.04	0.95	0.16	0.44	0.11	0.48

*the Mann–Whitney *U* test versus control. †the Mann–Whitney *U* test versus POAG. HTG, high-tension glaucoma; NTG, normal-tension glaucoma; OHT, ocular hypertension; POAG, primary open-angle glaucoma; SG, secondary glaucoma.

more likely to occur in POAG compared with controls. Likewise, there were significant associations between severity of hemorrhages ($P < 0.0001$), dilated capillaries ($P = 0.002$), and avascular zones ($P = 0.002$) and odds of POAG compared with controls, with POAG patients having 18.2 times greater odds of exhibiting two or more hemorrhages. OHT was associated with the presence of one or more hemorrhages (OR = 1.4, $P = 0.006$) and increasing numbers of hemorrhages ($P = 0.01$) compared with controls. SG was not associated with any nailfold abnormalities. When POAG was compared with OHT using the multivariable-adjusted model (Table 5), the odds of one or more hemorrhages (OR = 3.5, $P = 0.001$) and the association between increasing numbers of hemorrhages (OR = 11.8, $P < 0.0001$) was greater in POAG. Likewise, when POAG was compared with SG, the odds of one or more hemorrhages (OR = 5.1, $P = 0.001$) and the association between increasing numbers of hemorrhages was greater in POAG (OR = 9.1, $P = 0.001$).

To examine the association between IOP and nailfold microvascular abnormalities in POAG, univariate and multivariable-adjusted logistic regression was performed in

Table 3: Descriptive analyses of nailfold capillary abnormalities.

Nailfold microvascular feature	Control	POAG ($n = 206$)		HTG ($n = 173$)		NTG ($n = 33$)		SG ($n = 29$)		P value vs. POAG	OHT ($n = 57$)		P value vs. POAG
	n (%)	n (%)	P value vs. control	n (%)	P value vs. control	n (%)	P value vs. control	n (%)	P value vs. control		n (%)	P value vs. control	
Hemorrhages /100 capillaries, n (%)													
0.0	156 (56.3)	29 (14.1)	<0.0001*	24 (13.9)	<0.0001*	5 (15.2)	<0.0001*	11 (37.9)	0.49*	0.001*	23 (40.4)	0.96*	<0.0001*
>0.0 and <1.0	58 (20.9)	57 (27.7)		50 (28.9)		7 (21.2)		9 (31.0)			18 (31.6)		
≥1.0 and <2.0	32 (11.6)	41 (19.9)		34 (19.7)		7 (21.2)		10 (34.5)			12 (21.1)		
≥2.0	31 (11.2)	79 (38.3)		65 (37.6)		12 (42.4)		4 (13.8)			4 (7.0)		
Any hemorrhages	121 (43.7)	177 (85.9)	<0.0001†	149 (86.1)	<0.0001†	28 (84.8)	<0.0001†	18 (62.1)	0.06†	0.001†	34 (59.6)	0.03†	<0.0001†
Dilated capillaries /100 capillaries, n (%)													
0.0	185 (66.8)	108 (52.4)	0.03*	97 (56.1)	0.10	11 (33.3)	0.01*	19 (65.5)	0.59*	0.11*	30 (52.6)	0.14*	0.92*
>0.0 and <1.0	32 (11.6)	36 (17.5)		28 (16.2)		8 (24.2)		7 (24.1)			8 (14.0)		
≥1.0 and <2.0	25 (9.0)	24 (11.7)		18 (10.4)		6 (18.2)		5 (17.2)			8 (14.0)		
≥2.0	32 (12.6)	38 (18.4)		30 (17.3)		8 (24.2)		2 (6.9)			11 (19.3)		
Any dilated capillaries	92 (33.2)	98 (47.6)	<0.0001†	76 (43.9)	0.02†	22 (66.7)	<0.0001†	10 (34.5)	0.89†	0.19†	27 (47.4)	0.05†	0.05†
Avascular zones /100 capillaries, n (%)													
0.0	253 (91.3)	166 (80.6)	0.0007*	142 (82.1)	0.002*	24 (72.7)	0.03*	27 (93.1)	0.49*	0.45*	48 (84.2)	0.21*	0.29*
>0.0 and <0.75	11 (4.0)	12 (5.8)		8 (4.6)		4 (12.1)		0 (0.0)			5 (8.8)		
≥0.75	13 (4.7)	28 (13.6)		23 (13.3)		5 (15.2)		2 (6.9)			4 (7.0)		
Any avascular zones	24 (8.7)	40 (19.4)	0.001†	31 (17.9)	0.004†	9 (27.3)	0.004†	2 (6.9)	0.98†	0.10†	9 (15.8)	0.12†	0.53†

*the Mantel–Haenszel–Cochran test. † the χ^2 test. HTG, high-tension glaucoma; NTG, normal-tension glaucoma; OHT, ocular hypertension; POAG, primary open-angle glaucoma; SG, secondary glaucoma.

POAG patients examining the association between nailfold microvascular abnormalities and IOP, previous glaucoma surgery, or surgical success in those who had undergone surgery (Table 7). There were no significant differences between POAG patients who had undergone IOP-reducing surgery and those who had not. Among those who had undergone surgery, there were no differences between patients whose surgery was classified as successful versus unsuccessful. Additionally, there were no significant correlations between IOP and hemorrhages, dilated capillaries, or avascular zones in any group (Supplementary Table 3).

To evaluate the influence of POAG severity, nailfold capillary abnormalities were compared between POAG patients with early and intermediate-to-late stage disease (Supplementary Table 4). The presence of one or more hemorrhages was significantly more common in intermediate-to-late POAG in the univariate analysis (OR = 2.5, $P = 0.03$) but not after adjustments in the multivariable model (OR = 2.2, $P = 0.08$), suggesting that other factors such as age or comorbidities may influence the difference between groups. There was, however, a significant correlation between visual field loss and hemorrhages across all POAG patients ($P = 0.03$) (Supplementary Table 3).

4. Discussion

In this study, POAG patients exhibited increased levels of nailfold hemorrhages, dilated capillaries, and avascular zones. The presence of any hemorrhage or 2 or more

Table 4: Univariate logistic regression analysis of nailfold capillary abnormalities in relation to control subjects.

Nailfold microvascular feature	POAG ($n = 206$)		HTG ($n = 173$)		NTG ($n = 33$)		SG ($n = 29$)		OHT ($n = 57$)	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Hemorrhages/100 capillaries										
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <1.0	5.3 (3.1–9.1)	<0.0001	5.6 (3.2–9.9)	<0.0001	3.8 (1.1–12.3)	<0.0001	2.2 (0.9–5.6)	0.30	2.1 (1.1–4.2)	0.05
≥1.0 and <2.0	6.7 (3.6–12.3)		6.7 (3.5–12.7)		6.6 (2.0–22.1)		2.1 (0.7–6.6)		2.5 (1.1–5.4)	
≥2.0	14.2 (7.9–25.2)		14.1 (7.7–25.9)		14.6 (4.9–43.4)		1.9 (0.6–6.3)		0.9 (0.3–2.8)	
Any hemorrhages	7.9 (5.0–12.5)	<0.0001	8.0 (4.9–13.1)	<0.0001	7.2 (2.7–19.3)	<0.0001	2.1 (1.0–4.6)	0.06	1.9 (1.1–3.4)	0.03
Dilated capillaries/100 capillaries										
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <1.0	1.9 (1.1–3.3)	0.02	1.7 (0.9–2.9)	0.14	4.2 (1.6–11.3)	0.002	2.1 (0.8–5.5)	0.20	1.5 (0.6–3.7)	0.23
≥1.0 and <2.0	1.8 (1.0–3.2)		1.4 (0.7–2.6)		5.4 (2.0–14.7)		0.4 (0.1–3.0)		2.0 (0.8–4.8)	
≥2.0	1.8 (1.0–3.0)		1.6 (0.9–2.8)		2.9 (1.0–8.3)		0.6 (0.1–2.5)		1.9 (0.9–4.2)	
Any dilated capillaries	1.8 (1.3–2.6)	0.001	1.6 (1.1–2.3)	0.02	4.0 (1.9–8.6)	<0.0001	1.1 (0.5–2.4)	0.89	1.8 (1.0–3.2)	0.05
Avascular zones/100 capillaries										
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <0.75	1.7 (0.7–3.9)	0.001	1.3 (0.5–3.3)	0.005	3.8 (1.1–13.0)	0.02	---	---	2.4 (0.8–7.2)	0.27
≥0.75	3.3 (1.7–6.5)		3.2 (1.5–6.4)		4.1 (1.3–12.3)		---		1.6 (0.5–5.2)	
Any avascular zones	2.5 (1.5–4.4)	0.001	2.3 (1.3–4.1)	0.004	4.0 (1.7–9.5)	0.004	0.8 (0.2–3.5)	0.74	2.0 (0.9–4.5)	0.12

CI, confidence interval; HTG, high-tension glaucoma; NTG, normal-tension glaucoma; OHT, ocular hypertension; OR, odds ratio; POAG, primary open-angle glaucoma; SG, secondary glaucoma.

hemorrhages was found to be highly significant risk factors for POAG. OHT patients exhibited a trend towards increased hemorrhages characterized by moderate frequency but low severity, and SG patients exhibited no significant microvascular abnormalities. Compared directly with OHT and SG, hemorrhages were both more frequent and more severe in POAG. No associations were found between nailfold capillary abnormalities and IOP, glaucoma surgery, or surgical success in POAG, and hemorrhages were only moderately associated with disease severity.

Studies demonstrating systemic findings in POAG have reported an increased prevalence of diabetes [2], cardiovascular system irregularities such as vasospasm [10, 16], altered hemorheological properties such as increased platelet activation/aggregation [17–21], and reduced cerebrospinal fluid pressure [22]. Additionally, POAG has been associated with alterations in systemic blood flow (both high and low blood pressure) and ocular perfusion pressure [23]. These properties suggest a possible vascular etiology to the development and progression of POAG. Vascular alterations such as vasospasm are associated with altered ocular blood flow and consequently decreased autoregulation of ocular perfusion pressure [10]. Decreased autoregulation of ocular blood flow and ocular perfusion pressure have been demonstrated in POAG [10, 24, 25] and can lead to ischemia, hypoxia, and ultimately retinal ganglion cell death. There have also been associations reported between optic disc hemorrhages and both nailfold hemorrhages and avascular zones in POAG [8], suggesting a comparable microvascular disruption at the systemic and ocular levels. Nailfold capillaroscopy may provide an easily accessible method of assessing risk of POAG based on systemic microvascular manifestations.

The exact cause of nailfold microvascular abnormalities in POAG remains unknown. An increased number of extravascular hemorrhages suggest a defect in the capillary wall and potentially present an opportunity for intervention. The endothelial cells lining the capillary wall are joined at their borders, a thin basal lamina, and are not supported by an outer tunic of smooth muscle cells. The luminal diameter ranges from 3 to 10 μm, and blood flow is regulated by

Table 5: Univariate and multivariable-adjusted logistic regression analysis of nailfold capillary abnormalities in relation to POAG compared with SG and OHT.

Nailfold microvascular feature	POAG ($n = 206$) vs. SG ($n = 29$)			POAG ($n = 206$) vs. OHT ($n = 57$)		
	Univariate OR (95% CI)	Multivariate OR (95% CI)	P value	Univariate OR (95% CI)	Multivariate OR (95% CI)	P value
Hemorrhages/100 capillaries						
0.0	1.0 (ref)			1.0 (ref)		
>0.0 and <1.0	2.4 (0.9–6.5)	3.2 (1.1–9.7)	0.004	2.5 (1.2–5.4)	2.0 (0.8–4.9)	<0.0001
≥1.0 and <2.0	3.1 (1.0–9.9)	5.1 (1.4–18.5)		2.7 (1.2–6.3)	2.7 (1.1–6.8)	
≥2.0	7.5 (2.2–25.4)	9.1 (2.5–33.7)		15.7 (5.0–49.2)	11.8 (3.5–39.5)	
Any hemorrhages	3.7 (1.6–8.7)	5.1 (1.9–13.4)	0.001	4.1 (2.1–7.8)	3.5 (1.7–7.3)	0.001
Dilated capillaries/100 capillaries						
0.0	1.0 (ref)			1.0 (ref)		
>0.0 and <1.0	0.9 (0.4–2.3)	1.1 (0.4–3.0)	0.18	1.3 (0.5–3.0)	1.9 (0.7–5.0)	0.53
≥1.0 and <2.0	4.2 (0.5–33.1)	4.5 (0.6–36.8)		0.8 (0.3–2.0)	1.0 (0.3–2.8)	
≥2.0	3.3 (0.7–15.0)	3.2 (0.7–15.8)		1.0 (0.4–2.1)	1.5 (0.6–3.9)	
Any dilated capillaries	1.7 (0.8–3.9)	1.9 (0.8–4.5)	0.15	1.0 (0.6–1.8)	1.1 (1.0–1.1)	0.28
Avascular zones/100 capillaries						
0.0	1.0 (ref)			1.0 (ref)		
>0.0 and <0.75	———	———	0.08	0.7 (0.2–2.1)	0.8 (0.2–2.6)	0.29
≥0.75	3.3 (0.7–14.3)	3.3 (0.7–15.4)		2.0 (0.7–6.1)	2.3 (0.7–7.6)	
Any avascular zones	3.3 (0.7–14.3)	3.3 (0.7–15.4)	0.08	0.3 (0.6–2.8)	1.4 (0.6–3.5)	0.41

The model adjusts for age (in years), sex, race, family history of glaucoma, hypertension, use of antiplatelet medication, and study site. CI, confidence interval; OHT, ocular hypertension; OR, odds ratio; POAG, primary open-angle glaucoma; SG, secondary glaucoma.

Table 6: Multivariable-adjusted logistic regression analysis of nailfold capillary abnormalities in relation to control subjects.

Nailfold microvascular feature	All POAG ($n = 206$)		HTG ($n = 173$)		NTG ($n = 33$)		SG ($n = 29$)		OHT ($n = 57$)	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Hemorrhages/100 capillaries										
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <1.0	5.6 (2.8–11.0)	<0.0001	6.5 (3.0–14.0)	<0.0001	4.6 (1.2–18.1)	<0.0001	1.4 (0.4–4.6)	0.65	2.7 (1.3–5.8)	0.01
≥1.0 and <2.0	6.7 (3.1–14.6)		7.8 (3.3–18.3)		5.8 (1.3–25.5)		2.4 (0.6–8.9)		3.3 (1.4–8.2)	
≥2.0	18.3 (8.5–39.4)		18.7 (3.3–18.3)		19.0 (5.0–72.0)		1.2 (0.3–5.3)		1.0 (0.3–3.5)	
Any hemorrhages	8.3 (4.6–15.0)	<0.0001	9.1 (4.7–17.8)	<0.0001	8.2 (2.6–26.3)	<0.0001	1.6 (0.6–4.1)	0.34	1.4 (0.7–2.8)	0.006
Dilated capillaries/100 capillaries										
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <1.0	2.5 (1.3–4.9)	0.002	2.3 (1.1–5.0)	0.02	3.4 (1.0–11.2)	0.01	2.5 (0.8–8.0)	0.46	1.3 (0.5–3.4)	0.26
≥1.0 and <2.0	2.0 (0.9–4.5)		1.4 (0.6–3.5)		5.1 (1.4–17.9)		0.6 (0.1–6.8)		2.0 (0.7–5.2)	
≥2.0	3.1 (1.5–6.1)		2.9 (1.3–6.4)		4.7 (1.4–15.7)		1.1 (0.2–6.1)		2.2 (0.9–5.2)	
Any dilated capillaries	2.5 (1.6–4.2)	<0.0001	2.2 (1.3–3.9)	0.003	4.2 (1.7–10.6)	0.001	1.6 (0.6–4.2)	0.36	1.8 (0.9–3.4)	0.08
Avascular zones/100 capillaries										
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <0.75	3.2 (1.1–9.2)	0.002	2.6 (0.7–9.4)	0.006	5.6 (1.3–24.0)	0.05	———	0.46	4.5 (1.4–15.0)	0.05
≥0.7	4.0 (1.6–9.9)		4.6 (1.7–12.6)		3.3 (0.8–14.1)		2.3 (0.3–18.0)		2.0 (0.6–7.2)	
Any avascular zones	3.7 (1.8–7.6)	<0.0001	3.7 (1.6–8.5)	0.002	4.2 (1.3–13.1)	0.02	2.3 (0.3–18.0)	0.46	3.0 (1.2–7.6)	0.03

The model adjusts for age (in years), sex, race, family history of glaucoma, hypertension, use of antiplatelet medication, and study site. CI, confidence interval; HTG, high-tension glaucoma; NTG, normal-tension glaucoma; OHT, ocular hypertension; OR, odds ratio; POAG, primary open-angle glaucoma; SG, secondary glaucoma.

Table 7: Multivariable-adjusted logistic regression analysis of nailfold capillary abnormalities in relation to IOP (mm Hg), previous glaucoma surgery, and surgical outcomes in POAG patients.

Nailfold microvascular feature	IOP >15 ($n = 129$) vs. IOP ≤15 ($n = 77$)		Previous surgery ($n = 55$) vs. no surgery ($n = 118$)		Successful ($n = 19$) vs. unsuccessful ($n = 36$) surgery	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Hemorrhages/100 capillaries						
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <1.0	1.7 (0.6–4.8)	0.07	0.6 (0.2–2.0)	0.42	3.3 (0.1–80.1)	0.17
≥1.0 and <2.0	0.7 (0.2–1.9)		1.4 (0.4–4.8)		1.2 (0.0–33.9)	
≥2.0	0.6 (0.2–1.6)		0.9 (0.3–2.7)		7.4 (0.3–178.3)	
Any hemorrhages	0.8 (0.3–2.0)	0.71	0.9 (0.3–2.5)	0.80	3.4 (0.2–60.4)	0.37
Dilated capillaries/100 capillaries						
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <1.0	1.1 (0.5–2.6)	0.99	2.2 (0.9–5.4)	0.38	1.0 (0.1–8.1)	0.06
≥1.0 and <2.0	1.0 (0.4–2.6)		1.4 (0.5–4.1)		5.0 (0.8–32.9)	
≥2.0	0.9 (0.4–2.2)		1.5 (0.5–4.4)		13.0 (0.9–192.1)	
Any dilated capillaries	1.0 (0.5–1.9)	0.96	1.7 (0.9–3.5)	0.12	3.6 (0.9–14.8)	0.08
Avascular zones/100 capillaries						
0.0	1.0 (ref)		1.0 (ref)		1.0 (ref)	
>0.0 and <0.75	1.1 (0.3–4.4)	0.67	0.3 (0.0–2.6)	0.23	———	0.27
≥0.75	0.7 (0.3–1.6)		0.5 (0.1–1.6)		3.3 (0.4–28.4)	
Any avascular zones	0.8 (0.4–1.7)	0.54	0.4 (0.1–1.2)	0.09	3.3 (0.4–28.4)	0.27

The model adjusts for age (in years), sex, race, family history of glaucoma, hypertension, use of antiplatelet medication, and study site. CI, confidence interval; IOP, intraocular pressure; OR, odds ratio; POAG, primary open-angle glaucoma; Ref, reference.

pericapillary contractile cells called pericytes. The rate of capillary endothelial replication is age-dependent and may have a half-life of one to three years [26]. In addition, circulating endothelial progenitor cells normally recruited to the capillary wall to maintain the endothelial barrier function are reduced in POAG [27]. Endothelial progenitor cell enrichment therapy has thus been suggested as a measure to target ischemic endothelial damage in POAG [28]. Other environmental cues such as the extracellular matrix and basal lamina also influence capillary function and may play a role in nailfold capillary abnormalities.

The elevated risk of POAG associated with OHT [29] makes the evaluation of nailfold capillaroscopy in these subjects, which has never previously been reported, particularly intriguing. The fact that hemorrhage prevalence increased in a stepwise fashion from control (44%) to OHT (60%) to POAG (86%) could be explained by this increased risk. Combined with the finding that 81% of early-stage POAG patients already exhibited hemorrhages, these results strongly suggest that nailfold hemorrhages occur early in the majority of POAG of cases and likely precede measurable changes in functional and structural diagnostic criteria such as visual field, cup-disc ratio, and retinal nerve fiber layer thickness. Interestingly, OHT status was more predictive of hemorrhages than IOP itself, suggesting that hemorrhages may be useful in predicting which OHT patients progress to POAG and which do not. This study was cross sectional in design; however, longitudinal studies would be needed to fully evaluate the time course of nailfold microvascular abnormalities in relation to POAG and the association between those abnormalities and risk of progression in controls and OHT patients.

Despite being sufficient in power analyses, we recognize that some groups consisted of disproportionate sample sizes and that this may have limited the reliability of certain comparisons. Specifically, there was a comparably low number of SG patients enrolled and a high ratio of HTG to NTG patients in the POAG sample. Future studies would need to specifically target the less-common SG population.

Nailfold hemorrhages measured using nailfold capillaroscopy appear to be a fast, easy, and reliable clinical risk factor for POAG. A large majority of early-stage POAG patients and nearly all intermediate/late-stage POAG patients exhibit hemorrhages, which do not appear to be associated with known risk factors such as IOP or common comorbidities such as hypertension. In contrast, the evaluation of other reported systemic vascular risk factors for the disease may be costly and require elaborate and time-consuming procedures, limiting their usefulness in a routine clinical setting. Nailfold capillaroscopy may serve not only as a useful assessment of risk in POAG but also as a research tool facilitating the identification of disease mechanisms and potential therapeutic targets.

5. Conclusions

In conclusion, POAG patients exhibited increased levels of all nailfold microvascular abnormalities measured. Nailfold hemorrhages, in particular, may serve as a useful clinical risk factor for the development and/or progression of POAG.

Data Availability

The datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this research.

Acknowledgments

This research was supported by Zaparackas and Knepper Ltd., Testog Immunodiagnostics Inc., and our generous patients.

Supplementary Materials

Supplementary Table 1: demographic and clinical features of POAG patients classified as high-tension (HTG) or normal-tension (NTG) glaucoma. Supplementary Table 2: mean numbers of capillaries counted and the effect of normalization to counts per 100 capillaries for hemorrhages. Supplementary Table 3: correlations between nailfold microvascular outcomes per 100 capillaries with IOP or visual field loss. Supplementary Table 4: univariate and multivariable-adjusted logistic regression analysis of nailfold capillary abnormalities in relation to intermediate-to-late ($n = 95$) vs. early POAG ($n = 111$). (*Supplementary Materials*)

References

[1] R. N. Weinreb, T. Aung, and F. A. Medeiros, "The pathophysiology and treatment of glaucoma," *JAMA*, vol. 311, no. 18, pp. 1901–1911, 2014.

[2] H. Hou, T. Shoji, L. M. Zangwill et al., "Progression of primary open-angle glaucoma in diabetic and nondiabetic patients," *American Journal of Ophthalmology*, vol. 189, pp. 1–9, 2018.

[3] G. Fuchsjäger-Mayrl, B. Wally, M. Georgopoulos et al., "Ocular blood flow and systemic blood pressure in patients with primary open-angle glaucoma and ocular hypertension," *Investigative Ophthalmology and Visual Science*, vol. 45, no. 3, pp. 834–839, 2004.

[4] S. Orgül, H. J. Kaiser, J. Flammer, and P. Gasser, "Systemic blood pressure and capillary blood-cell velocity in glaucoma patients: a preliminary study," *European Journal of Ophthalmology*, vol. 5, no. 2, pp. 88–91, 1995.

[5] L. R. Pasquale, "Vascular and autonomic dysregulation in primary open-angle glaucoma," *Current Opinion in Ophthalmology*, vol. 27, no. 2, pp. 94–101, 2016.

[6] N. M. Pfahler, M. Miazga, I. Bielskus et al., "Systemic manifestations of microvascular disease in primary open-angle glaucoma," in *In Glaucoma Research And Clinical Advances: 2018 to 2020*, P. A. Knepper and J. R. Samples, Eds., pp. 147–163, Kugler Publications, Amsterdam, Netherland, 2018.

[7] K. A. Green and P. A. Knepper, "Biomarkers in primary open-angle glaucoma," in *In Glaucoma Research And Clinical Advances: 2016 to 2018*, P. A. Knepper and J. R. Samples, Eds., pp. 173–184, Kugler Publications, Amsterdam, Netherland, 2016.

[8] H.-Y. L. Park, S. H. Park, Y. S. Oh, and C. K. Park, "Nail bed hemorrhage," *Archives of Ophthalmology*, vol. 129, no. 10, pp. 1299–1304, 2011.

[9] L. R. Pasquale, A. Hanyuda, A. Ren et al., "Nailfold capillary abnormalities in primary open-angle glaucoma: a multisite study," *Investigative Opthalmology & Visual Science*, vol. 56, no. 12, pp. 7021–7028, 2015.

[10] M. Emre, S. Orgül, K. Gugleta K, and J. Flammer, "Ocular blood flow alteration in glaucoma is related to systemic vascular dysregulation," *British Journal of Ophthalmology*, vol. 88, no. 5, pp. 662–666, 2004.

[11] E. Hodapp, R. K. Parrish II, and D. R. Anderson, *Clinical Decisions in Glaucoma*, The C.V. Mosby Co., London, UK, 1993.

[12] M. Hasegawa, "Dermoscopy findings of nail fold capillaries in connective tissue diseases," *The Journal of Dermatology*, vol. 38, no. 1, pp. 66–70, 2011.

[13] C. C. Cousins, M. L. Alosco, H. C. Cousins et al., "Nailfold capillary morphology in alzheimer's disease dementia," *Journal of Alzheimer's Disease*, vol. 66, no. 2, pp. 601–611, 2018.

[14] P. A. Knepper, N. M. Pfahler, J. McGuire, I. Bielskus, M. Giovingo, and N. J. Volpe, "The link between primary open angle glaucoma and Alzheimer's disease," in *In Glaucoma Research And Clinical Advances: 2018 to 2020*, P. A. Knepper and J. R. Samples, Eds., pp. 171–185, Kugler Publications, Amsterdam, Netherland, 2018.

[15] N. M. Pfahler, I. Bielskus, M. C. Giovingo, L. R. Pasquale, N. J. Volpe, and P. A. Knepper, "Systemic capillary abnormalities in age-related macular degeneration," *Investigative Ophthalmology and Visual Science*, vol. 60, no. 9, p. 1199, 2019.

[16] G. Gramer, B. H. F. Weber, and E. Gramer, "Migraine and vasospasm in glaucoma: age-related evaluation of 2027 patients with glaucoma or ocular hypertension," *Investigative Opthalmology & Visual Science*, vol. 56, no. 13, pp. 7999–8007, 2015.

[17] J. S. Pober and W. C. Sessa, "Inflammation and the blood microvascular system," *Cold Spring Harbor Perspectives in Biology*, vol. 7, no. 1, Article ID a016345, 2015.

[18] S. H. Shim, J. M. Kim, H.-Y. Woo, K. U. Shin, J. W. Koh, and K. H. Park, "Association between platelet function and disc hemorrhage in patients with normal-tension glaucoma: a prospective cross-sectional study," *American Journal of Ophthalmology*, vol. 160, no. 6, pp. 1191–1199, 2015.

[19] M. Giovingo, K. Carey, I. Bielskus, M. Miazga, N. M. Pfahler, and P. A. Knepper, "The clot may thicken in primary open angle glaucoma," *Investigative Ophthalmology and Visual Science*, vol. 58, no. 8, p. 4597, 2017.

[20] N. M. Pfahler, I. Bielskus, M. Miazga et al., "A novel method to reduce superactivated platelets in POAG and alzheimer's disease," *Investigative Ophthalmology and Visual Science*, vol. 58, no. 8, p. 4598, 2017.

[21] J. Haney, I. Bielskus, N. M. Pfahler et al., "Flip or flop: calcium activated chloride channels in the phosphatidylserine flip: superactivated platelets and POAG," *Investigative Ophthalmology and Visual Science*, vol. 59, no. 9, p. 5096, 2018.

[22] J. P. Berdahl, R. R. Allingham, and D. H. Johnson, "Cerebrospinal fluid pressure is decreased in primary open-angle glaucoma," *Ophthalmology*, vol. 115, no. 5, pp. 763–768, 2008.

[23] J. M. Tielsch, J. Katz, A. Sommer, H. A. Quigley, and J. C. Javitt, "Hypertension, perfusion pressure, and primary open-angle glaucoma," *Archives of Ophthalmology*, vol. 113, no. 2, pp. 216–221, 1995.

[24] A. P. Cherecheanu, G. Garhofer, D. Schmidl, R. Werkmeister, and L. Schmetterer, "Ocular perfusion pressure and ocular blood flow in glaucoma," *Current Opinion in Pharmacology*, vol. 13, no. 1, pp. 36–42, 2013.

[25] D. Schmidl, G. Garhofer, and L. Schmetterer, "The complex interaction between ocular perfusion pressure and ocular

blood flow-relevance for glaucoma," *Experimental Eye Research*, vol. 93, no. 2, pp. 141–155, 2011.

[26] S. M. Schwartz and E. P. Benditt, "Aortic endothelial cell replication. I. effects of age and hypertension in the rat," *Circulation Research*, vol. 41, no. 2, pp. 248–255, 1977.

[27] G. P. Fadini, C. Pagano, I. Baesso et al., "Reduced endothelial progenitor cells and brachial artery flow-mediated dilation as evidence of endothelial dysfunction in ocular hypertension and primary open-angle glaucoma," *Acta Ophthalmologica*, vol. 88, no. 1, pp. 135–141, 2010.

[28] T. Asahara, A. Kawamoto, and H. Masuda, "Concise review: circulating endothelial progenitor cells for vascular medicine," *Stem Cells*, vol. 29, no. 11, pp. 1650–1655, 2011.

[29] M. O. Gordon, J. A. Beiser, J. D. Brandt et al., "The ocular hypertension treatment study," *Archives of Ophthalmology*, vol. 120, no. 6, pp. 714–720, 2002.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 8642135, 12 pages
https://doi.org/10.1155/2020/8642135

Research Article

Hydrogen Sulfide and β-Synuclein Are Involved and Interlinked in the Aging Glaucomatous Retina

Hanhan Liu,[1] Karl Mercieca,[2,3] Fabian Anders,[1] and Verena Prokosch [1]

[1]*Experimental Ophthalmology, Department of Ophthalmology, University Medical Center of the Johannes Gutenberg University, Mainz 55131, Germany*
[2]*Manchester University Hospitals NHS Trust, Manchester Royal Eye Hospital, Manchester M13 9WH, UK*
[3]*Faculty of Biology, Medicine and Health School of Health Sciences, University of Manchester, Manchester M13 9WH, UK*

Correspondence should be addressed to Verena Prokosch; vprokosch@gmx.de

Received 27 December 2019; Revised 14 February 2020; Accepted 11 March 2020; Published 14 April 2020

Guest Editor: Barbara Cvenkel

Purpose. Glaucoma, one of the leading causes of irreversible blindness worldwide, is a group of disorders characterized by progressive retinal ganglion cell (RGC) loss. Synucleins, a family of small proteins, have been of interest in studies of neurodegeneration and CNS. However, their roles and functions in glaucoma are still not completely understood and remain to be explored. Our previous studies showed that α-synuclein and H_2S play a pivotal role in glaucoma. This study aims to (1) elucidate the potential roles and functions of synucleins in glaucoma throughout aging, (2) investigate the interaction between the synucleins and H_2S, and better understand the mechanism of H_2S in neuroprotection. *Methods*. The chronic IOP elevation model was carried out in 12 animals at different ages (3 months and 14 months), and RGCs were quantified by Brn3a staining. Mass spectrometric-assisted proteomics analysis was employed to measure synuclein levels and H_2S producing proteins in retina. Secondly, the acute IOP elevation model was carried out in 12 juvenile animals, with or without intravitreal injection of GYY4137 (a H_2S donor). RGCs were quantified along with the abundancy of synucleins. *Results*. RGCs and β-synuclein (SNCB) are significantly changed in old animals. Under chronic IOP elevation, there is a significant RGC loss in old animals, whereas no significant change in young animals; SNCB is significantly downregulated and 3MST is significantly upregulated in young animals due to IOP, while no significant changes in old ones are notable. Under acute IOP elevation (approx. 55 mmHg), a significant RGC loss is observed; exogenous H_2S significantly reduced RGC loss and downregulated SNCB levels. *Conclusion*. The present study indicates a strong link between ageing and SNCB regulation. In young animals SNCB is downregulated going along with less RGC loss. Furthermore, increasing endogenous H_2S is effective to downregulate SNCB and is neuroprotective against acute IOP elevation.

1. Introduction

Glaucoma, one of the leading causes of irreversible blindness worldwide [1], is a group of disorders characterized by progressive retinal ganglion cell (RGC) loss and axon atrophy, which leads to gradually visual field loss [2]. By far the only known modifiable risk factor of glaucoma is intraocular pressure (IOP); however, lowering IOP is not able to halt the deterioration of glaucoma in most patients in clinic practice, indicating again the multifactorial pathogenesis and the complexity of glaucoma [3]. The other main risk factor is age. Alternative approaches independent of IOP and probably combating aging as well as focusing on the pathophysiological processes are in demand to ameliorate glaucoma neuropathy. Other pathophysiological processes including oxidative stress, inflammatory reaction, glial activation, vascular dysfunctions, and abnormal protein accumulation are proven to be closely involved [4–7].

Hydrogen sulfide (H_2S) has been recognized as the third endogenous gaseous signaling molecule alongside carbon monoxide (CO) and nitric oxide (NO) [8]. As a potent reductant, H_2S plays critical roles in multiple physiological and pathological processes, it works to alleviate inflammatory responses and oxidative stress and restores energy

shortage [9–11]. H_2S has shown profound therapeutic efficiency potential in neurodegenerative diseases in CNS [12–15]. Research studies focused on H_2S in connection with glaucoma have increasingly emerged in last few years; our knowledge on this topic is still lacking and remains to be thoroughly expanded. Alteration of endogenous H_2S level in retina is correlated with different pathological situations, and its exogenous donors exhibited potential in protecting retinal ganglion cells against assaults, such as diabetic retinopathy, ischemia-reperfusion injury, and *N*-methyl-D-aspartic acid- (NMDA-) induced excitatory neurotoxicity [16–18]. In our previous study, alteration of endogenous H_2S synthases is observed in a glaucoma animal model; furthermore, we observed that GYY4137, a slow-release H_2S donor, effectively protected RGCs against different glaucomatous injuries in vitro and in vivo [19]. The neuroprotective effect of H_2S was partly attributed to its capability of vasorelaxation, antioxidative stress, neuroendocrine regulation, and inflammation suppression [20–22], but the internal mechanism underlying it is still unclear.

Synuclein is a family of small proteins including α (SNCA), β (SNCB), and γ (SNCG) synucleins [23, 24] and is involved in various neurodegenerations in the CNS. Specifically, SNCA is a major constituent of Lewy bodies (LB) and pathological neuronal inclusion bodies found in Parkinson's disease (PD), Alzheimer's disease (AD), and other neurodegenerative disorders [25, 26]. Mutations of SNCA play a central role in PD pathology, and misfolding and aggregation of SNCA directly linked to microglial activation, followed by inflammation and oxidative stress resulting in neurodegeneration [27]. Synucleins are present in the retina and optic nerve [28] and are associated with glaucomatous alterations in the optic nerve [29]. SNCA autoantibody was found to be downregulated in serum and upregulated in aqueous humor of glaucoma patients [30], and in our previous study, intravitreal injection of SNCA antibodies is found to be neuroprotective in a glaucoma animal model [31].

β-Synuclein shares a similar protein structure to SNCA [32], but lacks the nonamyloid-β component domain [33]. Its expression is documented to be increased in cerebrospinal fluid in patients with neurodegenerative diseases and in neuronal retina and visual cortex of rats and nonhuman primates with age and external stress [34–36]. SNCB is thought to function as a physiological inhibitor of SNCA in neurodegenerative diseases [36, 37], and it retains antiapoptotic ability in a dose-dependent manner [23], and β-synuclein-derived peptides behave as antiaggregating agents [25]_ENREF_7.

While SNCA and SNCB are mainly associated with diseases in the CNS, γ-synuclein is first identified as breast-specific gene protein 1 [38], but it is also involved in axonal spheroid-like lesions in Parkinson's disease, deposition in glial cells in glaucoma, and motor neuron dysfunction and death [29, 39, 40].

Because of the pivotal role of SNCA in neurodegeneration in CNS, it has been extensively studied in CNS. But its role in retina or glaucoma, as well as SNCB and SNCG's roles and functions in glaucoma is still sparse and remain to be thoroughly explored.

Studies have shown that H_2S and synucleins are involved in several mutual pathophysiological processes, such as microglia activation, p53-mediated apoptosis, inflammatory response, and free radical reactions [8, 11, 41, 42]. Purpose of this study is to first elucidate the potential roles and functions of synucleins in glaucomatous neuropathy, following this, to investigate the potential of H_2S to regulate it and to better understanding the mechanism of H_2S in neuroprotection.

2. Method

2.1. Animal Treatment and Ethical Statement. Female Sprague-Dawley rats ($n = 24$,250–300 g) were used for this study. All experimental procedures were conducted in accordance with the Association of Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the Institutional Animal Care and Use Committee. The use of animals for research purposes was approved by the Health Investigation Office Rhineland-Palatinate (permission number: 14-1-085; approvals date: 13 October 2014). All animals were housed at the Translational Animal Research Center (TARC) of the University Medical Center of Johannes Gutenberg University Mainz. Food and water were provided ad libitum with a day- and night-circle of 12 hours, respectively.

During experimental interventions, it was prioritized to minimize the discomfort and pain from the animals. For anesthesia, a mixture of medetomidine hydrochloride (Dorbene vet., Pfizer, New York, NY, USA) and Ketamine (Inresa Arzneimittel, Freiburg, Germany) was administered intraperitoneally, and oxybuprocain (Novesine, OmniVision, Puchheim, Germany) was applied topically onto the ocular surface. Novaminsulfon (Novalgin, Ratiopharm, Ulm, Germany) was injected subcutaneously and added to drinking water after the operation to reduce postoperation pain. All animals were observed directly after each intervention and following daily by TARC staff, in terms of their health condition and general behavior.

2.2. Study Design and Induction of Glaucoma Models

2.2.1. Induction of Chronic IOP Elevation. The chronic IOP elevation model was carried out in 12 Sprague-Dawley rats at different ages (3 months and 14 months), through episcleral vein occlusion (EVO), described in our previous work, to create a constant IOP elevation for a period of seven weeks [19]. Only left eyes were operated. Briefly, the animals were anesthetized as described above; connective tissue of the eye was carefully opened and the three of the five episcleral vein trunks were cauterized using a medical cauterization device (Bovie Medical Corporation, USA). The IOP was measured before the EVO and on a weekly basis afterwards.

IOP measurement was achieved using a rodent-customized rebound Tonolab (iCare, Finland). Measurements were carried out between 9am and 11am, animals were awake and gently fixated through hand-holding during the measurement. Per eye, ten IOP readings were taken per

measurement and subsequently averaged. Animals with fluctuating IOPs or no signs of IOP elevation as a result of the EVO were excluded from the study.

2.2.2. Induction of Acute IOP Elevation. The retinal ischemia-reperfusion injury model is a well-known animal model to mimic clinical manifestations such as retinal vascular occlusion diseases and acute glaucoma and has been widely used for studying retinal neuronal cell damage after ischemic injury. Acute IOP elevation was induced in 12 animals. The animals were anesthetized as described above; before the intervention, 3 μl of saline or GYY4137 (Sigma-Aldrich; Darmstadt, Germany), a slow-releasing H_2S donor, was injected posterior to the pars plana into the vitreous with a Hamilton syringe (Sigma-Aldrich; Darmstadt, Germany) and 33-gauge needle, the injection volume was 3 μl for optimal distribution of the compound [43]. To avoid injection reflux, the needle was kept intravitreal for a period of 15 s. Assuming the vitreous volume of an adult rat eye to be approximately 56 μl [43], the final intraocular concentration of GYY4137 was approximately 100 nM.

Immediately after that, an anterior chamber was carefully cannulated from the superotemporal cornea with a 30-gauge infusion needle; care was taken not to injure the central cornea, lens, and other surrounding tissue. The needle was connected to a plastic container of sterile saline solution. IOP was raised to 55 mmHg by elevating the saline container for 60 min; IOP measurement was conducted by rebound Tonolab (iCare, Vantaa, Finland), designed for rodents. During the procedure, the iris turned pale and the retina lost its red reflex, thus confirmed the ischemia condition. Retinae from untouched contralateral eyes were recruited as baseline control. Animals were euthanized 24 h after the reperfusion injury.

2.3. Preparation of Retinal Explants and Quantification of Retinal Ganglion Cells. Sprague-Dawley rats were euthanized under CO_2 atmosphere. Eyes were enucleated immediately postmortem, and retinae were explanted and flat-mounted as previously described [19]. Briefly, retinal explants were flat-mounted with the ganglion cell side up on millipore filters (Millipore, Millicell, Cork, Ireland), and the vitreous body was removed.

One quarter of each treated retinal explant was carefully separated under the microscope for subsequent immunohistochemical staining against the brain-specific homeobox/POU domain protein 3A (Brn3a). Brn3a immunodetection is a powerful tool to assess RGC survival in several mouse and rat injury models, as shown by Nadal-Nicolas et al. [44, 45]. In brief, retinal tissue was fixed in 4% formalin solution for 30 min (Carl Roth, Karlsruhe, Germany), transferred in 30% sucrose solution, and finally frozen in methylbutane for 10 seconds (Merck, Germany) and then stained subsequently as previously described [46]. Immunofluorescent RGCs were further visualized with a fluorescent microscope (Carl Zeiss, Ltd., Hertfordshire, UK), images were taken with a magnification of 20-fold, 16 images were captured from each quarter of retinal flat-mounts, and great effort was taken to ensure the proportion of central and peripheral retinal images was identical in every individual retinal piece. Total numbers of Brn3a positive cells were counted with the assistance of ImageJ (ImageJ Fiji v_1).

2.4. Optical Coherence Tomography. Spectral domain-optic coherence tomography (SD-OCT) (Heidelberg Engineering, Germany) was employed in this study to measure the thickness of the retinal nerve fiber layer (RNFL), inner nuclear layer (INL), and outer nuclear layer (ONL). For all measurements, eye-track and progression analysis was applied. Optic nerve head was centralized in the fundus picture; 100 frames of 12-degree circular B-scan were captured and subsequently overlaid. The thickness of different retinal segments was analyzed from the OCT B-scan with the assistance of Heidelberg Eye Explorer software. Manual adjustments were applied to ensure a correct representation of different retinal segments in the software. Baseline OCT measurement was taken before surgical intervention and the final measurement was taken 7 weeks after chronic IOP elevation.

2.5. Mass Spectrometry. Remaining retinal tissues of all animals were further utilized for proteomic investigations using an electron spray ionization LTQ Orbitrap mass spectrometer (Thermo Fisher, USA) with an upstream connected liquid chromatography device (LC-ESI-MS).

2.5.1. Sample Preparation. Retina samples were rinsed in ice-cold PBS to remove blood contaminants and weighed; subsequently, the samples were lysed by T-PER Tissue Protein Extraction Reagent (Thermo Scientific Inc., Waltham, MA, USA) and BBY24 M Bullet Blender Storm (Next Advance Inc., Averill Park, NY, USA). According to the manufacturer's instruction, 100 μl T-PER reagent was added to the remaining retinal sample; subsequently, the sample was homogenized by the Bullet Blender Storm and centrifuged at 3,000 ×g for 10 minutes.

The supernatant was collected and pooled together and subsequently cleaned with the Amicon Ultra 0.5 mL centrifugal filters with 3K cutoff (Merck Millipore, Carrigtwohill, Ireland). The protein concentration for each eluate was determined with BCA Protein Assay Kit (Pierce, Rockford, IL). From each sample, 50 μg of the total protein mixture was transferred into 1 × LDS sample buffer (NuPAGE, Thermo Fisher) and subsequently put under reducing condition heated at 80°C for 15 min and separated on a 8% Bis-Tris gel ((Invitrogen, Karlsruhe, Germany) for 30 minutes at 180 V in 1 × MES buffer. SeeBlue Plus 2 (Invitrogen, Karlsruhe, Germany) was used as a molecular mass marker. Colloidal Blue Staining Kit (Invitrogen, Karlsruhe, Germany) was used to stain the gel. Protein lanes were sliced into 20 bands per replica and cut into small pieces and destained with the mixture composed of 1 : 2 (vol/vol) of 100 mM ammonium bicarbonate (NH_4HCO_3) and acetonitrile, accordingly 10 mM 1,4-dithiothreitol (DTT) in 100 mM ammonium bicarbonate was employed to disulfide

bonds and 55 mM iodoacetamide (IAA) in 100 mM NH_4HCO_3 for alkylation. Pure acetonitrile was utilized for gel dehydration prior to digesting with sequence grade-modified trypsin (Promega, Madison, USA) in 10 mM NH_4HCO_3 and 10% acetonitrile at 37°C overnight. The tryptic peptides were firstly extracted with acetonitrile and then incubated for 30 min at 37°C with a mixture of 5% formic acid and acetonitrile 1 : 2 (vol/vol); the supernatant was pooled and dried in SpeedVac (Eppendorf, Darmstadt, Germany). SOLA SPE plates and cartridges (Thermo Scientific Inc., Waltham, MA, USA) were utilized for further purification of the peptides following manufacture's instruction. The eluate was dried in SpeedVac and stored at −20°C.

2.5.2. Liquid Chromatography- (LC-) Electrospray Ionization- (ESI-) MS/MS. The LC-ESI-LTQ-Orbitrap MS system in our laboratory is well established and optimized to improve sequence coverage and reduce ion suppression effects, details were described in our previous studies [47, 48]. The LC system contains a Rheos Allegro pump (Thermo scientific, Rockford, USA) paired with an HTS PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). The system encompassed a 30 × 0.5 mm BioBasic C18 precolumn (Thermo Scientific, Rockford, USA) connected to a 150 × 0.5 mm BioBasic C18 column (Thermo Scientific, Rockford, USA); the C18 is the hydrophobic alkyl chain which has reversible hydrophobic interactions with the peptides. The reverse phase aqueous solvent A is made of LC-MS grade water with 0.1% (v/v) formic acid, and the organic solvent B is made of LC-MS grade acetonitrile with 0.1% (v/v) formic acid. The gradient had a running time of 90 minutes per gel band as follows: 0–50 min, 10–35% B; 50–70 min, 35–55% B; 70–75 min, 55–90% B; 75–80 min, 90% B; 80–83 min, 90–10% B; and 83–90 min, 10% B [47, 49]. An ESI-LTQ Orbitrap XL-MS system (Thermo Scientific, Bremen, Germany) collects the continuum MS data [50]. The general parameters of the instrument were set as described in detail previously [50]. In brief, positive ion electrospray ionization mode is employed with a spray voltage of 2.15 kV and a heated capillary temperature of 220°C. Data were acquired in an automatic dependent mode switching between Orbitrap-MS and LTQ MS/MS. The Orbitrap resolution was 30000 at m/z 400 with survey full scan MS spectra. Target automatic gain control (AGC) was set at 1.0×10^6 ions. Polydimethylcyclosiloxane (PCM) at m/z 445.120025 ions in real time is utilized for internal recalibration and the lock mass option was enabled in MS mode [51]. Top five most intense precursor ions were selected and obtained as tandem data and further subjected for fragmentation by collision-induced dissociation (CID). The normalized collision energy (NCE) was set to 35% with activation time of 30 ms with repeat count of 3 and dynamic exclusion duration of 600 s. The resulting fragmented ions were recorded in the LTQ.

Obtained raw files were analyzed by MaxQuant v.1.5.3.30 (Max-Planck-Gesellschaft, Germany). Parameters were set to a false discovery rate of 0.01 with a minimum peptide length of six. Only unique peptides accounted for the follow-up label-free quantification process. Retinal samples of chronic IOP elevation model were measured individually, and those from acute IOP elevation model were pooled into three biological replicates for the measurement.

2.6. Statistics. In all experiments, the control data derived from the contralateral eyes of the respective experimental group did not show any signs of IOP elevation during the whole study. All obtained data regarding are presented as mean ± SD values unless otherwise stated. The averaged RGC density of retinal whole-mounts was calculated per mm^2. All data were analyzed statistically using grouped parametric t-tests for Gaussian distributions, if not, Mann–Whitney U testing was used. All statistical calculations and display of the figures were carried out using Prism 8 software (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Chronic IOP Elevation over 7 Weeks due to EVO. The intraocular pressure (IOP) of all operated eyes increased significantly three weeks after the episcleral vein occlusion. No noticeable difference in IOP was observed between age groups. The untreated contralateral eyes remained unaffected in terms of IOP changes [52] (see Figure 1) ($^{***}p < 0.001$, $n = 12$, mean ± SD).

3.2. RGC Density Decreases and Its Susceptibility to Elevated IOP Increases with Age. A significant decrease in RGC density can be observed between young (3 months old, 1655 ± 57.08 RGC/mm^2) and old animals (14 months old, 1243 ± 43.58 RGC/mm^2, $^{***}p < 0.001$). The chronic IOP elevation over a period of 7 weeks resulted in significant RGC loss in old animals with a reduction of 26% ($^{*}p < 0.05$), while in young animals only 4% of RGC loss is observed (see Figure 2) [52].

3.3. Morphological Alterations in Retina due to Aging and Chronic Elevated IOP. Measurement of the retinal nerve fiber layer thickness showed comparable results to the RGC density quantification. The thinning of the RNFL was about 8% in young animals and 13% in old animals. Furthermore, an age-related thinning of the RNFL and ONL could be observed; the decrease was about 16% and 13%, respectively ($^{**}p < 0.01$, $n = 12$, mean ± SEM) (see Figure 3).

3.4. Alteration of Endogenous H_2S in Glaucoma Model. In mammalian cells, the endogenous H_2S is generated on three major pathways: cystathionine-γ-synthase (CSE), cystathionine-β-lyase (CBS), and 3-mercapto-methylthio pyruvate aminotransferase (3MST). 3MST pathway is the dominating way to produce H_2S in mammalian retina as 3MST is located in the retinal neurons [53]. In this study, 3-mercaptopyruvate sulfurtransferase in retina is over 2-fold upregulated in young animals due to chronic IOP elevation, while nonsignificant alteration in old animals is visible.

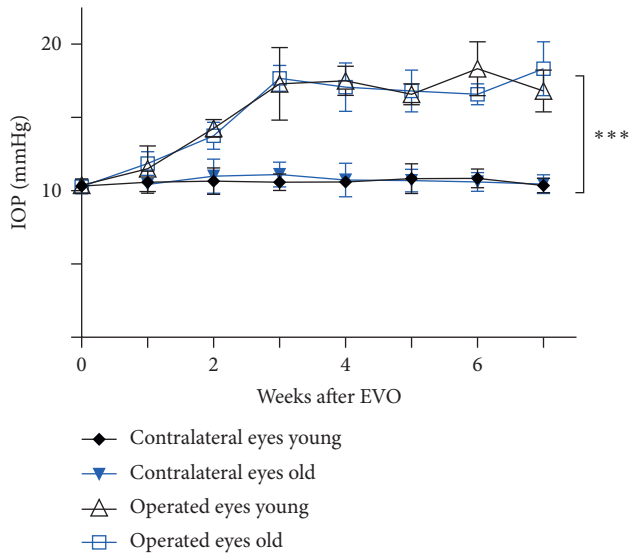


Figure 1: Overview of IOP after episcleral vein occlution for 7 weeks. The intraocular pressure (IOP) of all operated eyes increased significantly ($^{***}p < 0.001$, $n = 12$, mean ± SD) three weeks after the intervention. No noticeable difference in IOP was observed between age groups. The untreated contralateral eyes remained unaffected in terms of IOP changes.

There is no significant alteration in expression due to aging between groups (see Figure 4).

3.5. Acute Elevated IOP (I/R) Induced Significant Cell Loss in Juvenile Animals, while H_2S Treatment Protected RGC. Compared with the contralateral control (1382.8 ± 235.4 RGC/mm^2), 60 min of acute IOP elevation resulted in significant RGC loss in the experimental eye (714.3 ± 223.4 RGC/mm^2); pretreatment with 100 nM GYY4137 significantly reduced RGC loss (1083.5 ± 243.1 RGC/mm^2). There was no significant difference in RGC numbers between control and H_2S-treated group, as RGCs were preserved (see Figure 5) ($^{***}p < 0.0005$, $^{*}p < 0.05$, $n = 12$, means ± SD).

3.6. Altered Synuclein Levels in Acute Elevated IOP Model with or without H_2S Treatment. According to label-free quantification process following LC-ESI-LTQ-Orbitrap mass spectrometry, acute elevated IOP has the tendency to downregulate the β-synuclein (not statistically significant), while administration of H_2S significantly reduced the abundancy of β-synuclein (FC = −1.749, $^{*}p < 0.05$, $n = 6$, mean ± SEM). Either I/R injury or H_2S has significant impact on α-/γ-synuclein (see Figure 6).

3.7. Altered Synuclein Levels in Animals at Different Ages with or without Chronic Elevated IOP. Label-free quantification process following LC-ESI-LTQ-Orbitrap mass spectrometry shows that β-synuclein is significantly more abundant than α-/γ-synuclein in retina in both young and old animals. Furthermore, a significant downregulation of β-synuclein (FC = −0.679) is observed with aging, while nonsignificant age-related alteration in α-/γ-synuclein. The chronic IOP elevation over a period of 7 weeks resulted in significant downregulation of β-synuclein (FC = −−1.428) in young animals, while nonsignificant change in old animals. Elevated IOP does not show significant impact on α-/γ-synuclein in both animal groups (see Figure 7) ($^{****}p < 0.0005$, $^{*}p < 0.05$, $n = 12$, mean ± SEM).

4. Discussion

In our previous work, we demonstrated that exogenous H_2S supplement and α-synuclein antibodies significantly improved the RGC survival in different experimental glaucoma [19, 31]. However, the underlying mechanisms remained to be explored. In this study, we investigated the level changes of synucleins in the retina in different glaucoma animal models, a chronic progressive model of glaucoma at different age stages and an acute IOP elevation induced by ischemia-reperfusion injury. Furthermore, as synucleins and H_2S are involved in several mutual pathophysiological processes, we expect to explore the correlation between H_2S and synucleins in order to better understand the mechanism H_2S's neuroprotective properties in experimental glaucoma.

In this study, we had the following findings:

(1) In rat retina, SNCB has significantly higher abundancy than α-/γ-synuclein

(2) SNCB decreases with age in rat retina

(3) In respond to chronic elevated IOP, SNCB is significantly downregulated in juvenile animals while no significant change is observed in old animals

(4) Downregulation of SNCB and upregulation of H_2S is correlated with reduced cell loss due to chronic elevated IOP in juvenile animals

(5) Exogenous H_2S significantly reduced cell loss due to acute elevated IOP

(6) Exogenous H_2S significantly downregulates SNCB in rat retina

In first part of this study, glaucoma was mimicked through mildly elevated IOP for seven weeks. Subsequent loss of RGCs is in agreement with thinning of the retinal nerve fiber layer, which suggests that EVO is a sufficient glaucoma model. As predominantly elder people are affected in glaucoma, older animals showed a higher susceptibility to IOP elevation resulting in significant loss of RGCs and RNFL thickness, while younger animals seemed to show resistance against mildly elevated IOP.

Comparing to young animals, thickness of both ONL and RNFL is decreased considerably in old animals. It has been shown that the cyclic light intensities under which the rats are reared have impacts on the rod outer segment length and photoreceptor cell density [54]. Especially in albino animals such as Sprague-Dawley rats, the retina of caged rodents develops light damage in old age. Therefore, thinning in ONL and RNFL indicates an overall decrease of retinal neurons, the observed chronic RGC decline in aged animals is likely due to chronic light damage.

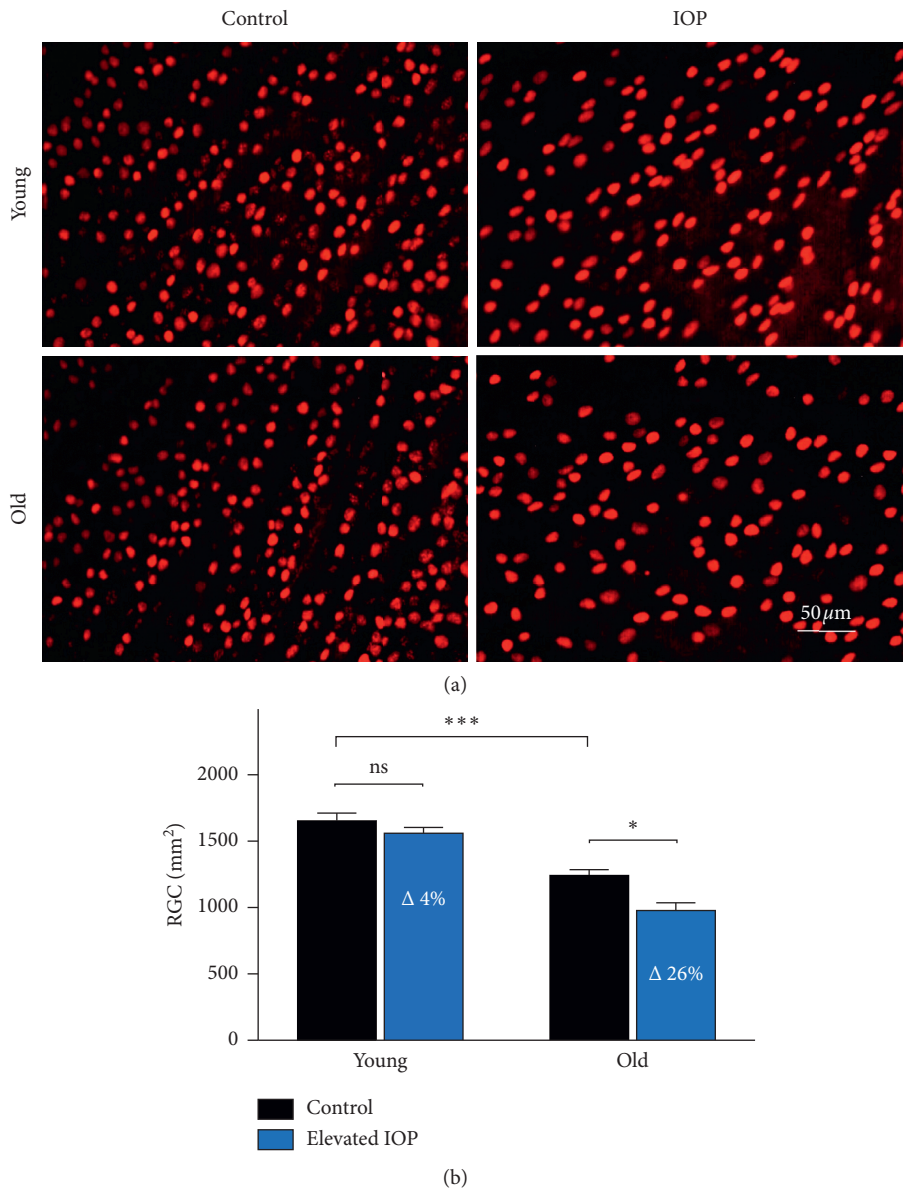


Figure 2: RGC quantification in animals at different ages with or without chronic elevated IOP. (a) Representative fluorescence microscopy of Brn3a staining of retina explants from animals at different ages, with or without elevated IOP. The number of RGCs correlates with the age of the animals and IOP elevation. (b) Independent from IOP, a significant decrease of RGCs could be observed between young and old animals. Elevation of the IOP for 7 weeks leads to a 26% loss of RGCs in old animals, while nonsignificant 4% loss in young animals ($^{***}p < 0.001$, $^{*}p < 0.05$, $n = 12$, mean ± SEM).

Secondly, we employed label-free quantification process following LC-ESI-LTQ-Orbitrap mass spectrometry to measure the abundance of synucleins and 3MST in the retina. We found that SNCB has significantly more abundant retina than other family members; furthermore, its level is significantly altered due to aging and elevated IOP, while the other two family members did not show noticeable changes, which indicates that SNCB might have a more pivotal role to play in neurodegenerations in retina than other family members.

In physiological aging, the abundance of SNCB declines, which is correlated with the decreased RGC density and increased susceptibility to IOP. As under physiological conditions, SNCB is thought to be neuroprotective by functioning as a physiological inhibitor of SNCA and behaving as antiaggregating agents. Studies in autopsy brains of PD, dementia with Lewy bodies, and AD suggest that decreased amount of SNCB may lead to relative loss of protective functions of SNCB against neurotoxicity caused by SNCA [55]. Furthermore, downregulation of SNCB could occur not only in aggregation of SNCA, but also in other types of neurodegenerative disease [56].

SNCB's downregulation with aging increases RGC's susceptibility to glaucomatous assaults secondary to elevated IOP, such as elevated mechanic stress, insufficient retinal perfusion, and increased oxidative stress.

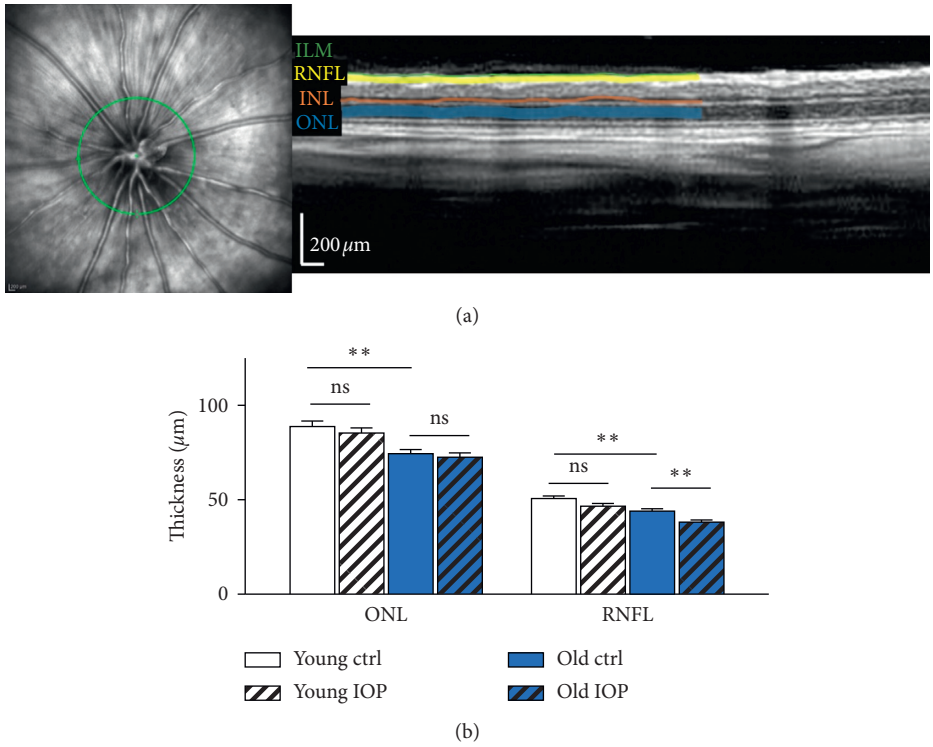


Figure 3: Decrease of the retinal layer thickness as a result of aging and elevated IOP. (a) Exemplary display of the fundus pictures with optic nerve head in the center, and on the right is the OCT B-scan of different retinal layers, internal limiting membrane (ILM), retinal nerve fiber layer (RNFL), inner nuclear layer (INL), and outer nuclear layer (ONL). (b) Compared to young animals, significant thinning is observed in both ONL and RNFL in animals. Elevated IOP only resulted in significant thinning of RNFL while ONL remained unaffected (** $p < 0.01$, $n = 12$, mean ± SEM).

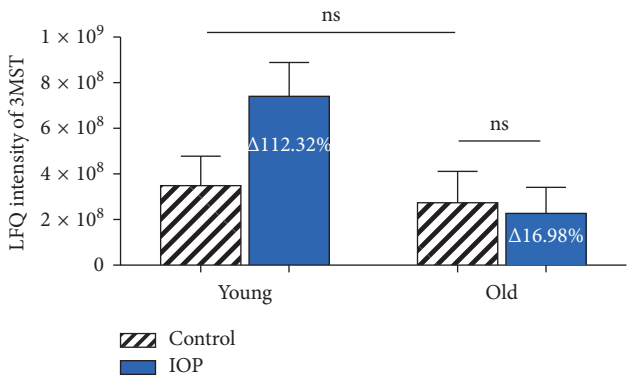


Figure 4: LFQ intensity of 3-mercaptopyruvate sulfurtransferase in retina from animals at different ages with or without chronic elevated IOP. Elevation of the IOP for 7 weeks leads to over 2-fold upregulation of 3MST in young animals, while nonsignificant alteration in old animals. No significant alteration is observed between young and old animals.

Under pathological conditions, chronic elevated IOP, SNCB in juvenile animals is downregulated, and the downregulation of SNCB is correlated with reduced RGC loss. While in aged animals, there is no significant alteration of SNCB in response to assault, but more significant RGC loss.

According to mass spectrometry results, the amount of 3MST in rat retina is not significantly altered through the process of aging. When exposed to elevated IOP over a period of 7 weeks, 3MST was significantly upregulated in juvenile animals, while no significant change is observed in old animals. Associating with the data from immunofluorescence staining of RGC, it suggests that upregulation of 3MST, a key H_2S-producing enzyme, is correlated with reduced RGC loss induced by elevated IOP. The self-regulation of H_2S is decreased with aging. Therefore, we assume that downregulating SNCB and upregulating endogenous H_2S level are neuroprotective against elevated IOP, and the function of regulating them is weakened with aging, which renders RGC's vulnerability.

Furthermore, acute IOP elevation is induced in juvenile animals, which led to significant RGC loss and SNCB downregulation. Although juvenile animals are more resilient than old animals to mildly elevated IOP (ca. 18 mmHg), but when it reaches a threshold, acute IOP elevation (IOP ca. 55 mmHg) led to a significant RGC loss. Downregulation of SNCB might be therefore a self-protective mechanism presenting from the beginning of the IOP elevation, but an exhaustion of the functional reserve eventually led to RGC loss.

Our data on SNCB abundance and RGC loss agree with recent studies in retina, showing that the protective property of SNCB is exerted in a dose-dependent manner [23], which means overexpression and accumulation of SNCB increase oxidative stress and inflammatory responses, and

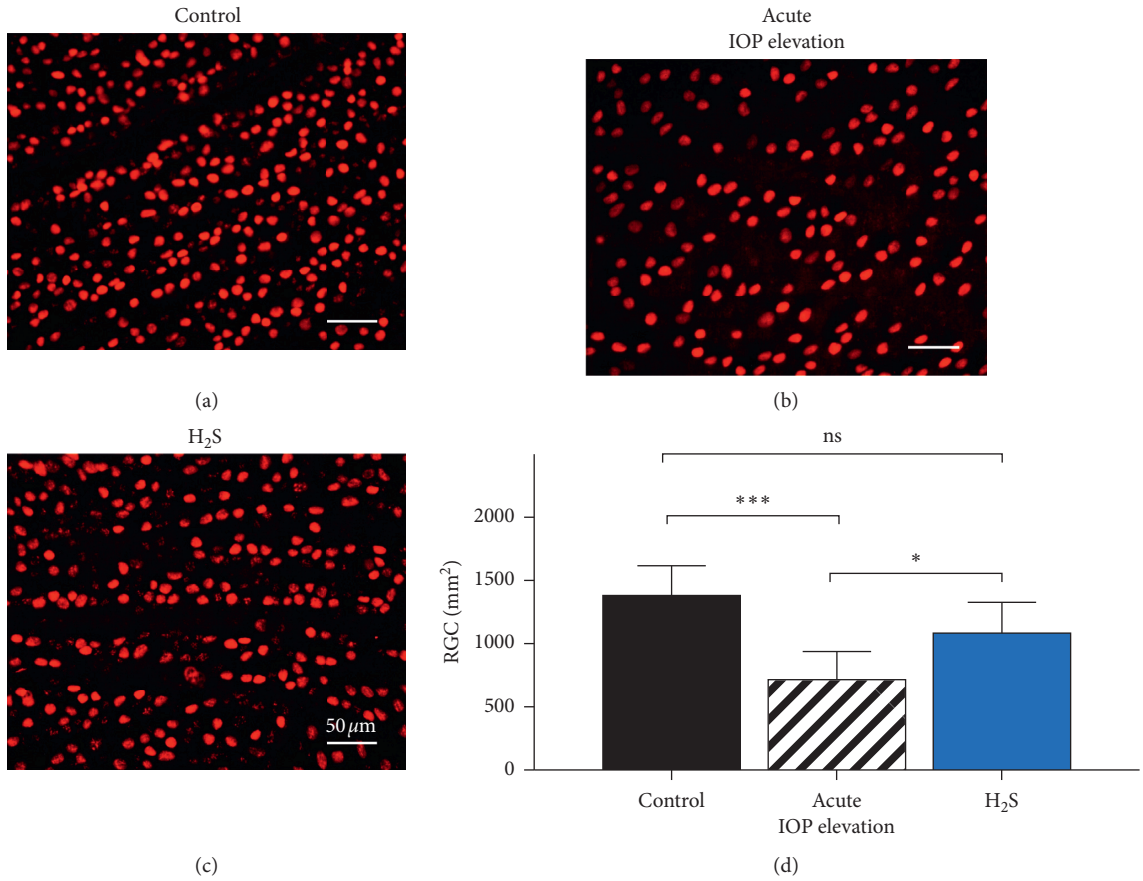


Figure 5: Effect of exogenous H_2S on RGC loss due to acute IOP elevation. (a–c) Representative fluorescence microscopy of Brn3a staining of retinal explants 24 hours after inducing acute IOP elevation in vivo. (a) Contralateral eye as control. (b) Acute IOP elevation. (c) Acute IOP elevation+100 nM GYY4137. (d) Compared with the contralateral control (1382.8 ± 235.4 RGC/mm^2), 60 min of acute IOP elevation resulted in significant RGC loss in the experimental eye (714.3 ± 223.4 RGC/mm^2); pretreatment with GYY4137 significantly reduced RGC loss ($^{***}p < 0.0005$, $^{*}p < 0.05$, $n = 12$, means ± SD).

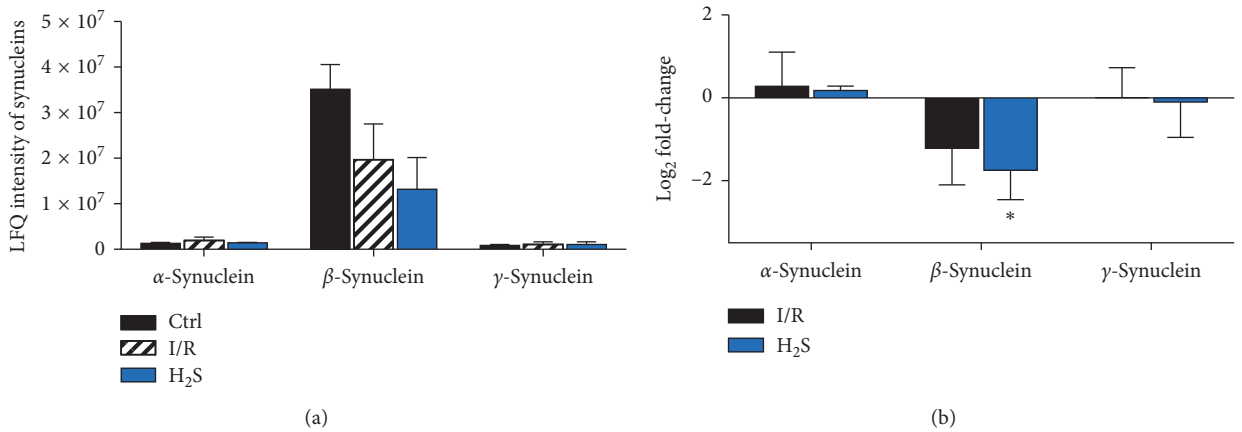


Figure 6: LFQ intensity and alteration of synucleins in the I/R animal model with or without H_2S treatment. (a) LFQ intensity of synucleins following LC-ESI-LTQ-Orbitrap mass spectrometry. (b) Acute elevated IOP has the tendency to downregulate the β-synuclein (not statistically significant), while administration of H_2S significantly reduced the abundancy of β-synuclein (FC = −1.749, $^{*}p < 0.05$, $n = 6$, mean ± SEM). Either I/R injury or H_2S has significant impact on α-/γ-synuclein.

furthermore promote the apoptosis, while lower concentrations of SNCB show antiapoptotic effect [36, 57]. In various aspects of neurodegeneration, accumulation of SNCB is present, such as in dystrophic neurites in the hippocampal region in brains from PD and DLB patients, which suggest that accumulation of SNCB is involved in the

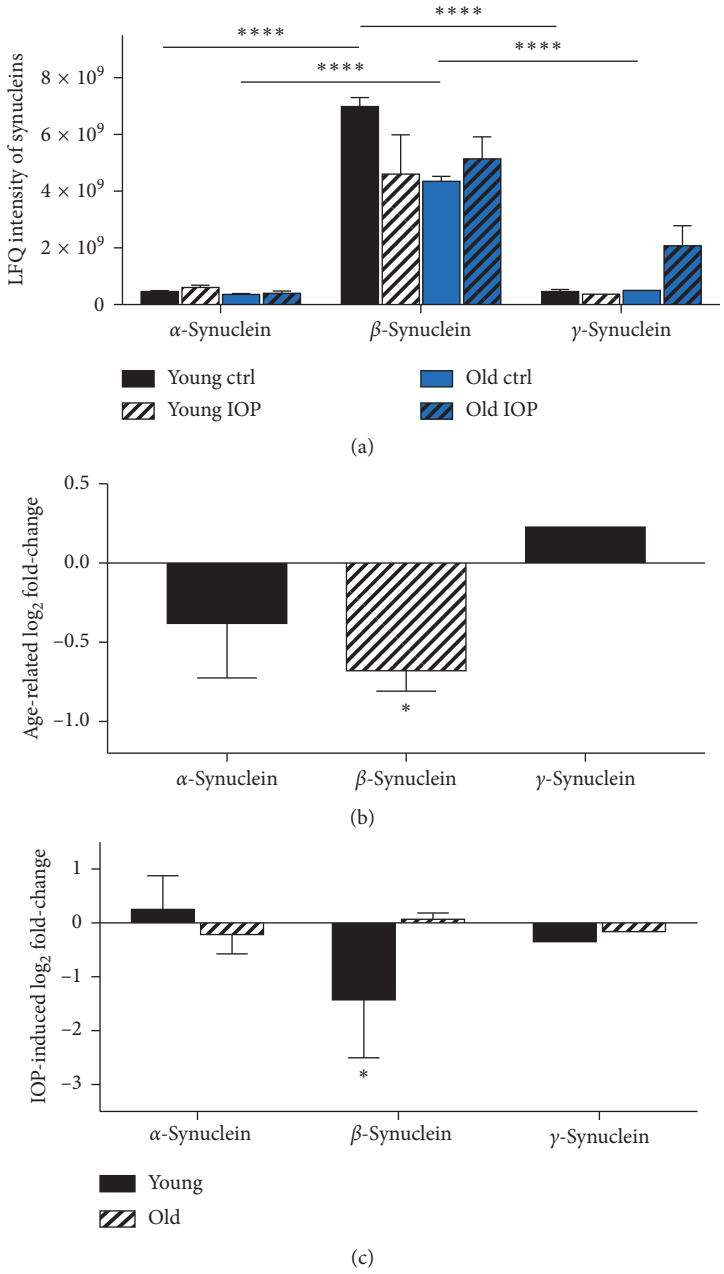


Figure 7: LFQ intensity and alteration of synucleins in retina from animals at different ages with or without chronic elevated IOP. (a) LFQ intensity of synucleins following LC-ESI-LTQ-Orbitrap mass spectrometry. In both young and old animals, β-synuclein is significantly more abundant than α-synuclein and γ-synuclein in retina. (b) Independent from IOP, β-synuclein is significantly downregulated due to aging, while nonsignificant alteration in α-/γ-synuclein is observed. (c) Elevation of the IOP for 7 weeks leads to significant downregulation of β-synuclein in young animals, while nonsignificant change in old animals. No significant alteration of α-/γ-synuclein is observed in both groups ($^{****}p < 0.001$, $^{*}p < 0.05$, $n = 12$, mean ± SEM).

axonal pathology [39]. SNCB was found to form toxic cytosolic inclusions in a similar manner to SNCA and shares similar toxicity mechanisms, including vesicular trafficking impairment and induction of oxidative stress [58]. Overexpression of SNCB in cultured primary cortical neurons led to cell loss and signs of metabolic impairment, in a similar manner to overexpressing SNCA neurons [59].

Treatments targeting SNCA to reduce its levels and toxicity have shown positive results in rescuing neuronal cells and halting the neurodegeneration process in preclinical studies [56, 60]. For example, in our previous study, intravitreal injection of SNCA antibodies is found to be neuroprotective in a glaucoma animal model [31].

Thus, it is reasonable to target the pathogenic SNCB and to decrease the intracellular SNCB as novel strategies for therapeutic intervention in neurodegeneration. Removal of pathogenic SNCB or to reduce its abundancy may be effective to rescue neuron and halt the progression of glaucoma.

H_2S has shown profound involvement in various retinal neuropathy processes; in previous studies by different groups including us, exogenous donors exhibited therapeutic potential in conditions of several retinal diseases [11, 19]. The underlying mechanism, through which H_2S exerts its neuroprotection, was partly attributed to its capability of vasorelaxation, antioxidative stress, neuroendocrine regulation, and inflammation suppression [20–22]. Moreover, H_2S is involved in several mutual pathophysiological processes with SNCB, such as microglia activation, p53-mediated apoptosis, inflammatory response, and free radical reactions [8, 11, 41, 42].

Quantification of Brn3a positive RGCs showed that administration of exogenous H_2S correlated positively with RGC survival improvement in acute IOP elevation. The mass spectrometric-assisted proteomics analysis of the retinal tissue demonstrated that administration of H_2S also further downregulated SNCB.

We may suggest that downregulating SNCB partly contributes to the neuroprotection by H_2S under glaucomatous condition. The extent to which internal mechanism and/or inflammatory factors, signaling pathways, or the disruption of vascular function participate in the process is to be elucidated.

5. Conclusion

In conclusion, our results indicate that SNCB can transform from a neuroprotective to a neurodegenerative molecule. In physiological process, SNCB is neuroprotective; its level and the function of its self-regulation decreases with aging, which increases RGC's susceptibility to glaucomatous assaults. In pathological conditions, SNCB is neurotoxic; downregulation of SNCB is a self-protective mechanism, which presents from the beginning of IOP elevation, and the exhaustion of its functional reserve leads to irreversible neurodegeneration. Furthermore, increasing endogenous H_2S is effective to downregulate SNCB and improve RGC survival against acute IOP elevation. Further detailed and in-depth investigation is required for comprehension of the roles of SNCB and H_2S in glaucoma.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) with a grant PR1569/1-1.

References

[1] S. Resnikoff, D. Pascolini, S. P. Mariotti, and G. P. Pokharel, "Global magnitude of visual impairment caused by uncorrected refractive errors in 2004," *Bulletin of the World Health Organization*, vol. 86, no. 1, pp. 63–70, 2008.

[2] H. A. Quigley, "Number of people with glaucoma worldwide," *British Journal of Ophthalmology*, vol. 80, no. 5, pp. 389–393, 1996.

[3] E. E. Chang and J. L. Goldberg, "Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement," *Ophthalmology*, vol. 119, no. 5, pp. 979–986, 2012.

[4] E. C. Johnson and J. C. Morrison, "Friend or foe? Resolving the impact of glial responses in glaucoma," *Journal of Glaucoma*, vol. 18, no. 5, pp. 341–353, 2009.

[5] G. N. Wilson, D. M. Inman, C. M. Dengler Crish, M. A. Smith, and S. D. Crish, "Early pro-inflammatory cytokine elevations in the DBA/2J mouse model of glaucoma," *Journal of Neuroinflammation*, vol. 12, p. 176, 2015.

[6] G. Tezel, "Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences," *Progress in Retinal and Eye Research*, vol. 25, no. 5, pp. 490–513, 2006.

[7] P. T. Lansbury and H. A. Lashuel, "A century-old debate on protein aggregation and neurodegeneration enters the clinic," *Nature*, vol. 443, no. 7113, pp. 774–779, 2006.

[8] R. Tabassum, N. Y. Jeong, and J. Jung, "Therapeutic importance of hydrogen sulfide in age-associated neurodegenerative diseases," *Neural Regeneration Research*, vol. 15, no. 4, pp. 653–662, 2020.

[9] F. N. Salloum, "Hydrogen sulfide and cardioprotection—mechanistic insights and clinical translatability," *Pharmacology & Therapeutics*, vol. 152, pp. 11–17, 2015.

[10] S. Panthi, H. J. Chung, J. Jung, and N. Y. Jeong, "Physiological importance of hydrogen sulfide: emerging potent neuroprotector and neuromodulator," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 9049782, 11 pages, 2016.

[11] S. Huang, P. Huang, H. Yu et al., "Extracellular signal-regulated kinase 1/2 pathway is insufficiently involved in the neuroprotective effect by hydrogen sulfide supplement in experimental glaucoma," *Investigative Opthalmology & Visual Science*, vol. 60, no. 13, pp. 4346–4359, 2019.

[12] Y. Liu, Y. Deng, H. Liu, C. Yin, X. Li, and Q. Gong, "Hydrogen sulfide ameliorates learning memory impairment in APP/PS1 transgenic mice: a novel mechanism mediated by the activation of Nrf2 [Pharmacol. Biochem. Behav. 150-151 (2016)," *Pharmacology Biochemistry and Behavior*, vol. 153, pp.191, 2017.

[13] Y. Liu, Y. Deng, H. Liu, C. Yin, X. Li, and Q. Gong, "Hydrogen sulfide ameliorates learning memory impairment in APP/PS1 transgenic mice: a novel mechanism mediated by the activation of Nrf2," *Pharmacology Biochemistry and Behavior*, vol. 150-151, pp. 207–216, 2016.

[14] L. Xie, S. Yu, K. Yang, C. Li, and Y. Liang, "Hydrogen sulfide inhibits autophagic neuronal cell death by reducing oxidative stress in spinal cord ischemia reperfusion injury," *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 8640284, 15 pages, 2017.

[15] M. R. Sarookhani, H. Haghdoost-Yazdi, A. Sarbazi-Golezari, A. Babayan-Tazehkand, and N. Rastgoo, "Involvement of adenosine triphosphate-sensitive potassium channels in the neuroprotective activity of hydrogen sulfide in the 6-hydroxydopamine-induced animal model of Parkinson's

disease," *Behavioural Pharmacology*, vol. 29, no. 4, pp. 336–343, 2018.

[16] K. Sakamoto, Y. Suzuki, Y. Kurauchi, A. Mori, T. Nakahara, and K. Ishii, "Hydrogen sulfide attenuates NMDA-induced neuronal injury via its anti-oxidative activity in the rat retina," *Experimental Eye Research*, vol. 120, pp. 90–96, 2014.

[17] J. Biermann, W. A. Lagreze, N. Schallner, C. I. Schwer, and U. Goebel, "Inhalative preconditioning with hydrogen sulfide attenuated apoptosis after retinal ischemia/reperfusion injury," *Molecular Vision*, vol. 17, pp. 1275–1286, 2011.

[18] Y.-F. Si, J. Wang, J. Guan, L. Zhou, Y. Sheng, and J. Zhao, "Treatment with hydrogen sulfide alleviates streptozotocin-induced diabetic retinopathy in rats," *British Journal of Pharmacology*, vol. 169, no. 3, pp. 619–631, 2013.

[19] H. Liu, F. Anders, S. Thanos et al., "Hydrogen sulfide protects retinal ganglion cells against glaucomatous injury in vitro and in vivo," *Investigative Opthalmology & Visual Science*, vol. 58, no. 12, pp. 5129–5141, 2017.

[20] G. Yang, L. Wu, B. Jiang et al., "H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine -lyase," *Science*, vol. 322, no. 5901, pp. 587–590, 2008.

[21] Y. Kaneko, Y. Kimura, H. Kimura, and I. Niki, "L-cysteine inhibits insulin release from the pancreatic -cell: possible involvement of metabolic production of hydrogen sulfide, a novel gasotransmitter," *Diabetes*, vol. 55, no. 5, pp. 1391–1397, 2006.

[22] H. Zhang and M. Bhatia, "Hydrogen sulfide: a novel mediator of leukocyte activation," *Immunopharmacology and Immunotoxicology*, vol. 30, no. 4, pp. 631–645, 2008.

[23] C. A. da Costa, E. Masliah, and F. Checler, "β-synuclein displays an antiapoptotic p53-dependent phenotype and protects neurons from 6-Hydroxydopamine-induced caspase 3 activation," *Journal of Biological Chemistry*, vol. 278, no. 39, pp. 37330–37335, 2003.

[24] A. Surguchov, "Intracellular dynamics of synucleins," *International Review of Cell and Molecular Biology*, vol. 320, pp. 103–169, 2015.

[25] M. Windisch, B. Hutter-Paier, E. Rockenstein, M. Hashimoto, M. Mallory, and E. Masliah, "Development of a new treatment for Alzheimer's disease and Parkinson's disease using anti-aggregatory beta-synuclein-derived peptides," *J Mol Neurosci*, vol. 19, no. 1-2, pp. 63–69, 2002.

[26] N. Hattori, Y. Machida, and K. Noda, "[Pathogenesis of Parkinson's disease: a common pathway between alpha-synuclein and parkin and the mechanism of lewy bodies formation," *Rinsho Shinkeigaku*, vol. 45, no. 11, pp. 905–907, 2005.

[27] D. Beraud, M. Twomey, B. Bloom et al., "Alpha-synuclein alters toll-like receptor expression," *Frontiers in Neuroscience*, vol. 5, p. 80, 2011.

[28] A. Surguchov, B. McMahan, E. Masliah, and I. Surgucheva, "Synucleins in ocular tissues," *Journal of Neuroscience Research*, vol. 65, no. 1, pp. 68–77, 2001.

[29] I. Surgucheva, B. McMahan, F. Ahmed, S. Tomarev, M. B. Wax, and A. Surguchov, "Synucleins in glaucoma: implication of γ-synuclein in glaucomatous alterations in the optic nerve," *Journal of Neuroscience Research*, vol. 68, no. 1, pp. 97–106, 2002.

[30] N. Boehm, D. Wolters, U. Thiel et al., "New insights into autoantibody profiles from immune privileged sites in the eye: a glaucoma study," *Brain, Behavior, and Immunity*, vol. 26, no. 1, pp. 96–102, 2012.

[31] J. Teister, F. Anders, S. Beck et al., "Decelerated neurodegeneration after intravitreal injection of alpha-synuclein antibodies in a glaucoma animal model," *Scientific Reports*, vol. 7, no. 1, p. 6260, 2017.

[32] P. H. Jensen, E. S. Sorensen, T. E. Petersen, J. Gliemann, and L. K. Rasmussen, "Residues in the synuclein consensus motif of the alpha-synuclein fragment, NAC, participate in transglutaminase-catalysed cross-linking to Alzheimer-disease amyloid beta A4 peptide," *Biochemical Journal*, vol. 310, no. 1, pp. 91–94, 1995.

[33] I. F. Tsigelny, P. Bar-On, Y. Sharikov et al., "Dynamics of α-synuclein aggregation and inhibition of pore-like oligomer development by β-synuclein," *FEBS Journal*, vol. 274, no. 7, pp. 1862–1877, 2007.

[34] M. R. Bohm, H. Melkonyan, and S. Thanos, "Life-time expression of the proteins peroxiredoxin, beta-synuclein, PARK7/DJ-1, and stathmin in the primary visual and primary somatosensory cortices in rats," *Frontiers in Neuroanatomy*, vol. 9, p. 16, 2015.

[35] M. R. R. Böhm, S. Mertsch, S. König, T. Spieker, and S. Thanos, "Macula-less rat and macula-bearing monkey retinas exhibit common lifelong proteomic changes," *Neurobiology of Aging*, vol. 34, no. 11, pp. 2659–2675, 2013.

[36] K. Hadrian, H. Melkonyan, S. Schlatt et al., "Age-related distribution and potential role of SNCB in topographically different retinal areas of the common marmoset *Callithrix jacchus*, including the macula," *Experimental Eye Research*, vol. 185, Article ID 107676, 2019.

[37] M. Hashimoto, E. Rockenstein, M. Mante, M. Mallory, and E. Masliah, "β-synuclein inhibits α-synuclein aggregation," *Neuron*, vol. 32, no. 2, pp. 213–223, 2001.

[38] H. Ji, Y. E. Liu, T. Jia et al., "Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing," *Cancer Research*, vol. 57, no. 4, pp. 759–764, 1997.

[39] J. E. Galvin, K. Uryu, V. M.-Y. Lee, and J. Q. Trojanowski, "Axon pathology in Parkinson's disease and lewy body dementia hippocampus contains alpha -, beta -, and gamma -synuclein," *Proceedings of the National Academy of Sciences*, vol. 96, no. 23, pp. 13450–13455, 1999.

[40] N. Ninkina, O. Peters, S. Millership, H. Salem, H. van der Putten, and V. L. Buchman, "γ-synucleinopathy: neurodegeneration associated with overexpression of the mouse protein," *Human Molecular Genetics*, vol. 18, no. 10, pp. 1779–1794, 2009.

[41] M. Kumar and R. Sandhir, "Hydrogen sulfide suppresses homocysteine-induced glial activation and inflammatory response," *Nitric Oxide*, vol. 90, pp. 15–28, 2019.

[42] F. Longhena, G. Faustini, V. Brembati, M. Pizzi, and A. Bellucci, "The good and bad of therapeutic strategies that directly target alpha-synuclein," *IUBMB Life*, vol. 72, no. 4, pp. 590–600, 2019.

[43] B. A. Berkowitz, R. A. Lukaszew, C. M. Mullins, and J. S. Penn, "Impaired hyaloidal circulation function and uncoordinated ocular growth patterns in experimental retinopathy of prematurity," *Investigative Ophthalmology & Visual Science*, vol. 39, no. 2, pp. 391–396, 1998.

[44] F. M. Nadal-Nicolás, P. Sobrado-Calvo, M. Jiménez-López, M. Vidal-Sanz, and M. Agudo-Barriuso, "Long-term effect of optic nerve axotomy on the retinal ganglion cell layer," *Investigative Opthalmology & Visual Science*, vol. 56, no. 10, pp. 6095–6112, 2015.

[45] F. M. Nadal-Nicolas, M. Salinas-Navarro, M. Jimenez-Lopez et al., "Displaced retinal ganglion cells in albino and pigmented rats," *Frontiers in Neuroanatomy*, vol. 8, p. 99, 2014.

[46] F. Anders, J. Teister, A. Liu et al., "Intravitreal injection of beta-crystallin B2 improves retinal ganglion cell survival in an

experimental animal model of glaucoma," *PLoS One*, vol. 12, no. 4, Article ID e0175451, 2017.

[47] N. Perumal, S. Funke, N. Pfeiffer, and F. H. Grus, "Proteomics analysis of human tears from aqueous-deficient and evaporative dry eye patients," *Scientific Reports*, vol. 6, Article ID 29629, 2016.

[48] N. Perumal, S. Funke, D. Wolters, N. Pfeiffer, and F. H. Grus, "Characterization of human reflex tear proteome reveals high expression of lacrimal proline-rich protein 4 (PRR4)," *Proteomics*, vol. 15, no. 19, pp. 3370–3381, 2015.

[49] C. Manicam, N. Perumal, N. Pfeiffer, F. H. Grus, and A. Gericke, "First insight into the proteome landscape of the porcine short posterior ciliary arteries: key signalling pathways maintaining physiologic functions," *Scientific Reports*, vol. 6, Article ID 38298, 2016.

[50] C. Manicam, N. Perumal, J. Wasielica-Poslednik et al., "Proteomics unravels the regulatory mechanisms in human tears following acute renouncement of contact lens use: a comparison between hard and soft lenses," *Scientific Reports*, vol. 8, no. 1, p. 11526, 2018.

[51] J. V. Olsen, L. M. F. de Godoy, G. Li et al., "Parts per million mass accuracy on an orbitrap mass spectrometer via lock mass injection into a C-trap," *Molecular & Cellular Proteomics*, vol. 4, no. 12, pp. 2010–2021, 2005.

[52] F. Anders, C. Mann, A. Liu et al., "Correlation of crystallin expression and RGC susceptibility in experimental glaucoma rats of different ages," *Current Eye Research*, vol. 43, no. 10, pp. 1267–1273, 2018.

[53] Y. Mikami, N. Shibuya, Y. Kimura, N. Nagahara, M. Yamada, and H. Kimura, "Hydrogen sulfide protects the retina from light-induced degeneration by the modulation of Ca^{2+} Influx," *Journal of Biological Chemistry*, vol. 286, no. 45, pp. 39379–39386, 2011.

[54] M. J. Castelhano-Carlos and V. Baumans, "The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats," *Laboratory Animals*, vol. 43, no. 4, pp. 311–327, 2009.

[55] E. Rockenstein, L. A. Hansen, M. Mallory, J. Q. Trojanowski, D. Galasko, and E. Masliah, "Altered expression of the synuclein family mRNA in lewy body and Alzheimer's disease," *Brain Research*, vol. 914, no. 1-2, pp. 48–56, 2001.

[56] M. Fujita, A. Sekigawa, K. Sekiyama, Y. Takamatsu, and M. Hashimoto, "Possible alterations in β-synuclein, the non-amyloidogenic homologue of α-synuclein, during progression of sporadic α-synucleinopathies," *International Journal of Molecular Sciences*, vol. 13, no. 9, pp. 11584–11592, 2012.

[57] K. Brockhaus, M. R. R. Böhm, H. Melkonyan, and S. Thanos, "Age-related beta-synuclein alters the p53/mdm2 pathway and induces the apoptosis of brain microvascular endothelial cells in vitro," *Cell Transplantation*, vol. 27, no. 5, pp. 796–813, 2018.

[58] S. Tenreiro, R. Rosado-Ramos, E. Gerhardt et al., "Yeast reveals similar molecular mechanisms underlying alpha- and beta-synuclein toxicity," *Human Molecular Genetics*, vol. 25, no. 2, pp. 275–290, 2016.

[59] G. Taschenberger, J. Toloe, J. Tereshchenko et al., "β-synuclein aggregates and induces neurodegeneration in dopaminergic neurons," *Annals of Neurology*, vol. 74, no. 1, pp. 109–118, 2013.

[60] M. Tolmasov, R. Djaldetti, N. Lev, and Y. Gilgun-Sherki, "Pathological and clinical aspects of alpha/beta synuclein in Parkinson's disease and related disorders," *Expert Review of Neurotherapeutics*, vol. 16, no. 5, pp. 505–513, 2016.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 9329310, 6 pages
https://doi.org/10.1155/2020/9329310

Review Article

Innovative IOP-Independent Neuroprotection and Neuroregeneration Strategies in the Pipeline for Glaucoma

James C. Tsai

Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York Eye and Ear Infirmary of Mount Sinai, New York, NY, USA

Correspondence should be addressed to James C. Tsai; jtsai@nyee.edu

Received 28 January 2020; Accepted 23 March 2020; Published 10 April 2020

Academic Editor: Steffen Heegaard

While sustained reduction of intraocular pressure (IOP) has been shown to halt and/or delay the progressive death of retinal ganglion cells (RGCs) in glaucoma, there exists great interest in the development and validation of IOP-independent therapeutic strategies for neuroprotection and/or neuroregeneration. Multiple etiologies for RGC death have been implicated in glaucoma including defective axonal transport, ischemia, excitotoxicity, reactive oxygen species, trophic factor withdrawal, and loss of RGC electrical activity. However, IOP lowering with medical, laser, and surgical therapies is itself neuroprotective, and investigators are seeking to identify agents that are able to confer neuroprotection independent of IOP reduction, as well as providing for regeneration of nonviable RGCs and their axons to restore and/or maintain functional vision. These innovative strategies in the pipeline include investigation of neurotrophic factors, gene therapy, immune system modulation, and novel neuroregeneration pathways. Alongside this new knowledge, enhanced opportunities for discovery of vision preservation and/or restoration therapies must be weighed against the potential disadvantages of perturbing the complex central nervous system environment.

1. Introduction

Glaucoma, a multifactorial disease, is the second leading cause of blindness worldwide, affecting an estimated 76.0 million people worldwide by 2020 and increasing to 111.8 million by 2040 [1]. In addition, a high percentage of patients with glaucoma are unaware of their visual loss until permanent damage has occurred; for example, the percentage of undiagnosed patients in the United States ranges from 56% to 92% [2]. While glaucoma was historically defined as a disease of elevated intraocular pressure (IOP) greater than 21 mm Hg (the statistical upper 95th percentile of IOP in normal subjects), population-based studies have shown that one-third or more of persons with open angle glaucoma (OAG) have normal levels of IOP [3]. Thus, the current definition of primary open angle glaucoma (POAG) is no longer contingent on the presence of "elevated IOP" but rather "a progressive, chronic optic neuropathy in adults where IOP and other currently unknown factors contribute to a characteristic acquired atrophy of the optic nerve and loss of retinal ganglion cells (RGCs)" [4].

Over the past several decades, multiple prospective studies have validated intraocular pressure (IOP) to be the most important risk factor for the development and progression of optic nerve damage in primary open angle glaucoma (POAG). The Ocular Hypertension Treatment Study (OHTS) demonstrated that the 5-year conversion rates of ocular hypertension to POAG were more than twice as high as for placebo-treated patients vs. medically treated patients (9.9% vs. 4.4%, respectively) [5]. Based on multivariate analysis, the Early Manifest Glaucoma Trial (EMGT) reported that the progression risk of early onset open angle glaucoma (OAG) was reduced by 50% with treatment, and the risk of glaucoma progression was decreased by 10% for each mm of Hg of initial IOP reduction [6]. In patients with OAG who present with normal IOP levels, the Collaborative Normal Tension Glaucoma Study (CNTGS) showed that only 12% of treated eyes had either progression of

glaucomatous optic disc cupping or visual field loss compared with a 35% rate in control eyes ($p < 0.0001$) [7].

Currently accepted strategies for treatment of POAG, whether medical, laser, or incisional surgical modalities, aim to lower IOP below a presumed threshold level [4, 8, 9]. Though these IOP-lowering therapies have been proven to slow and/or halt progression of the glaucomatous damage and therefore are neuroprotective in nature, slow progress has been made in developing IOP-independent neuroprotection and/or neuroregeneration strategies [10, 11]. While neuroprotection strategies to enhance the ability of target cells in the central nervous system (CNS) to withstand pathological insults have shown substantive promise in animal models, none have been reported to be clinically effective in human clinical trials [11]. In addition, early stage intervention to prevent disease development and/or progression would likely require evolution of improved algorithms and technologies to enable and/or enhance this earlier detection. Novel experimental strategies are exploring disease modification/intervention to prevent against optic nerve head (ONH) damage and/or retinal ganglion cell (RGC) death without depending on IOP lowering. One upstream goal would target intervention at an early stage of the glaucomatous disease process to halt or slow down the underlying neurodegenerative process. Various etiologies for RGC death have been implicated including defective axonal transport, ischemia, excitotoxicity, reactive oxygen species, trophic factor withdrawal, and loss of RGC electrical activity [12, 13]. Technological advances allowing for in-vivo imaging of RGCs may assist in clear identification of dying RGCs, providing opportunities to evaluate interventions with appropriate clinical applications. For example, human clinical trials are now underway utilizing a noninvasive real-time imaging technique with the fluorescent biomarker annexin A5 to detect in-vivo rates of apoptosis in RGCs [14].

Though IOP lowering with conventional therapies is itself neuroprotective, the development of non-IOP dependent neuroprotective therapies is definitely possible. The overarching goal would be to intervene with these agents at the earliest stage of the disease and/or its progression. In addition, regeneration of nonviable RGCs and their axons would be highly desirable to restore and maintain functional vision. However, randomized clinical trials investigating oral memantine and topical brimonidine have not demonstrated a clear neuroprotection benefit in patients with glaucoma [11, 15]. For the latter pharmacologic agent, patients with low-pressure glaucoma had slightly less visual field progression with topical administration of the alpha2-adrenergic agonist brimonidine tartrate 0.2% compared to the beta-adrenergic antagonist timolol maleate 0.5% [15]. However, significantly more brimonidine-treated patients (28.3%) discontinued study participation due to drug-related adverse events as compared to the timolol-treated patients (11.4%) [15].

1.1. Neurotrophic Factors. Neurotrophic factors have shown promise in retarding progression of neurodegenerative diseases. Active preclinical and clinical studies are ongoing investigating ciliary neurotrophic growth factor (CNTF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotropic factor (GDNF), and others [16, 17]. Exogenous application of BDNF to the retina and viral vector has been shown to induce amplification of BDNF expression in retinal neurons and to be effective in neuroprotection of RGCs [17]. One study showed that in an axotomy-induced cell death model in the adult C57BL/6J mouse, GDNF and CNTF potently and synergistically rescued RGCs when compared to control retinas up to 8 weeks after the lesion [18].

Human CNTF was investigated in a phase 1 clinical safety trial with delivery of cells (designated NTC-201 and derived originally from the human retinal pigment epithelium cell line ARPE-19) transfected with the human CNTF gene and insulated within surgically implanted capsules [19]. The study enrolled 10 participants who received vitreous implanted CNTF encapsulated devices that were semipermeable to allow the neurotrophic factor to reach the retina in therapeutic levels for retinal degeneration. The study conclusion was that CNTF is safe for human retina and may have future applications for a wide range of retinal degenerative diseases including glaucoma [19]. In contrast, BDNF cannot successfully cross the blood-brain barrier, and thus, it is not surprising that there are no studies to date reporting BDNF's therapeutic effects for retinal degeneration [16]. Erythropoietin (EPO), a naturally occurring cytokine used to treat anemia by inhibiting apoptosis in erythrocyte progenitors, has been shown to be neuroprotective [20–22]. Experimental studies have reported that intravitreal injections of EPO rescue RGCs and prevent caspase-3 activation in axotomized rats (Sprague-Dawley), as well as retard against RGC loss in a rat model of ocular hypertension [20, 21]. In the DBA/2J mouse model of glaucoma, EPO was shown to promote RGC survival without affecting the IOP [23]. Furthermore, EPO-induced neuroprotection has been demonstrated to follow a bell-shaped dose-response curve in vitro and in vivo [20]. Additional studies have reported that one-time intravitreal administration of EPO (at doses up to 625 ng) does not cause adverse effects on retinal vasculature, retinal anatomy, or retinal function as assessed by electroretinography (ERG) in Sprague-Dawley rats and New Zealand white rabbits [24, 25].

1.2. Gene Therapy. Another approach to prevent and/or retard RGC loss is via gene therapy to deliver antiapoptotic neurotrophic proteins [16]. As noted above, a human Phase 1 safety trial demonstrated the successful delivery of CNTF by cells transfected with the human gene CNTF gene [19]. In brown Norway rat RGCs, the gene for Bcl-XL (amplified from C57BL/6J mouse whole-brain cDNA), a prosurvival and antiapoptotic protein, has been successfully delivered via adeno-associated virus (AAV) and HIV-Tat-derived fusion proteins [26]. In addition, an AAV vector (AAV-BDNF-WPRE) capable of efficient transfection of Wistar rat RGCs has been developed to deliver BDNF [27]. In the study, rats that had overexpression of the AAV-BDNF gene were more resistant to RGC death in an hypertensive model

of glaucoma. To counteract the reduction of BDNF effect over time as a result of downregulation of tropomyosin-related receptor-B (TrkB) receptors, a novel AAV2 gene therapy vector has been designed to code both the BDNF ligand and the TrkB receptor and delivered via intravitreal injection to C57BL/6 mice [28].

There has been great interest in understanding Wallerian degeneration of axons and synapses, an active process that is intricately associated with RGC death as described in C57/BL6J mice [29]. The slow Wallerian degeneration (Wld(s)) gene specifically delays axonal and synaptic degeneration in multiple neurodegenerative conditions. Since altered mitochondrial responses to degenerative stimuli likely play an important role in the neuroprotective Wld(s) phenotype, targeting proteins involved in this phenotype may lead to novel therapies in glaucoma as noted in Wld^S mice [30, 31]. In addition, the neuroprotective effects of the Wld(s) gene are correlated with proteasome expression rather than inhibition of apoptosis [32].

An alternative to viral vector delivered gene therapy is the use of stem cells to transfer these specific genes. One study reported the feasibility of mesenchymal stem cell-based delivery of BDNF gene to Sprague-Dawley rat retina [33]. Following subretinal injections of rat bone marrow mesenchymal stem cells administered to axotomized rat retina, significant expression of BDNF was observed for 4 weeks following transplantation of these stem cells [33]. A more recent publication reported the development of CD-1 mouse multipotent retinal stem cell (MRSC)-derived RGCs that express key RGC characteristics with the potential for neuroprotection and regeneration of damaged RGCs [34]. In this study, three-dimensional (3D) cocultures were used to validate the model for transfection efficiency and BDNF bioactivity measurements [34].

1.3. Immune System Modulation. Tumor necrosis factor-alpha (TNF-α) has been shown to cause a cascade of events that lead to loss of RGCs. In a C57BL/6 mouse model of glaucoma, increased levels of TNF-α were demonstrated to cause microglial activation, loss of oligodendrocytes in the optic nerve, and loss of RGCs in an irradiation-induced murine model of ocular hypertension (OHT) [35]. In contrast, no increased death of RGCs above baseline was observed when animals were treated with TNF-α inhibitors, and/or knockout mice unable to produce TNF-α were studied [35]. Based on their experimental results, the investigators concluded that blocking TNF-α signaling or inflammation may be someday proven helpful in treating glaucoma. Based on the above findings, future treatments for glaucoma may involve modulation of the TNF-α pathway including direct blockage of TNF-α function and inhibition of downstream microglial activation [35]. These various possibilities include blocking antibodies that interfere with TNF-α, soluble receptor(s), and a TNF-α-converting enzyme inhibitor(s). A more recent study reported that antagonism of the TNF-α signaling pathway delays axotomy-induced RGC loss in a C57Bl/6 mouse model of traumatic neuropathy though the effect was not as favorable as observed with activation of survival pathways by BDNF [36]. Furthermore, combination treatment with BDNF and the small cell permeable molecule (R7050) that inhibited TNF-α/TNF receptor 1 (TNFR1) did not demonstrate superiority to BDNF alone and did not improve RGC survival [36].

In the near future, the method of T-cell-based vaccination for morphological and functional neuroprotection may be possible since this therapeutic option has been shown to be effective in retarding RGC cell loss in an inbred Lewis and Sprague-Dawley rat model of glaucoma [37]. In experimental animals with chronically elevated IOP, vaccination with the synthetic copolymer glatiramer acetate (Cop-1) was shown to be protective against IOP-induced loss of RGCs by eliciting a systemic T-cell-mediated response capable of cross-reacting with self-antigens in the eye [38]. However, no benefit was observed when Cop-1 was administered in inbred Lewis and Sprague-Dawley rats deprived of T-cells, thus supporting the hypothesis that the effect was T-cell mediated [38]. A recent interim report of a randomized placebo-controlled double-masked clinical trial in 38 patients with primary angle closure glaucoma failed to show any difference in visual field progression (or RNFL thickness change) between the Cop-1 and placebo groups [39]. However, there was slight improvement of the mean deviation (MD) at week 16 in the Cop-1 patient group compared to worsening of MD in the placebo group [39]. While promising, exploration of these T-cell-mediated immune response pathways should be equalized with the potential risk(s) of inducing autoimmune disease.

2. Neuroregeneration

In the adult mammalian CNS, the growth of injured axons is very limited following pathologic insult. For patients with glaucomatous neuropathy, the eventual goal for therapy will likely be one of visual restoration (partial and/or complete). Theoretically, this approach would be possible through neuroregeneration, in which there is reversal of the process of RGC death through regeneration of functional axons and restoration and/or recovery of the appropriate visual input. In experimental animal models, a variety of methods have been developed to deliver stem cells to replace RGCs and their axons, with the goal of ultimately re-establishing functional vision [40, 41]. To date, however, no IOP-independent neuroprotection and/or visual function enhancement trials have been successfully conducted in humans affected with glaucoma [41].

A recently described scientific protocol in rodents provides a novel and cost-effective means to differentiate human embryonic stem cells (hESCs) into RGC-like neurons, thus broadening the scope for future cell replacement therapy in glaucoma [36, 42]. In this protocol, human RGC-like cells were observed to migrate successfully into the rat ganglion cell layer approximately one week following cellular transplantation via intravitreal injection [42]. In Sprague-Dawley rat eyes with unilateral optic nerve crush injury, topical administration of a pharmacologic inhibitor of Rho-associated protein kinase (ROCK) and norepinephrine transporter (Net) has been shown to promote RGC

survival and regeneration [43]. While the ROCK/Net inhibitor (AR-13324) lowered IOP as expected, the investigators observed RGC survival and optic nerve axonal regeneration at significantly higher levels compared with placebo [43].

Another therapeutic strategy for neuroregeneration in glaucoma involves modulation of axonal outgrowth in the CNS. A novel approach involves neuroprotection and neurorestoration via inhibition of the myelin-associated Nogo receptor pathway. Three CNS myelin proteins, Nogo-A, Nogo-B, and Nogo-C, stimulate the Nogo receptor, thereby inhibiting neurite outgrowth by causing growth cones to collapse through activation of ROCK [44]. The use of anti-Nogo antibodies has been shown to upregulate CNS regeneration, as well as improve sensory and motor function in both rats and primates with spinal cord injuries [44]. In spinal-injured Sprague-Dawley rats, administration of the soluble function-blocking NgR ectodomain (NgR(310)ecto-Fc protein) causes axonal sprouting with subsequent correlation to improved spinal cord electrical conduction and improved locomotion [45]. Soluble LINGO-1 (LINGO-1-Fc), acting as an antagonist of these axonal outgrowth inhibition pathways by blocking LINGO-1 binding to NgR1, significantly improves functional recovery and promotes axonal sprouting after spinal cord injury in Sprague-Dawley rats [46]. In Sprague-Dawley rat glaucoma models, a human NgR1 blocking protein (NgR1(310)-Fc) injected intravitreally promoted RGC axonal regeneration following optic nerve crush injury, as well as demonstrated a neuroprotective effect in a microbead glaucoma model [47].

Changes in Krüppel-like transcription factors (KLFs) are known to be associated with decreases in intrinsic axon growth capacity during development of adult mammalian CNS neurons. KLFs are also involved in regulating axon growth in CNS neurons including RGCs. Investigators have reported that knockdown of KLF9 via short hairpin RNA (shRNA) promotes long-distance optic nerve regeneration in adult Sprague-Dawley rats and mice of both sexes [48]. Moreover, a novel physiologic role for the interaction of KLF9 and JNK3 (c-Jun N-terminal kinase 3) has been discovered to be critical for KLF9 axon-growth-suppressive activity [48].

3. Conclusion

Recent discoveries of the molecular mechanisms underlying POAG have provided new insights into the disease's complex pathogenesis. This enhanced understanding offers opportunities for the development of novel sight preserving and/or vision restoration therapeutic strategies for this blinding disease. Moreover, the refinement of animal models of glaucoma that more closely mimic the pathophysiology of the human disease will assist in facilitating development of these novel strategies. In addition, the restoration of vision after de novo genesis of photoreceptors in congenitally blind mice ($Gnat1^{rd17}$ $Gnat2^{cpfl3}$ double mutant mice) offers promise that a similar regenerative process could be achieved for RGCs [49]. Finally, any exploration of these novel pathways for neuroprotection and neuroregeneration should be balanced and weighed against the real risk(s) of disrupting and damaging the complex CNS milieu in vivo (e.g., induction of autoimmune disease with manipulation of the T-cell-mediated immune response).

Conflicts of Interest

Over the past 12 months, Dr. Tsai has received consulting honoraria from Eyenovia and ReNetXBio.

Acknowledgments

This work was funded and supported by proceeds from the Delafield-Rodgers Professorship held by Dr. Tsai.

References

[1] Y. C. Tham, X. Li, T. Y. Wong, H. A. Quigley, T. Aung, and C. Y. Cheng, "Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systemic review and meta-analysis," *Ophthalmology*, vol. 121, no. 11, pp. 2081–2090, 2014.

[2] R. Varma, M. Ying-Lai, B. A. Francis et al., "Prevalence of open-angle glaucoma and ocular hypertension in latinos: the Los Angeles Latino Eye study," *Ophthalmology*, vol. 111, no. 8, pp. 1439–1448, 2004.

[3] B. E. Klein, R. Klein, W. E. Sponsel et al., "Prevalence of glaucoma: the Beaver Dam Eye Study," *Ophthalmology*, vol. 99, no. 10, pp. 1499–1504, 1992.

[4] American Academy of Ophthalmology Preferred Practice Patterns Committee Glaucoma Panel, *Preferred Practice Patterns. Primary Open-Angle Glaucoma*, American Academy of Ophthalmology, San Francisco, CA, USA, 2016.

[5] M. A. Kass, D. K. Heuer, E. J. Higginbotham et al., "The ocular hypertension treatment study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma," *Archives of Ophthalmology*, vol. 120, no. 6, pp. 701–713, 2002.

[6] M. C. Leske, A. Heijl, M. Hussein, B. Bengtsson, L. Hyman, and E. Komaroff, "Factors for glaucoma progression and the effect of treatment," *Archives of Ophthalmology*, vol. 121, no. 1, pp. 48–56, 2003.

[7] Collaborative Normal-Tension Glaucoma Study Group, "Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures," *American Journal of Ophthalmology*, vol. 126, no. 4, pp. 487–497, 1998.

[8] American Academy of Ophthalmology Preferred Practice Patterns Committee Glaucoma Panel, *Preferred Practice Patterns. Primary Open-Angle Glaucoma Suspect Preferred Practice Pattern*, American Academy of Ophthalmology, San Francisco, CA, USA, 2016.

[9] C. A. Girkin, *Glaucoma. American Academy of Ophthalmology: Basic and Clinical Science Course. Section 10: Glaucoma*, American Academy of Ophthalmology, San Francisco, CA, USA, 2017.

[10] J. C. Tsai, "Canadian journal of ophthalmology lecture: translational research advances in glaucoma neuroprotection," *Canadian Journal of Ophthalmology*, vol. 48, no. 3, pp. 141–145, 2013.

[11] T. Z. Khatib and K. R. Martin, "Neuroprotection in glaucoma: towards clinical trials and precision medicine," *Current Eye Research*, vol. 45, no. 3, pp. 327–338, 2020.

[12] E. E. Chang and J. L. Goldberg, "Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement," *Ophthalmology*, vol. 119, no. 5, pp. 979–986, 2012.

[13] J. L. Goldberg, "Role of electrical activity in promoting neural repair," *Neuroscience Letters*, vol. 519, no. 2, pp. 134–137, 2012.

[14] T. E. Yap, P. Donna, M. T. Almonte, and M. F. Cordeiro, "Real-time imaging of retinal ganglion cell apoptosis," *Cells*, vol. 7, no. 6, p. 60, 2018.

[15] T. Krupin, J. M. Liebmann, D. S. Greenfield, R. Ritch, and S. Gardiner, "A randomized trial of brimonidine versus timolol in preserving visual function: results from the low-pressure glaucoma treatment study," *American Journal of Ophthalmology*, vol. 151, no. 4, pp. 671–681, 2011.

[16] L. Fu, S. S. Kwok, Y. K. Chan et al., "Therapeutic strategies for attenuation of retinal ganglion cell injury in optic neuropathies: concepts in translational research and therapeutic implications," *Biomed Reserch International*, vol. 2019, Article ID 8397521, 10 pages, 2019.

[17] A. Kimura, K. Namekata, X. Guo et al., "Neuroprotection, growth factors and BDNF-TrkB signalling in retinal degeneration," *International Journal of Molecular Sciences*, vol. 17, no. 9, p. 1584, 2016.

[18] K. Flachsbarth, W. Jankowiak, K. Kruszewski, S. Helbing, S. Bartsch, and U. Bartsch, "Pronounced synergistic neuroprotective effect of GDNF and CNTF on axotomized retinal ganglion cells in the adult mouse," *Experimental Eye Research*, vol. 176, pp. 258–265, 2018.

[19] P. A. Sieving, R. C. Caruso, W. Tao et al., "Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase 1 trial of CNTF delivered by encapsulated cell intraocular implants," *Proceedings of the National Academy of Sciences*, vol. 103, no. 10, pp. 3896–3901, 2006.

[20] J. Weishaupt, G. Rohde, E. Pölking et al., "Effect of erythropoietin axotomy-induced apoptosis in rat retinal ganglion cells," *Investigative Ophthalmology & Visual Science*, vol. 45, no. 5, pp. 1514–1522, 2004.

[21] J. C. Tsai, L. Wu, B. Worgul, M. Forbes, and J. Cao, "Intravitreal administration of erythropoietin and preservation of retinal ganglion cells in an experimental rat model of gaucoma," *Current Eye Research*, vol. 30, no. 11, pp. 1025–1031, 2005.

[22] J. C. Tsai, B. J. Song, L. Wu, and M. Forbes, "Erythropoietin: a candidate neuroprotective agent in the treatment of glaucoma," *Journal of Glaucoma*, vol. 16, no. 6, pp. 567–571, 2007.

[23] L. Zhong, J. Bradley, W. Schubert et al., "Erythropoietin promotes survival of retinal ganglion cells in DBA/2J glaucoma mice," *Investigative Ophthalmology & Visual Science*, vol. 48, no. 3, pp. 1212–1218, 2007.

[24] J. C. Tsai, "Safety of intravitreally administered recombinant erythropoietin (an AOS thesis)," *Transactions of the American Ophthalmological Society*, vol. 106, pp. 459–472, 2008.

[25] B. J. Song, H. Cai, J. C. Tsai, S. Chang, M. Forbes, and L. V. Del Priore, "Intravitreal recombinant erythropoietin: a safety study in rabbits," *Current Eye Research*, vol. 33, no. 9, pp. 750–760, 2008.

[26] R. Diem, N. Taheri, G. P. Dietz et al., "HIV-Tat-mediated bcl-XL delivery protects retinal ganglion cells during experimental autoimmune optic neuritis," *Neurobiology of Disease*, vol. 20, no. 2, pp. 218–226, 2005.

[27] K. R. Martin, H. A. Quigley, D. J. Zack et al., "Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model," *Investigative Ophthalmology & Visual Science*, vol. 44, no. 10, pp. 4357–4365, 2003.

[28] A. Osborne, A. X. Z. Wang, A. Tassoni, P. S. Widdowson, and K. R. Martin, "Design of a novel gene therapy construct to achieve sustained brain-derived neurotrophic factor signaling in neurons," *Human Gene Therapy*, vol. 29, no. 7, pp. 828–841, 2018.

[29] A. Catenaccio, M. Llavero Hurtado, P. Diaz, D. J. Lamont, T. M. Wishart, and F. A. Court, "Molecular analysis of axonal-intrinsic and glial-associated co-regulation of axon degeneration," *Cell Death & Disease*, vol. 8, no. 11, p. e3166, 2017.

[30] M. A. Avery, T. M. Rooney, J. D. Pandya et al., "WldS prevents axon degeneration through increased mitochondrial flux and enhanced mitochondrial Ca^{2+} buffering," *Current Biology*, vol. 22, no. 7, pp. 596–600, 2012.

[31] T. M. Wishart, J. M. Paterson, D. M. Short et al., "Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow wallerian degeneration (wlds) gene," *Molecular & Cellular Proteomics*, vol. 6, no. 8, pp. 1318–1330, 2007.

[32] Y. Simonin, M. Ferrer-Alcon, A. Ferri, and A. C. Kato, "The neuroprotective effects of the WldS gene are correlated with proteosome expression rather than apoptosis," *European Journal of Neuroscience*, vol. 25, no. 8, pp. 2269–2274, 2007.

[33] H. Y. Park, J. H. Kim, H. Sun Kim, and C. K. Park, "Stem cell-based delivery of brain-derived neurotrophic factor gene in the rat retina," *Brain Research*, vol. 1469, pp. 10–23, 2012.

[34] D. W. Chen, L. Narsineni, and M. Foldvari, "Multipotent stem cell-derived retinal ganglion cells in 3D culture as tools for neurotrophic factor gene delivery system development," *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 21, Article ID 102045, 2019.

[35] T. Nakazawa, C. Nakazawa, A. Matsubara et al., "Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed ganglion cell loss in a mouse model of glaucoma," *Journal of Neuroscience*, vol. 26, no. 49, pp. 12633–12641, 2006.

[36] F. Lucas-Ruiz, C. Galindo-Romero, M. Salinas-Navarro et al., "Systemic and intravitreal antagonism of the TNFR1 signaling pathway delays axotomy-induced retinal ganglion cell loss," *Frontiers in Neuroscience*, vol. 13, p. 1096, 2019.

[37] M. Schwartz, "Neurodegeneration and neuroprotection in glaucoma: development of a therapeutic neuroprotective vaccine: the Friedenwald lecture," *Investigative Ophthalmology & Visual Science*, vol. 44, no. 4, pp. 1407–1411, 2003.

[38] S. Bakalash, G. B. Shlomo, E. Aloni et al., "T-cell-based vaccination for morphological and functional neuroprotection in a rat model of chronically elevated intraocular pressure," *Journal of Molecular Medicine*, vol. 83, no. 11, pp. 904–916, 2005.

[39] K. R. Fan, M. Baskaran, M. E. Nonpiur et al., "Investigating the neuroprotective effect of copolymer-1 in acute primary angle closure—interim report of a randomized placebo-controlled double-masked clinical trial," *Acta Ophthalmologica*, vol. 97, no. 6, pp. e827–e832, 2019.

[40] H. A. Quigley and D. S. Iglesia, "Stem cells to replace the optic nerve," *Eye*, vol. 18, no. 11, pp. 1085–1088, 2004.

[41] J. H. Stern, Y. Tian, J. Funderburgh et al., "Regenerating eye tissues to preserve and restore vision," *Cell Stem Cell*, vol. 22, no. 6, pp. 834–849, 2018.

[42] X. Zhang, K. Tenerelli, S. Wu et al., "Cell transplantation of retinal ganglion cells derived from hESCs," *Restorative Neurology and Neuroscience*, pp. 1–10, 2019.

[43] P. X. Shaw, A. Sang, Y. Wang et al., "Topical administration of a Rock/net inhibitor promotes retinal ganglion cell survival

and axon regeneration after optic nerve injury," *Experimental Eye Research*, vol. 158, pp. 33–42, 2017.

[44] R. Mohammed, K. Opara, R. Lall, U. Ojha, and J. Xiang, "Evaluating the effectiveness of anti-nogo treatment in spinal cord injuries," *Neural Development*, vol. 15, no. 1, 2020.

[45] S. Li, B. P. Liu, S. Budel et al., "Blockade of nogo-66, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein by soluble nogo-66 receptor promotes axonal sprouting and recovery after spinal injury," *Journal of Neuroscience*, vol. 24, no. 46, pp. 10511–10520, 2004.

[46] B. Ji, M. Li, W. T. Wu et al., "LINGO-1 antagonist promotes functional recovery and axonal sprouting after spinal cord injury," *Molecular and Cellular Neuroscience*, vol. 33, no. 3, pp. 311–320, 2006.

[47] X. Wang, J. Lin, A. Arzeno et al., "Intravitreal delivery of human NgR-fc decoy protein regenerates axons after optic nerve crush and protects ganglion cells in glaucoma models," *Investigative Ophthalmology & Visual Science*, vol. 56, no. 2, pp. 1357–1366, 2015.

[48] A. Apara, J. Galvao, Y. Wang et al., "KLF9 and JNK3 interact to suppress axon regeneration in the adult CNS," *The Journal of Neuroscience*, vol. 37, no. 40, pp. 9632–9644, 2017.

[49] K. Yao, S. Qui, Y. V. Wang et al., "Restoration of vision after de novo genesis of rod photoreceptors in mammalian retinas," *Nature*, vol. 560, no. 7719, pp. 484–488, 2018.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 5901537, 5 pages
https://doi.org/10.1155/2020/5901537

Review Article

Influence of Cost of Care and Adherence in Glaucoma Management: An Update

Frances Meier-Gibbons[1] **and Marc Töteberg-Harms**[2]

[1]*Eye Center Rapperswil, Merkurstr. 50, Rapperswil-Jona 8640, Switzerland*
[2]*UniversityHospital Zurich, Department of Ophthalmology, Frauenklinikstr. 24, Zurich 8091, Switzerland*

Correspondence should be addressed to Frances Meier-Gibbons; frances.meier@bluewin.ch

Received 18 January 2020; Accepted 5 March 2020; Published 8 April 2020

Guest Editor: Barbara Cvenkel

The costs for glaucoma care are rising worldwide. The main reason is the increase of life expectancy and the increasing variety of diagnostic tests and therapeutically options by implants and devices. How can we influence the increase in costs? Does a relationship exist between the rising costs and the behavior of patients especially in regard to adherence of patients to the prescribed therapy? Are there ways to improve adherence? The costs of a disease can be estimated by adding the direct costs and the indirect costs deriving from the disease. Many studies have been looking at the direct costs, for example, the costs of diagnostic tests and treatment modalities. Unfortunately, not many studies investigated the indirect costs, i.e., costs related to the need of a person to accompany the patient during his or her outpatient visits or the costs deriving from loss of work capacity because of the disease itself or the outpatient visits. Adherence or the synonym compliance has been discussed since many years, and it seems that it remains a major problem in the management of many chronic diseases. Despite all efforts to improve adherence, the adherence rate in chronic diseases such as glaucoma or arterial hypertension remains considerably low. One of the main factors in improving adherence is raising patient's awareness of the disease by providing general understanding of their disease. Other important factors are simplified therapeutic regimens, e.g., fixed combination drops, sustained drug release techniques, or new glaucoma surgical procedures with a more favorable risk profile.

1. Introduction

Glaucoma is one of the major causes for irreversible blindness worldwide. Quigley [1] estimated in 2006 that, by 2020, 80 million people will be affected. Tham [2] in 2014 mentioned that the number of patients with glaucoma will be 111.8 million by 2040. Glaucoma in early stages is mainly asymptomatic and thus, many patients are not aware of their disease. In the developed world, about half of the patients do not know that they suffer from glaucoma.

The prevalence of glaucoma is rising in a nonlinear fashion with age. The number of elderly people is rising and therefore, more patients will suffer from glaucoma in the future. The increasing digitalization and education of the population plays an important role. Many patients over the age of 65 work on a computer and drive their cars nowadays. On the other hand, the new technical possibilities for detecting a disease and the therapeutic options to treat it lead to an increasing demand from patients. Having the possibilities of detecting and treating the disease is one part of glaucoma management, and the other part is the behavior of the patients. Glaucoma, as many chronic diseases, has a low adherence and persistence rate which may lead to a progression of the disease and hence, an increase of costs [3]. In an earlier review [4], we studied the possible connections between adherence and costs of glaucoma care and concluded that improving adherence could reduce the costs of the disease by reducing the progression of the disease. The WHO has shown that the average healthcare costs have increased worldwide since 2009 despite a decrease in the real growth rates per capita, which is partially influenced by the economic crisis [5]. All the factors mentioned above

influence the rising healthcare costs around the world. The question arises, if this vicious cycle can be interrupted or slowed down.

2. Materials and Methods

A literature search was performed using "PubMed," search strings were "adherence" and "glaucoma" or "costs of glaucoma care." Only publications in English, published until Dec 31, 2019, were included.

3. Results

The results were divided into (1) costs of glaucoma care, (2) patients adherence, and (3) improvement of adherence. (4) The last paragraph discussed the question whether a connection between costs and adherence exists.

3.1. Costs of Glaucoma Care. Schmier [6] mentioned in 2007 that studies published on costs in glaucoma care focus mainly on direct costs, i.e., costs of diagnostic tests and therapies (drugs, surgery, or laser) and the costs for transportation of the patient to his or her visits. The indirect costs, which are as important as direct costs, however, are seldom looked at in studies. The indirect costs include the costs for the accompanying persons and the costs for loss of work productivity, for example, days lost at work, but also the loss of work productivity for accompanying persons. Other indirect costs derive from the consequences of an advanced disease stage, for example, inability to drive, increased risk of falls, and depression triggered by the disease, for example. Another aspect, the quality of life of a patient, is also seldom addressed in studies. A European study [7] showed that the costs of glaucoma care have a significant linear trend parallel to the increasing severity of the disease. It is important to recognize that glaucoma is a chronic disease, which progresses in every patient. It is crucial to differentiate between fast progressors and slow progressors to keep costs under control [8] because the frequency of outpatient visits can vary between fast and slow progressors.

Looking at the studies on direct costs of glaucoma care in different countries, the main message was that the aspect of costs should be discussed with the patient especially in regard to the price of antiglaucomatous drugs, which are quite expensive in some countries (e.g., the United States). Costs for antiglaucomatous drugs must be judged in comparison to the median income and not as a total amount. An important study concerning the aspect of costs came from Nigeria, where the costs for glaucoma drugs was 50% of the monthly income of a middle-class family, but 100% of a lower-class family [9].

In the recent years, generic medications have taken over the antiglaucomatous drug market. The price of a generic drug is much lower compared to the branded drug, but many studies [10,11] have shown that they are not equal: only the main substance needs to be identical to the branded drug. The remaining, especially the preservative agent, but also the consistency of the container and the size of the drop can differ. In addition, the concentration of the main substance is allowed to vary within certain limits as well. Switching from a branded drug to a generic drug means introducing a new medication with more costs in regard to follow-up visits and to informing the patients about this topic. It is crucial to make ophthalmologist and pharmacists aware about the differences between branded and generic drugs. Interestingly, a large difference in the prescription habits of generic drugs exists between different countries. In Europe, the northern countries prescribe more generic drugs than the southern countries [12].

3.2. Patient's Adherence. Adherence or the synonym compliance is defined as the cooperation of the patient with the recommendation given by the treating doctor. The term persistence on the other hand describes the length of time the patient uses the medication as prescribed [13].

Glaucoma, as other chronic diseases, has a low adherence and persistence rate. Many patients, especially in the early phases of their disease, do not realize the consequences of progression. The so-called "white coat adherence" is seen frequently: the patient applies the drops only a few days before he or she visits his or her doctor and stops to take them shortly after the visit again. Many studies have discussed the theme adherence, but as Cate [14] pointed out in 2015, there are inconsistencies among different monitoring strategies and adherence measures. Common obstacles to adherence can be grouped into four categories: situational and environmental factors; medication regimens; patient's factors; and doctor's factors [15]. Newman-Casey [16] mentioned among the most often cited factors for nonadherence psychological factors (for example, low self-efficacy and forgetfulness), difficulty with drug administration (especially in patients with rheumatic diseases or in patients with dementia), and medication scheme. Hasebe [17] and Movahedinejad [18] found similar reasons and added that an important factor was the lack of awareness regarding the complications of progressive glaucoma. A practical comment was published by Muir [19], who said that adherence involves four steps.

The patient needs to get the medication, he or she has to be physically able to apply the drop in the eye, and use the medication at the appropriate time. Lastly, he or she needs to repeat these three steps every day.

Interestingly, the patient's declaration about their adherence often differs with the rates actually measured. Gatwood [20] found a great discrepancy between the patient reported data and the actual measurements obtained by a wireless device.

Having access to electronic information should improve adherence; hence, Newman-Casey [21] and Fiscella [22] showed in studies that neither the availability of information sent via mail nor access to electronic information improved adherence.

An interesting study by Rees [23] looked at cultural differences to adherence. They found that in Western cultures the beliefs about glaucoma treatment were predictive of adherence.

It is well known that glaucoma treatment often leads to local and systemic side effects. Zimmermann [24] found in a study that up to two-thirds of glaucoma patients suffer from side effects. The side effects derive either from the medication itself or from the preserving agents of the drops. It is mandatory to find an adequate therapy and perhaps change from a preservative containing to a preservative free medication, which might improve the ocular surface and lead to less local side effects.

A special interest has been given lately to the glaucoma management of elderly patients. Different factors influence the behavior of an elderly patient: often, they take other medication to treat systemic diseases and the introduction of an antiglaucomatous drug needs to be discussed with the family doctor. Other influencing factors are rheumatic diseases or a possible dementia, interfering with reliable application of drugs.

In an elderly patient, other treatment options than local drugs must be discussed: a good alternative are laser treatments or surgical options [25].

We tend to undertreat elderly glaucoma patients because the lifespan is much longer than in earlier years and patients might realize progressive visual field defects, as seen in an increased tendency for falls and a reduced capacity for driving a car [25].

How can we improve the adherence of our patients?

To improve adherence, different factors need to be addressed: the medication itself, local factors, the application of the medication, the systemic factors, and last but not least the costs.

Many antiglaucomatous drugs lead to local side effects and may be exchanged by another drug of the same class with less side effects. Leung [26] and Fechtner [27] showed in studies that about 50% of patients using local antiglaucomatous medication suffered of more or less severe signs and symptoms of ocular surface disease (OSD) and eventually had to stop the medication.

Applying the drops the correct way is challenging. Patients with rheumatic disease are often not able to open drug containing bottles or single use units [28].

It is important to show the patient how to apply the drops correctly. Atey [29] showed in a study that an improvement of instillation of eye drops leads to a reduction of IOP. Some patient may misunderstand the instruction of the doctor: twice a day might mean at 8 am and at 8 pm, but patients may interpret the instruction another way and apply the drops in the morning and at lunch.

The systemic problems were addressed above: often, the patients with glaucoma have many other drugs prescribed by their general practitioner and it is important to talk to the general practitioner before introducing another topical medication.

The cost issue is an important factor and can lead to a reduced adherence if the patient cannot afford the medication prescribed [30].

As it is well known that glaucoma has a low adherence and persistence rate, much effort is given to ameliorate and simplify the administration of drugs: drug combinations replace the addition of bottles and longer-lasting products, for example, in the form of slow release drugs or intraocular injections, are studied.

3.3. Laser and Minimally Invasive Glaucoma Surgery to Improve Adherence. Multiple studies on laser trabeculoplasty and minimally invasive glaucoma surgery (MIGS) procedures have been published in the recent years. Recently, the LIGHT study got published [31]. The LIGHT trial was a randomized controlled trial with 36 months of follow-up comparing selective laser trabeculoplasty ($n = 356$) to drops ($n = 362$) in treatment-naïve patients. Eyes of patients in the selective laser trabeculoplasty group were within target intraocular pressure at more visits (93·0%) than in the eye drops group (91·3%) [31]. In addition, selective laser trabeculoplasty was more cost-effective in the United Kingdom compared to drops. The increasing interest in MIGS procedures is based on their favorable risk profile and at least moderate efficacy, which makes these procedures useful for moderate and early stages of glaucoma with mal-compliance or intolerability to topical therapy. The majority of MIGS procedures enhance conventional/trabecular outflow. Thus, a target pressure cannot be expected to be below 14–16 mmHg. In addition, MIGS procedures like the XEN gel stent (Allergan Inc.) or the PRESERFLO MicroShunt (Santen Inc.) bypass conventional and alternative outflow pathways and guide aqueous humor from the anterior chamber through the implant into the subconjunctival space. Generally, these procedures lower IOP independently from the patient's adherence to topical medications. Because the IOP lowering efficacy of a MIGS procedure is not dependent on the patient's adherence to the prescribed drops, they should logically enhance treatment success compared to topical therapy which on the other hand is dependent on the patient's adherence.

3.4. Does a Connection between Costs and Adherence Exist? Not many studies looked at the connection between costs and adherence. Disease progression and severity of the disease are probably the most relevant factors for adherence. However, costs of glaucoma medication, which increase in patients with nonadherence, should not be underestimated. As we mentioned above, Traverso discussed in a European study that the costs increased linearly with the severity of the disease [7]. A connection between costs and adherence exists probably via the progression of the disease: in a progressing disease, the costs are rising [7, 30]. However, more studies are needed to determine the influence of adherence on glaucoma progression and, thus, on the costs of glaucoma care. It is the duty of ophthalmologists, however, to improve patient's adherence to prescribed therapies [30].

4. Summary

Adherence has a major input on the outpatient care of glaucoma patients. In glaucoma, as in other chronic diseases, adherence is rather low. Interestingly, the adherence rate has not improved over the last decades despite better information of the patient about their disease and improvement

in medical and surgical therapies [32]. Low adherence may lead to progression of the disease and therefore to higher costs. More studies are needed to evaluate the influence of low adherence on progression of the disease and to calculate the costs deriving from progression.

However, the duty of the treating ophthalmologist is to improve patient's adherence, mainly by informing the patient and by finding an adequate glaucoma treatment, which fits into the patient's lifestyle.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] H. A. Quigley and A. T. Broman, "The number of people with glaucoma worldwide in 2010 and 2020," *British Journal of Ophthalmology*, vol. 90, no. 3, pp. 262–267, 2006.

[2] Y.-C. Tham, X. Li, T. Y. Wong, H. A. Quigley, T. Aung, and C.-Y. Cheng, "Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040," *Ophthalmology*, vol. 121, no. 11, pp. 2081–2090, 2014.

[3] F. Meier-Gibbons, "Current strategies for improving treatment adherence and persistence in glaucoma management," *View on Glaucoma*, vol. 13, no. 1, pp. 4–7, 2019.

[4] M. Töteberg-Harms, M. S. Berlin, and F. Meier-Gibbons, "Increasing healthcare costs," *Current Opinion in Ophthalmology*, vol. 28, no. 2, pp. 127–132, 2017.

[5] WHO, *Spending on health: a global overview*, WHO, Geneva, Switzerland, 2012, http://www.who.int/mediacentre/Factsheets/fs319/en/.

[6] J. K. Schmier, M. T. Halpern, and M. L. Jones, "The Economic Implications of Glaucoma," *Pharmacoeconomics*, vol. 25, no. 4, pp. 287–308, 2007.

[7] C. E. Traverso, J. G. Walt, S. P Kelly et al., "Direct costs of glaucoma and severity of the disease: a multinational long term study of resource utilisation in Europe," *British Journal of Ophthalmology*, vol. 89, no. 10, pp. 1245–1249, 2005.

[8] A. Tuulonen, P. J. Airaksinen, E. Erola et al., "The Finnish evidence-based guideline for open-angle glaucoma," *Acta Ophthalmologica Scandinavica*, vol. 81, no. 1, pp. 3–18, Feb 2003.

[9] A. O. Adio and A. A. Onua, "Economic burden of glaucoma in river state, Nigeria," *Clinical Ophthalmology*, vol. 6, pp. 2023–2031, 2012.

[10] A. A. Genazzani and F. Pattarino, "Difficulties in the production of identical drug products from a pharmaceutical technology viewpoint," *Drugs in R & D*, vol. 9, no. 2, pp. 65–72, 2008.

[11] Z. N. Mammo, J. G. Flanagan, D. F. James, and G. E. Trope, "Generic versus brand-name North American topical glaucoma drops," *Canadian Journal of Ophthalmology*, vol. 47, no. 1, pp. 55–61, 2012.

[12] R. D. Natale, "Trends and Discrepancies in Glaucoma Medical Therapy in Europe," *European Ophthalmic Review*, vol. 09, no. 02, pp. 130-131, 2015.

[13] EGS Guidelines 2014.

[14] H. Cate, D. Bhattacharya, A. Clark, R. Holland, and D. C. Broadway, "A comparison of measures used to describe adherence to glaucoma medication in a randomised controlled trial," *Clinical Trials: Journal of the Society for Clinical Trials*, vol. 12, no. 6, pp. 608–617, 2015.

[15] J. C. Tsai, C. A. McClure, S. E. Ramos, D. G. Schlundt, and J. W. Pichert, "Compliance barriers in glaucoma: a systematic classification," *Journal of Glaucoma*, vol. 12, no. 5, pp. 393–398, 2003.

[16] P. A. Newman-Casey, A. L. Robin, T. Blachley et al., "The most common barriers to glaucoma medication adherence: a cross-sectional survey," *Ophthalmology*, vol. 122, no. 7, pp. 1308–1316, 2015.

[17] Y. Hasebe, K. Kashiwagi, T. Tsumura et al., "Changes in adherence and associated factors among patients on newly introduced prostaglandin analog and timolol fixed-combination therapy," *Patient Preference and Adherence*, vol. 12, pp. 1567–1577, 2018.

[18] T. Movahedinejad and M. Adib-Hajbaghery, "Adherence to treatment in patients with open-angle glaucoma and its related factors," *Electronic physician*, vol. 8, no. 9, pp. 2954–2961, 2016.

[19] K. W. Muir and P. Lee, "Glaucoma medication adherence: room for improvement in both performance and measurement," *Archives of Ophthalmology*, vol. 129, no. 2, pp. 243–245, 2011.

[20] J. D. Gatwood, J. Johnson, and B. Jerkins, "Comparisons of self-reported glaucoma medication adherence with a new wireless device," *Journal of Glaucoma*, vol. 26, no. 11, pp. 1056–1061, 2017.

[21] P. A. Newman-Casey, O. J. Killeen, and M. Renner, "Access to ad experiences with e-health technology among glaucoma patients and their relationshiop with medication adherence," *Telemedicine and e-Health*, vol. 24, no. 12, pp. 1026–1035, 2018.

[22] R. Fiscella, E. Caplan, and P. Kamble, "The effect of an educational intervention on adherence to intraocular pressure-lowering medications in a large cohort of older adults with glaucoma," *Journal of Managed Care & Specialty Pharmacy*, vol. 24, pp. 1284–1294, 2018.

[23] G. Rees, X.-L. Chong, C. Y. Cheung et al., "Beliefs and adherence to glaucoma treatment," *Journal of Glaucoma*, vol. 23, no. 5, pp. 293–298, 2014.

[24] T. J. Zimmerman, S. R. Hahn, L. Gelb, H. Tan, and E. E. Kim, "The impact of ocular adverse effects in patients treated with topical prostaglandin analogs: changes in prescription patterns and patient persistence," *Journal of Ocular Pharmacology and Therapeutics*, vol. 25, no. 2, pp. 145–152, 2009.

[25] D. Raczyńska, L. Glasner, and E. Serkies-Minuth, M. A. Wujtewicz and K. Mitrosz, Eye surgery in the elderly," *Clinical Interventions in Aging*, vol. 11, pp. 407–414, 2016.

[26] E. W. Leung, F. A. Medeiros, and R. N. Weinreb, "Prevalence of ocular surface disease in glaucoma patients," *Journal of Glaucoma*, vol. 17, no. 5, pp. 350–355, 2008.

[27] R. D. Fechtner, D. G. Godfrey, D. Budenz, J. A. Stewart, W. C. Stewart, and M. C. Jasek, "Prevalence of ocular surface complaints in patients with glaucoma using topical intraocular pressure-lowering medications," *Cornea*, vol. 29, no. 6, pp. 618–621, 2010.

[28] A. L. Hennessy, J. Katz, D. Covert, C. Protzko, and A. L. Robin, "Videotaped evaluation of eyedrop instillation in glaucoma patients with visual impairment or moderate to severe visual field loss," *Ophthalmology*, vol. 117, no. 12, pp. 2345–2352, 2010.

[29] T. M. Atey, W. Shibeshi, A. T Giorgis, and S. W. Asgedom, "The impact of adherence and instillation proficiency of topical glaucoma medications on intraocular pressure," *Journal of Ophthalmology*, vol. 2017, Article ID 1683430, 2017.

[30] F. Meier-Gibbons, M. S. Berlin, and M. Töteberg-Harms, "Influence of new treatment modalities on adherence in

glaucoma," *Current Opinion in Ophthalmology*, vol. 30, no. 2, pp. 104–109, 2019.

[31] G. Gazzard, E. Konstantakopoulou, D. Garway-Heath et al., "Selective laser trabeculoplasty versus eye drops for first-line treatment of ocular hypertension and glaucoma (LiGHT): a multicentre randomised controlled trial," *The Lancet*, vol. 393, no. 10180, pp. 1505–1516, 2019.

[32] G. F. Schwartz, "Compliance and persistency in glaucoma follow-up treatment," *Current Opinion in Ophthalmology*, vol. 16, no. 2, pp. 114–121, 2005.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 1651265, 8 pages
https://doi.org/10.1155/2020/1651265

Review Article

The Use of Generic Medications for Glaucoma

Andrew J. Tatham

Princess Alexandra Eye Pavilion, University of Edinburgh, Chalmers Street, Edinburgh EH3 9HA, UK

Correspondence should be addressed to Andrew J. Tatham; andrewjtatham@gmail.com

Received 25 December 2019; Accepted 2 March 2020; Published 7 April 2020

Academic Editor: Steffen Heegaard

The use of generic medicines has grown considerably in recent years providing considerable cost savings. In England, generic items represented 11.7% of prescriptions for glaucoma and ocular hypertension in 2009, increasing to 55.2% of prescriptions in 2018. Generics can be brought to the market quickly and at low cost as manufacturers are not required to repeat animal or clinical research on active ingredients already approved for safety and efficacy. Although there is no regulatory requirement for studies comparing branded and generic eye drops, several randomised crossover studies have been performed comparing branded and generic prostaglandin analogues. While most have shown similar intraocular pressure lowering, studies are of short duration and have not evaluated visual field endpoints. Furthermore, differences in inactive ingredients, pH, viscosity, levels of particulate matter, and degradation over time have been reported. Other potential problems with generic eye drops include differences in bottle design affecting adherence, problems with supply, and the possibility that reduced revenue for innovator companies will lead to reduced investment in new drug development. This article reviews the potential advantages and disadvantages of generic antiglaucoma medications.

1. Introduction

Generic medications are defined by the World Health Organization (WHO) as pharmaceutical products intended to be interchangeable with an innovator product, manufactured without a licence from the innovator company and marketed after the expiry date of the patent or other exclusive rights [1]. They are required to have the same active ingredient, route of administration, dosing, and be manufactured to the same quality standards as the reference medication but may have different inactive ingredients and packaging [1, 2]. Generics can only be marketed once the period of exclusivity of innovator product has expired, which is typically 10 years from the date of first authorisation; before a generic medication can be marketed, it must be approved by the appropriate regulatory authority, for example, the European Medicines Agency (EMA) or United States (US) Food and Drugs Administration (FDA) [3]. Regulatory authorities conduct rigorous reviews to ensure generic drugs are of a high standard, conduct inspections of manufacturing facilities, and monitor drug safety after products are brought to market [4].

In recent years, the use of generic medicines has grown considerably, driven by their often substantially lower price compared to branded products. Generic items accounted for only 19% of prescription drugs sold in the US in 1984, increasing to 43% in 1996, and 89% in 2017 [5]. This has resulted in significant savings for healthcare systems, with an estimated saving in the US of $1.67 trillion between 2007 and 2016 [6]. The use of generics has also increased in ophthalmology, especially in the medical treatment of glaucoma. In England, generic items represented 11.7% of prescriptions for glaucoma and ocular hypertension in 2009, increasing to 55.2% of prescriptions in 2018 [7]. The first generic prostaglandin analogue (PGA), latanoprost, became available in early 2011.

There are however potential downsides to the use of generic medicines, including inconsistences in packaging and bottle design between products, differences in inactive ingredients which may cause unexpected issues with tolerability, and the likelihood that reduced revenue for innovator companies will lead to reduced investment in new drug development. In addition, some professionals and members of the public hold the view that generics are less

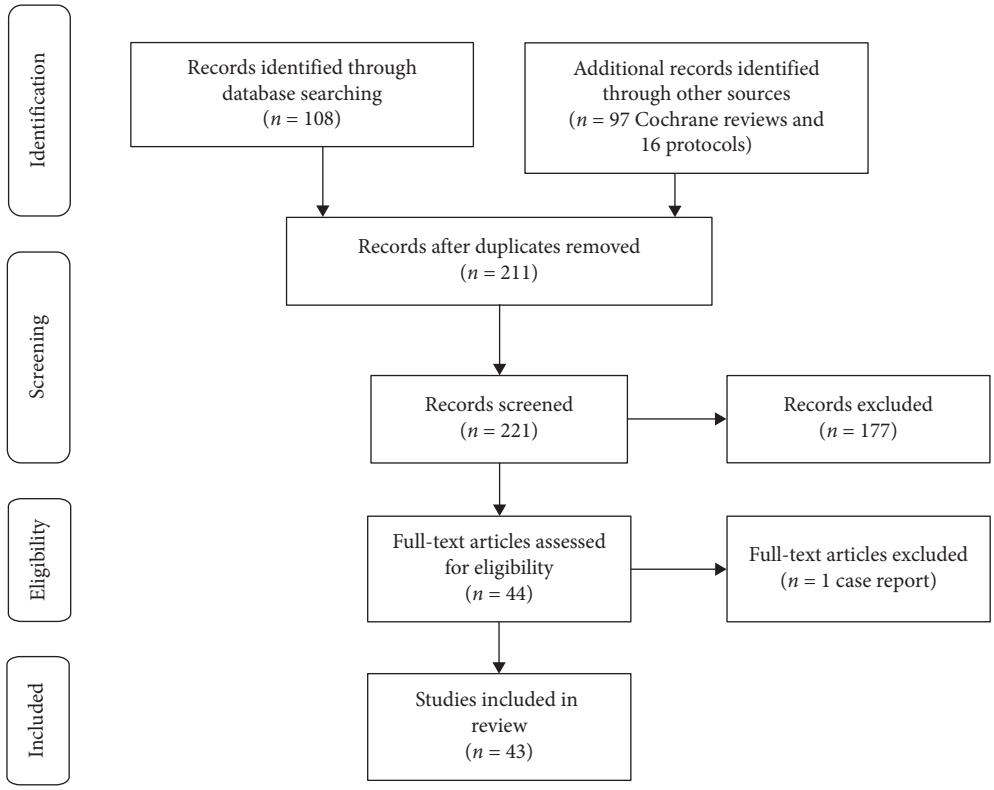


Figure 1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) diagram.

effective and of poorer quality than branded alternatives, which may reduce acceptance of generic drugs. As much debate surrounds the use of generic medicines, the aim of this article is to review of the use of generics for glaucoma and provide a balanced appraisal of potential advantages and disadvantages.

2. Methods

A PubMed database search was performed on 16th December 2019 using the following search terms: ((glaucoma[Title/Abstract]) AND generic[Title/Abstract]) AND ("1900/01/01"[Date - Publication]: "2019/12/16" (Date—Publication)). The Cochrane Library was also searched for meta-analyses or systemic reviews containing the keyword "generic" in their title or abstract. The PubMed search yielded 108 items, while 97 Cochrane reviews and 16 Cochrane protocols were identified (Figure 1). Article titles and abstracts were manually reviewed and of the 221 records screened, 178 were excluded as they were not deemed to relate to the use of generic medications in glaucoma. Though Cochrane protocols were identified proposing reviews of generic versus branded antiepileptic drug monotherapy in epilepsy (18th Sep 2015) and clozapine (generic versus branded) for schizophrenia (24th Jan 2019), no completed reviews were found and no Cochrane reviews or protocols were identified relating to generic ophthalmic medications. The full texts of the remaining 44 records were examined and one was excluded as it related to a single case report. Of the remaining articles, 15 primarily focused on cost analysis of glaucoma medications, 11 were experimental laboratory studies, for example, examining drug composition or bottle designs, 6 were randomised trials or switch studies, there were 4 reviews, and 7 studies of other design, for example, examining trends in prescribing patterns or comparing adherence between generic and branded medications.

2.1. Efficacy. Although there have been several meta-analyses conducted in areas outside ophthalmology showing no difference in outcome between branded and generic medications, including for cardiovascular drugs, antiepilepsy medications, and some antibiotics [8–10], our search found no meta-analyses or systematic reviews examining the efficacy of generic compared to branded antiglaucoma medications. The lack of studies is likely due to the fact that they are not required by regulatory authorities for market authorisation.

The World Health Organization (WHO) has developed global standards and requirements for the regulatory assessment, marketing authorisation, and quality control of generic medications [11]. These standards specify that generic products should fulfil three sets of criteria relating to (1) manufacture and quality control; (2) product characteristics and labelling; and (3) therapeutic equivalence, with assessment of equivalence normally requiring in vivo studies. Generic medications can be brought to market quickly as manufacturers are not required to repeat animal or clinical research on active ingredients already approved for safety and efficacy. However, generic products must have

pharmaceutical equivalence to the innovator product, meaning they contain the same amount of the same active substance(s) in the same dosage form, as well as bioequivalence, meaning bioavailability is within an acceptable limit. It is important to emphasise that pharmaceutical equivalence does not necessarily equate to bioequivalence as differences in excipients and/or the manufacturing process can lead to differences in absorption. Bioequivalence studies usually involve an assessment of rate and extent of absorption using the plasma concentration time curve. For example, the European Medicines Agency requires characteristics such as maximum serum concentration and AUC0-t (area under the plot of drug concentration over time curve from drug administration) to be within 80 to 125% of the reference product [12]. Classic bioequivalence studies cannot be performed on locally acting drugs such as eye drops as their active ingredients are not found in measurable quantities in the bloodstream and bloodstream levels are not related to efficacy [13]. Therefore, some products may be considered bioequivalent without the need for bioequivalence studies. The WHO list several examples of potentially exempt products including parenterally administered medications containing the same concentration of active substance with the same excipients; orally administered medications containing the same concentration of active substance, that do not contain an excipient known or suspected to affect absorption of the active ingredient; and, most relevant to this review, ophthalmic products prepared as aqueous solutions containing the same active substance in the same concentration and essentially the same excipients in comparable concentrations [11].

While there is no requirement for manufacturers to conduct head to head clinical studies of generic and branded topical ophthalmic products, several comparison studies have been published. The first study comparing the efficacy of branded Xalatan and generic latanoprost was published in 2007 [14]. Narayanaswamy and colleagues reported the results of an open-label randomised crossover study in which 30 participants received each treatment for a 12-week period. A larger percentage reduction in intraocular pressure (IOP) was observed when using Xalatan compared to the generic (38.66 ± 10.29% versus 25.42 ± 5.98%). In patients switching from generic latanoprost to Xalatan, there was an additional 4.3 ± 8.76% reduction in IOP, compared to an average 8.86±17.76% increase in IOP when switched from Xalatan to generic latanoprost. There was also a lower incidence of conjunctival hyperaemia and ocular irritation assumed to be due to higher amounts of particulate matter observed in the generic.

In contrast, subsequent studies have largely found similar efficacy between branded and nonbranded prostaglandin analogues (PGAs) [13–15]. A large double-masked study of 184 patients in Italy found noninferiority of generic latanoprost to Xalatan, with no difference in adverse events [15]. Similarly, Golan and colleagues conducted a randomised crossover study comparing Xalatan and a generic latanoprost, with patients masked to the medication they were receiving [16]. There were no significant differences in IOP lowering between groups but more ocular surface disease-type side effects were reported when using the generic. The differences in study results may reflect the large number of different generics available. The FDA hosts an "Orange Book" of approved drug products with therapeutic equivalence evaluations that can be used to verify whether particular generics have been approved for use in the US [17]. A search in December 2019 revealed 9 generic versions of latanoprost 0.005%.

Diagourtas and colleagues recently compared two generic PGAs available in Greece to branded latanoprost in 60 patients who had never received antiglaucoma treatment [13]. Although the study lasted only 16 weeks and did not include a crossover phase, patients were masked to the medication they were receiving. The generic drops produced similar IOP lowering compared to Xalatan, with percentage IOP reductions from 30.34 to 32.06%. The first study comparing the effectiveness of branded and generic travoprost was published in 2019 [18]. This prospective study of 70 patients, randomised patients to either branded Travatan *Z* (Alcon) or a generic travoprost (Sandoz Inc). Intraocular pressure was measured at baseline and after 3 weeks of treatment, after which patients switched medication, with a further IOP assessment at week 6. The IOP lowering effect of generic travoprost was found to be equivalent to Travatan. A questionnaire was used to assess tolerability, and this was found to be similar between formulations; however, this study had the disadvantage of patients not being masked to the study medications.

Although previous studies have compared the effect of branded and generic medications on ocular surface disease symptoms, their primary endpoint has been IOP. It is desirable to obtain data regarding more clinically relevant endpoints such as visual function and other patient reported outcomes. The only study identified in our literature search that examined a non-IOP endpoint was that of Kim and colleagues who used US commercial medical claims data to compare the hazard of needing a second glaucoma medication or surgical intervention for glaucoma in patients using generic latanoprost or a branded PGA [18]. The study identified 6,317 patients with primary open angle glaucoma using generic latanoprost and 3,703 using branded PGAs. Use of generic latanoprost was associated with a reduced hazard of undergoing a glaucoma procedure (HR = 0.72, 95% CI 0.62–0.84) but not with needing a second glaucoma medication (HR = 0.95, 95% CI 0.87–1.03), likely due to the reduced cost of generic medications leading to improved adherence.

2.2. Costs. The major advantage of generic over branded medications is lower cost. Over the last 20 years, there has been an increase in the number of antiglaucoma medications prescribed per head of population, with an associated increase in costs; however, an increase in the use of generic medications has slowed growth in expenditure. In England, between 2000 and 2012 there was a 67% increase in prescriptions for glaucoma issued in primary care, likely driven by improved case finding and an ageing population [19]. This was associated with an 88% increase in medication

costs, from £55.2 million annually in 2000 to £103.7 million annually in 2012 [19]. Between 2009 and 2018, the number of items prescribed grew from 1,382 per 10,000 people to 1,668 per 10,000 people [7]; however, prescribing costs remained relatively stable, largely due to increased use of generics. The proportion of generic medications prescribed during this time increased from 11.7% in 2009 to 55.2% in 2018, with the contribution of generic medications towards the total cost of glaucoma prescribing increasing from 4.4% to 37% [7].

There is though large variation in generic penetration between countries, likely in large part due to differences in price regulation and payment systems. European generic medicine pricing tends to follow either a free market approach, where manufacturers are relatively free to set prices, or a price-regulated system, where prices are set by law [20]. Penetration of generic medications is more successful in countries that permit free pricing of medicine as manufacturers of originator medicines tend to charge premium prices, attracting entry of generic products, whose manufacturers have room to profit while still undercutting the cost of the branded product. In countries with price regulation, the price of the originator medicine is driven down discouraging entry of generics and restricting price competition after patent expiry. In addition, countries with price-regulated systems often link the price of generic products to a reference price related to the branded equivalent, enabling manufacturers of originator medicines to lower prices to drive generic medicines out of the market [20].

Despite lower cost being a major advantage of generic medications, the medication prices are subject to fluctuation. For example, in 2018 there was a temporary 8-fold increase in the price of generic latanoprost in the UK due to a shortage [7]. The reasons for medication shortages are complex, but the FDA recently convened a drug shortages task force to examine the problem [21]. One hundred and sixty-three drugs were identified that went into shortage between 2013 and 2017, and these were compared to similar medicines not in short supply. Shortage drugs were more likely to be low priced and financially unattractive for manufacturers, with shortages often due to disruption in the supply chain. In many cases, manufacturers had discontinued the production of medications due to loss of profitability. The task force highlighted that driving down cost to the lowest possible price disincentivises investment by manufacturers which may increase the risk of manufacturing problems or prompt them to leave the market, and shortages were compounded by logistical and regulatory hurdles being too great for other companies to increase production during a shortage [21]. Drug shortages can have severe consequences; for example, a 2011 shortage of norepinephrine in the US was significantly associated with an increase in mortality in patients with septic shock [22]. The European Medicines Agency publishes information on specific medicine shortages affecting one or more European Union member states and includes links to national shortage registers [23].

It is also important to consider the possibility that cheaper medication costs may not automatically translate to cheaper overall costs. Dubois summarised several scenarios where use of generic eye drops may not automatically lead to a cost saving [24]. For example, if the switch to a generic leads to reduced adherence due to difficulty using a new bottle design, if the patient beings running out of medication early due to the generic bottle dispensing too large volume of medication, or if a difference in inactive ingredients leads to a higher rate of ocular surface disease. The use of generic medications may also increase the risk of dispensing errors, particularly with fixed dose combination medications, where one medication could be omitted from the repeat prescription or missed due to a dispensing error.

2.3. Tolerability and Differences in Formulation. The presumption that generics are equal to branded medications because the active ingredients are the same is also not necessarily true. Regulatory authorities require generic and branded medications to contain the same active ingredient but excipients may vary. Excipients are heterogenous inert pharmaceutical ingredients used in product formulations, for example, thickening agents and buffers. In most cases, they have limited or no pharmacological activity, but they can influence drug stability and bioavailability and these differences have the potential to affect efficacy [25, 26]. Kolko and colleagues examined the physical properties of 5 generic latanoprost solutions and found substantial differences to the branded version [26]. The pH of branded latanoprost was markedly lower than the generic products, and there was significant variation in viscosity. The difference in observed pH was unexpected as the advertised label pH of generic latanoprost is typically similar to Xalatan [27].

Kahook and colleagues also examined the composition of generic and branded PGAs and using mass spectroscopy found differences in the quantity of active ingredients and excipients [28]. Although the FDA requires concentrations of active ingredients to be within 10% of the labelled value, some generic medications had concentrations exceeding this [28]. Generic medications also had higher levels of particulate matter, the origin of which was presumed to be either contaminants, precipitates of active ingredients, or material from the eye drop container. Latanoprost can degrade at high room temperature, and there may be differences in degradation between formulations. Kahook found some bottles of generic latanoprost had a significant decrease in latanoprost over time, with loss of more than 10% of active ingredient after exposure to temperature levels at the higher end of their labelled indication (25°C for 30 days), raising questions about the stability of generic formulations. Degradation of benzalkonium chloride (BAK) was also observed. As the reduction in IOP with latanoprost is dose dependent, with an optimal concentration of 0.005% or 0.006%, changes in concentration due to instability may affect efficacy and may require higher concentrations of active ingredients at baseline to counter degradation.

Narayanaswamy also found generic latanoprost to have a higher pH and increased particulate matter compared to branded Xalatan, which was proposed to be a reason for lower therapeutic efficacy observed in their study [14]. The authors concluded that caution should be exercised when

switching from branded Xalatan to a generic due to potential changes in efficacy. Velpandian and colleagues examined the concentration of latanoprost in generic medications available in India [29]. The latanoprost content varied from 90 to 330% of the labelled claim, compared to 97% for branded latanoprost, a much greater degree of variability than in the US generics studied by Kahook [28]. There were also differences in degradation of latanoprost due to UV light and heat, made to simulate patient usage.

Leitritz and colleagues examined the concentration of latanoprost and BAK in 23 generic latanoprost formulations [30]. Although the pH of the generic drugs was similar to Xalatan (median 6.78, min 6.62, and max 6.81), all products contained less than the supposed 50 ug/mL of active ingredient. In contrast, most had higher concentrations of BAK than the original drug, with a mean 5.45% greater concentration (range -2.5% to 11.5%). Hallaji et al. also examined the preservative concentration of generic glaucoma eye drops compared to respective brands [31]. Most generics and branded products were found to have the same preservative. High performance liquid chromatography was used to measure the concentration of BAK in branded Xalatan 0.02% and 4 generic versions of latanoprost and found none varied by more than 10% from the concentration found in the branded product. The main exception was a slight difference in the sodium chlorite concentration of the preservative in Alphagan P 0.15% w/v (Allergan) and the generic equivalent [31].

2.4. Adherence and Ease of Use. A potential problem of generic eye drops is the difference in bottle design between manufacturers, which may adversely adherence [32]. Many patients with glaucoma struggle to correctly instil their eye drops and this problem likely to be worsened if they receive different bottles over time. Whereas the packaging of innovator products is carefully designed and evaluated in clinical trials, aiming to identify issues with bottle design, the same is not true of generic eye drops. Several studies have shown considerable variation in the force required to squeeze different bottles and successfully release a drop [26, 33, 34]. For example, Kolko and colleagues reported a considerable difference in force was needed to expel drops from a bottle of Xalatan compared to different generic latanoprost bottles, with Xalatan requiring the least pressure [26]. This is likely to be particularly important for patients who struggle to instil drops, such as those with arthritis or reduced strength.

A study examining patient experiences of the transition from Xalatan to generic latanoprost reported patients found drops from Xalatan bottles easier to instil, more comfortable in the eye and easier to open [35]. In 20% of patients, generic bottles failed to last for a full month. This study was though unmasked and may have been influenced by patients' perceptions of a preference towards a branded or familiar product.

Differences in bottle design also contribute to differences in drop size and volume and number of drops per bottle, consequently affecting the quantity of active ingredient delivered to the eye [26, 33, 34, 36]. A study by Mammo and colleagues found differences in the volume of eye drops delivered by a branded topical beta-blocker medication compared to generic versions, while others have reported similar findings with generic and branded PGAs [26, 34].

Differences in bottle design also complicate the use of drop delivery aids. The drop delivery aid for Xalatan (Xalease) does not fit generic versions, except the Pfizer generic, and therefore there is a danger that patients switched from branded to generic drops, or those switched between generics, may be unable to use their normal delivery aid [27]. Pharmacists frequently have little control over which generic product they stock or choose the cheapest generic available. Market forces can lead to frequent changes in the generic medications stocked, and patients who have been used to using the same medication for several years may find it confusing when changes are made.

Given the importance of eye drop bottle design to adherence and therefore efficacy, in addition to the effect on stability of the active ingredient, it would seem prudent that stricter regulation of bottle design be considered. For example, if all PGAs were housed in bottles of the same shape and rigidity, it would be easier for patients to use generic products made by different manufacturers, and there would be a greater likelihood of consistent drug delivery. Bottle design is intricately linked to bioavailability and therefore should be considered by the regulatory authorities when evaluating generic medications for market entry. In 2013, the European Medicines Agency released a concept paper on the development of product-specific guidance on demonstration of bioequivalence for generic medicines and subsequently several product-specific guidelines have been produced [37]. Product-specific guidelines for antiglaucoma eye drops may be worthwhile, especially if they stipulated consistency in bottle design.

Though differences in bottle design may be a barrier to adherence, in many healthcare systems cost may be the greater barrier, with the result that switching from branded to cheaper generic medications improves adherence. A previous study in the US reported 41% of patients had difficulty paying for their glaucoma medications [38]. Stein and colleagues examined the impact of the introduction of generic latanoprost on adherence in a large US managed care network [39]. Adherence rates were examined for 18-month periods before and after the introduction of generic latanoprost. When only branded PGAs were available, a subset of patients were noted to have poor adherence, which considerably improved when they were switched to generic latanoprost. This group obtained higher levels of adherence than those who had been maintained on branded PGAs. Although it was not possible to determine the reason for improved adherence with a switch to generics, it was thought to be due to lower costs to patients.

2.5. Perceptions of Generic Medications. A number of studies examining attitudes and awareness of generic medications among healthcare professionals and members of the public have shown a lack of confidence in generic prescribing. A

review article found 21 publications examining the topic of physician perception of generic medications and 36 reports on patient opinions [40]. The authors concluded that although opinions of generic medicines have improved over the years, some mistrust remains, particularly among patients. Patients tend to prefer branded medications and many do not consider generic medicines equivalent to branded products. There is also a belief that branded products have greater potency and fewer side effects. In several studies, patients seemed to be more accepting of generics for treatment of minor illnesses but preferred branded medicines for more serious problems. Patients also reported that variability in packaging and appearance made it more difficult to keep track of their medications, and some patients were found to be taking two or more equivalent medications due to differences in packaging [41].

It is likely that many of the negative perceptions of generic medications among patients are due to insufficient knowledge and information, and therefore healthcare professionals have an important role to communicate information about the equivalence of generic formulations, which is likely to improve confidence and adherence. A short explanation has been shown to improve the likelihood that a patient will accept a generic [42], and it is the authors view that a switch to a generic should not take place without the patient being informed. Acceptance of generics appears to be higher in patients with higher levels of education, while patients from lower socioeconomic demographics tend to have greater mistrust of generics, although this has not been a universal finding [40].

In the US, ophthalmologists have a higher rate of branded medicine prescribing than any other medical specialty, suggesting greater confidence in branded drugs; however, the use of generic medicines is increasingly rapidly [43]. The large increase in use of generic antiglaucoma medications suggests ophthalmologists now accept the use of generics and have changed their prescribing habits; however, change may also be driven by prescribing or dispensing rules. Changes to treatment guidelines are also likely to have contributed; for example, in the United Kingdom, the National Institute for Health and Care Excellence (NICE) glaucoma guidelines recommend that generic medication be considered first choice treatment [44]. Clinicians undertaking a large scale shift to generic prescribing should however be aware of the potential problems with the use of generic eye drops. Branded and generic medications are not identical. and patients should be informed of potential changes to their medication. It is also important to emphasise to patients that generics are authorised off patent versions of branded medications as not all patients appreciate the difference between generic and counterfeit medicines [45].

3. Conclusion

The bioavailability of medicines administered as eye drops is influenced by more than the concentration of the active ingredient, with bottle design one of the most important factors. Stricter regulation of bottle design should be considered to improve consistency of drug delivery, perhaps through product-specific guidance on demonstration of bioequivalence. Although several randomised crossover studies have shown similar efficacy of branded and generic prostaglandin analogues, this finding has not been universal and pharmacological studies have shown differences in the composition and properties of branded and generic antiglaucoma medications.

Generic medicines are here to stay, and unless new classes of superior antiglaucoma medications become available, generics will become increasingly common as older medications come off-patent. The shift to generic prescribing has a great potential for reducing healthcare-related costs; however, it is important that the limitations of generic medications are understood and addressed.

Conflicts of Interest

The author declares that there are no conflicts of interest.

References

[1] World Health Organisation (WHO), "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. Annex 7," in *WHO Technical Report Series No. 992*, World Health Organization, Geneva, Switzerland, 2015, https://www.who.int/medicines/areas/quality_safety/quality_assurance/Annex7-TRS992.pdf?ua=1.

[2] Food and Drugs Administration, *Generic Drugs Questions and Answers*, https://www.fda.gov/drugs/questions-answers/generic-drugs-questions-answers#q1, 2019.

[3] European Medicines Agency, "Generic and hybrid medicines," 2019, https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/generic-hybrid-medicines.

[4] L. B. Cantor, "Ophthalmic generic drug approval process," *Journal of Glaucoma*, vol. 6, no. 5, pp. 344–349, 1997.

[5] A. Cook, J. P. Acton, and E. Schwartz, *How Increased Competition from Generic Drugs Has Affected Prices and Returns in the Pharmaceutical Industry*, United States Congressional Budget Office, Washington, D.C., 1998, http://lcweb.loc.gov/catdir/toc/98-179043.html.

[6] Association for Accessible Medicines, "Generic drug access and savings in the US report," 2017, https://accessiblemeds.org/resources/blog/2017-generic-drug-access-and-savings-us-report.

[7] H. D. J. Hogg and A. Connor, "10-year trends in English primary care glaucoma prescribing," *Eye*, vol. 34, no. 1, pp. 192–196, 2019.

[8] A. S. Kesselheim, A. S. Misono, J. L. Lee et al., "Clinical equivalence of generic and brand-name drugs used in cardiovascular disease," *JAMA*, vol. 300, no. 21, pp. 2514–2526, 2008.

[9] A. S. Kesselheim, M. R. Stedman, E. J. Bubrick et al., "Seizure outcomes following the use of generic versus brand-name antiepileptic drugs," *Drugs*, vol. 70, no. 5, pp. 605–621, 2010.

[10] P. Tattevin, A.-C. Cremieux, C. Rabaud, and R. Gauzit, "Efficacy and quality of antibacterial generic products approved for human use: a systematic review," *Clinical Infectious Diseases*, vol. 58, no. 4, pp. 458–469, 2014.

[11] World Health Organization (WHO), "Expert committee on specifications for pharmaceutical preparations," in *WHO*

Technical Report Series, Vol. Vol 863, World Health Organization, Geneva, Switzerland, 1996.

[12] European Medicines Agency, *Guideline on the Investigation of Bioequivalence*, https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf, 2010.

[13] A. Diagourtas, K. Kagelaris, K. Oikonomakis, A. Droulias, N. Kokolakis, and D. Papaconstantinou, "Prospective study comparing Xalatan eye drops and two similar generics as to the efficacy and safety profile," *European Journal of Ophthalmology*, vol. 28, no. 4, pp. 378–384, 2018.

[14] A. Narayanaswamy, A. Neog, M. Baskaran et al., "A randomized, crossover, open label pilot study to evaluate the efficacy and safety of Xalatan in comparison with generic Latanoprost (Latoprost) in subjects with primary open angle glaucoma or ocular hypertension," *Indian Journal of Ophthalmology*, vol. 55, no. 2, pp. 127–131, 2007.

[15] M. Digiuni, G. Manni, M Vetrugno et al., "An evaluation of therapeutic noninferiority of 0.005% latanoprost ophthalmic solution and xalatan in patients with glaucoma or ocular hypertension," *Journal of Glaucoma*, vol. 22, no. 22, pp. 707–712, 2013.

[16] S. Golan, E. Rosenfeld, G. Shemesh, and S. Kurtz, "Original and generic latanoprost for the treatment of glaucoma and ocular hypertension: are they really the same?" *Clinical and Experimental Pharmacology and Physiology*, vol. 42, no. 2, pp. 220–224, 2015.

[17] Food and Drug Administration, "Orange book: approved drug products with therapeutic equivalence evaluations," 2019, https://www.accessdata.fda.gov/scripts/cder/ob/.

[18] D. H. Kim, V. M. Addis, W. Pan, and B. L. VanderBeek, "Comparative effectiveness of generic latanoprost versus branded prostaglandin analogs for primary open angle glaucoma," *Ophthalmic Epidemiology*, vol. 26, no. 1, pp. 63–71, 2019.

[19] A. J. Connor and S. G. Fraser, "Glaucoma prescribing trends in England 2000 to 2012," *Eye*, vol. 28, no. 7, pp. 863–869, 2014.

[20] S. Simoens, "A review of generic medicine pricing in Europe," *Generics and Biosimilars Initiative Journal*, vol. 1, no. 1, pp. 8–12, 2012.

[21] Food and Drug Administration, "Statement on FDA's new report regarding root causes and potential solutions to drug shortages," 2019, https://www.fda.gov/news-events/press-announcements/statement-fdas-new-report-regarding-root-causes-and-potential-solutions-drug-shortages.

[22] E. Vail, H. B. Gershengorn, M. Hua, A. J. Walkey, G. Rubenfeld, and H. Wunsch, "Association between US norepinephrine shortage and mortality among patients with septic shock," *JAMA*, vol. 317, no. 14, pp. 1433–1442, 2017.

[23] European Medicines Agency, "Shortages catalogue," 2019, https://www.ema.europa.eu/en/human-regulatory/post-authorisation/availability-medicines/shortages-catalogue.

[24] V. D. J. P. Dubois, "Are generic topical prostanoids the way forward in the care of glaucoma patients?-No," *Eye*, vol. 27, no. 9, pp. 1002-1003, 2013.

[25] A. A. Aref, "Generic drugs for the treatment of ocular conditions: changing the treatment landscape," *Expert Review of Clinical Pharmacology*, vol. 7, no. 5, pp. 551–553, 2014.

[26] M. Kolko and P. Koch Jensen, "The physical properties of generic latanoprost ophthalmic solutions are not identical," *Acta Ophthalmologica*, vol. 95, no. 4, pp. 370–373, 2017.

[27] L. Titcomb, "Help ensure that the change to generic latanoprost is free of problems," *The Pharamceutical Journal*, vol. 288, p. 709, 2012.

[28] M. Y. Kahook, R. D. Fechtner, L. J. Katz, R. J. Noecker, and D. A. Ammar, "A comparison of active ingredients and preservatives between brand name and generic topical glaucoma medications using liquid chromatography-tandem mass spectrometry," *Current Eye Research*, vol. 37, no. 2, pp. 101–108, 2012.

[29] T. Velpandian, A. Kotnala, N. Halder, A. K. Ravi, V. Archunan, and R. Sihota, "Stability of latanoprost in generic formulations using controlled degradation and patient usage simulation studies," *Current Eye Research*, vol. 40, no. 6, pp. 561–571, 2015.

[30] M. A. Leitritz, H.-P. Lipp, B. Voykov, and F. Ziemssen, "Originalpräparat versus generika-latanoprost," *Der Ophthalmologe*, vol. 112, no. 2, pp. 127–139, 2015.

[31] N. A. E. Hallaji, P. P. N. Rao, and G. E. Trope, "Preservative content in generic and brand name glaucoma eye drops," *Canadian Journal of Ophthalmology*, vol. 51, no. 6, p. 492, 2016.

[32] S. C. Patel and G. L. Spaeth, "Compliance in patients prescribed eyedrops for glaucoma," *Ophthalmic Surgery*, vol. 26, no. 3, pp. 233–236, 1995.

[33] A. J. Connor and P. S. Severn, "Force requirements in topical medicine use-the squeezability factor," *Eye*, vol. 25, no. 4, pp. 466–469, 2011.

[34] Z. N. Mammo, J. G. Flanagan, D. F. James, and G. E. Trope, "Generic versus brand-name North American topical glaucoma drops," *Canadian Journal of Ophthalmology*, vol. 47, no. 1, pp. 55–61, 2012.

[35] S. L. Painter and A. L. Mead, "Patient experience of the transition from Xalatan to generic latanoprost," *Eye*, vol. 28, no. 7, p. 911, 2014.

[36] L. Van Santvliet and A. Ludwig, "Determinants of eye drop size," *Survey of Ophthalmology*, vol. 49, no. 2, pp. 197–213, 2004.

[37] European Medicine Agency, "Concept paper on the development of product-specific guidance on demonstration of bioequivalence," 2013, https://www.ema.europa.eu/en/documents/scientific-guideline/concept-paper-development-product-specific-guidance-demonstration-bioequivalence_en.pdf.

[38] B. Sleath, A. L. Robin, D. Covert, J. E. Byrd, G. Tudor, and B. Svarstad, "Patient-reported behavior and problems in using glaucoma medications," *Ophthalmology*, vol. 113, no. 3, pp. 431–436, 2006.

[39] J. D. Stein, N. Shekhawat, N. Talwar, and R. Balkrishnan, "Impact of the introduction of generic latanoprost on glaucoma medication adherence," *Ophthalmology*, vol. 122, no. 4, pp. 738–747, 2015.

[40] S. S. Dunne and C. P. Dunne, "What do people really think of generic medicines? A systematic review and critical appraisal of literature on stakeholder perceptions of generic drugs," *BMC Med*, vol. 13, p. 173, 2015.

[41] H. Håkonsen and E.-L. Toverud, "Special challenges for drug adherence following generic substitution in Pakistani immigrants living in Norway," *European Journal of Clinical Pharmacology*, vol. 67, no. 2, pp. 193–201, 2011.

[42] B. Roman, "Patients' attitudes towards generic substitution of oral atypical antipsychotics," *CNS Drugs*, vol. 23, no. 8, pp. 693–701, 2009.

[43] P. A. Newman-Casey, M. A. Woodward, L. M. Niziol, P. P. Lee, and L. B. De Lott, "Brand medications and medicare Part D," *Ophthalmology*, vol. 125, no. 3, pp. 332–339, 2018.
[44] National Institute for Health and Care Excellence, "Glaucoma: diagnosis and management," 2017, https://www.nice.org.uk/guidance/ng81.
[45] L. C. Titcomb, "Are generic topical prostanoids the way forward in the care of glaucoma patients?-Yes," *Eye*, vol. 27, no. 9, pp. 999–1001, 2013.

Hindawi
Journal of Ophthalmology
Volume 2020, Article ID 7345687, 5 pages
https://doi.org/10.1155/2020/7345687

Research Article

Factors Predetermining Increased Aqueous Humour Flare in Long-Term Glaucoma Treatment

G. Pakuliene, L. Kuzmiene, and I. Januleviciene

Department of Ophthalmology, Lithuanian University of Health Sciences, Kaunas, Lithuania

Correspondence should be addressed to G. Pakuliene; giedrepakuliene@gmail.com

Received 17 December 2019; Accepted 28 January 2020; Published 23 March 2020

Guest Editor: Barbara Cvenkel

Glaucoma patients often require long-term or even lifelong medical antiglaucomatous treatment. Benzalkonium chloride (BAK) is the most frequently used preservative in medical glaucoma treatment. Laser flare photometry is the noninvasive quantitative measurement of anterior chamber protein level and helps tracking intraocular inflammation. The purpose of our study was to evaluate the ocular aqueous humour flare in glaucoma patients, scheduled for cataract surgery without any other ocular diseases, and the association with pseudoexfoliation (PEX) syndrome, number of medications used, and BAK. A prospective case-control age- and gender-matched study, including open-angle glaucoma patients (>2 years of treatment) with cataract, matched with cataract patients with no other ocular pathology (control group). We found that the aqueous humour flare was higher in the glaucoma group than in the control group. PEX syndrome increased the aqueous humour flare independently from glaucoma diagnosis. The number of used antiglaucomatous medications correlated moderately with the aqueous humour flare. The BAK index showed weak positive correlation with aqueous humour flare. A variety of factors can affect aqueous humour flare increase, including PEX syndrome, medical substance used to treat glaucoma, number of different medications, and presence of BAK. The combination of these factors is of key importance to long-term glaucoma treatment.

1. Introduction

Glaucoma patients often require long-term or even lifelong medical antiglaucomatous treatment [1]. Daily administration of ocular drops interferes with ocular surface integrity and increases the risk for adverse effects [2]. Both medical substance and preservative can contribute to toxicity-related ocular adverse effects [2]. This is even more important, if the patient requires ocular surgical treatment, after the history of long-term glaucoma medical treatment [3].

Benzalkonium chloride (BAK) is the most frequently used preservative in medical glaucoma treatment [4]. The inflammatory properties of BAK are very well presented by the contribution to dry eye disease and a variety of inflammatory cytokines found on ocular surface [5, 6]. Experimental animal studies show that topical administration of BAK on the ocular surface increases the corneal permeability and can lead to BAK presence intraocularly [7, 8]. BAK acts as a detergent and emulsifier, proposing the risk of intraocular inflammation, hence found intraocularly [6, 9, 10].

Laser flare photometry is the noninvasive quantitative measurement of anterior chamber protein level [11]. The technology allows tracking intracameral protein increase and inflammation [11]. The subclinical increase in aqueous humour flare using laser flare photometry in pseudoexfoliation syndrome (PEX) patients was observed back in 1992 [12]. Later on, the developing technology allowed to identify subtle differences in aqueous humour flare increase between different glaucoma patients, different medications, or with preservative presence in medications [13–16]. However, these studies confined to only one mentioned causative factor.

The purpose of our study was to evaluate ocular aqueous humour flare in glaucoma patients, scheduled for cataract surgery without any other ocular diseases, and the association with pseudoexfoliation (PEX) syndrome, number of medications used, and BAK.

2. Materials and Methods

We conducted a prospective case-control age- and gender-matched study. The case-control ratio was 1 : 2. The Kaunas Regional Biomedical Ethics Committee approved all study procedures. All of the participants signed an informed consent form. The study adhered to the tenants of Declaration of Helsinki.

The open-angle glaucoma group (treated for >2 years) with cataract was matched to the control group of cataract patients with no other ocular pathology. Inclusion criteria: >18 years old, intraocular pressure (IOP) <21.0 mmHg, no ocular hyperaemia or medication intolerance, and no previous ocular surgery.

The methods included full ophthalmic evaluation, Goldmann applanation tonometry for IOP, and ocular aqueous humour laser flare and cell photometry (Kowa FM-700 ver. 2.01.200000, Japan). Aqueous humour flare was analysed without pupil dilation [17, 18]. Ten measurements were obtained from each eye, and marginal values were eliminated to increase accuracy. Flare count was presented as photon count per millisecond (pc/ms). We additionally analysed the groups divided by presence of PEX syndrome, number of glaucoma medications used daily, and BAK index. The BAK index was calculated by adding up the used antiglaucomatous medications' BAK concentrations once or twice, depending on the daily prescription.

We used the following formula:

$$\text{Index (BAK)} = Xx1 + Yx2 + Zx2 + Qx0. \quad (1)$$

where X, Y, Z, and Q are BAK concentrations in medications, and it is multiplied by prescription once (1) or twice (2) daily, (0) if not prescribed.

To detect the difference of 3 pc/ms between the groups, we needed at least 20 participants in each group ($\alpha = 0.05$, $\beta = 0.1$, power 90%).

All of the participants answered the Ocular Surface Disease Questionnaire (OSDI©, Allergan, Ireland) for ocular surface complaints. We also performed Schirmer's test and tear break-up time (TBUT) for objective ocular surface evaluation. Schirmer's test was performed by adding a Schirmer's paper strip in the inferior fornix. Five minutes later, the strip was inspected for the length of moisture (mm) in the paper strip. This test demonstrated basal and reflex tear secretion. TBUT was performed by adding fluorescein dye in the inferior fornix of the eye. The ocular surface was observed under slit lamp with cobalt blue light. TBUT was measured in seconds until the tear film broke.

Statistical analysis was performed with SPSS v23.0 program package. We used Student's t test for two normally distributed independent samples and Mann–Whitney U test for two nonparametric independent samples. Spearman's rank correlation coefficient was used for nonparametric ranking correlations. We considered $p > 0.05$ statistically significant.

3. Results and Discussion

The glaucoma group included 22 subjects and 44 subjects in the control group. Demographic data are presented in Table 1.

3.1. Aqueous Humour Flare. The aqueous humour flare mean (SEM) in the glaucoma group was 18.9 (2.2) pc/ms and median 17.3 pc/ms, and accordingly 10.0 (0.76) pc/ms and median was 9.2 pc/ms in the control group ($p < 0.001$, Mann–Whitney U test) (Figure 1). There was no significant correlation between IOP and aqueous humour flare ($p > 0.05$, Spearman's rho).

PEX was found in 10 glaucoma and 9 control subjects. Aqueous humour flare mean (SEM) in the glaucoma (PEX+) group ($n = 10$) was 18.7 (2.8) pc/ms and median 17.8 pc/ms, while in the control group (PEX+) ($n = 9$) it was 14.8 (2.3) pc/ms and median 13.5 pc/ms, ($p = 0.234$, Mann–Whitney U test) (Figure 2). Aqueous humour flare mean (SEM) in the glaucoma group (PEX−) ($n = 12$) was 19.0 (3.4) pc/ms and median 17.0 pc/ms, and accordingly 8.6 (0.7) pc/ms and median 7.6 pc/ms in control (PEX−) ($n = 35$) ($p < 0.001$, Mann–Whitney U test) (Figure 3).

OSDI© scores were similar among control and glaucoma groups. The mean (SEM) total OSDI© score was 19.17 (2.9) in the control group and 22.19 (2.9) in the glaucoma group ($p = 0.174$, Mann–Whitney U test). Schirmer's test value mean (SEM) was 12.95 (1.3) in the control group and 10.05 (2.0) in the glaucoma group ($p = 0.222$, Student's t test). TBUT value mean (SEM) was 8.70 (0.8) and median 7.0 in the control group and mean (SEM) 8.50 (1.1) and median 7.0 in the glaucoma group ($p = 0.784$, Mann–Whitney U test).

3.2. BAK Index and Aqueous Humour Flare. We found weak positive correlation between aqueous humour flare and BAK index (Spearman's rho = 0.390, $p = 0.001$) (Figure 4); number of medications and aqueous humour flare.

We found moderate positive correlation between aqueous humour flare and the number of different types of antiglaucomatous medications used (Spearman's rho = 0.495, $p < 0.001$) (Figure 5). The majority of our participants with glaucoma ($n = 19$) received prostaglandin treatment with either latanoprost, bimatoprost, travoprost, or tafluprost; beta-blockers (timolol) ($n = 13$), alpha agonists (brimonidine) ($n = 4$), and carbonic anhydrase inhibitors (dorzolamide or brinzolamide) ($n = 8$). Monotherapy was prescribed to 7 of participants with glaucoma, 5 of which received only the prostaglandin inhibitor, and the remaining two received timolol.

Several studies showed increased aqueous humour flare in patients with PEX syndrome independently from glaucoma diagnosis [12, 15, 19]. Older of these studies did not show the aqueous humour flare difference between non-PEX controls and non-PEX glaucoma patients; Kahloun et al. were able to identify the difference, and our study results were consistent with their findings [12, 15, 19]. Kahloun et al. excluded participants who were treated with prostaglandins due to the ability of altering blood-aqueous barrier

Table 1: Demographic data of participants in control and glaucoma groups.

Demographic data	Glaucoma	Control	p
Number of participants	22	44	—
Male/female ratio (%)	32/68	32/68	—
Age mean (SEM) (years)	72.6 (8.2)	74.7 (8.9)	>0.05 (Student's t test)
IOP mean (SEM) (mmHg)	16.4 (0.6)	15.0 (0.4)	>0.05 (Mann–Whitney U test)

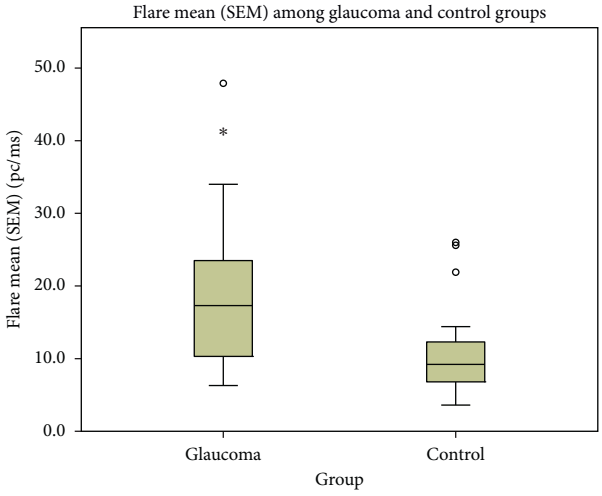


Figure 1: The graph shows aqueous humour flare mean (SEM) among glaucoma and control groups. The glaucoma group showed significantly higher aqueous humour flare than that of the control group ($p < 0.001$, Mann–Whitney U test). PEX syndrome and aqueous humour flare.

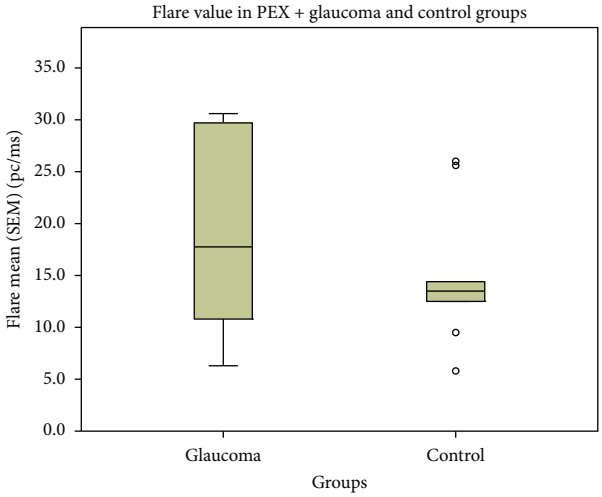


Figure 2: Flare value among (PEX–) control and glaucoma groups. The mean values did not differ significantly; however, the glaucoma (PEX–) group showed a higher aqueous humour flare tendency than the control group.

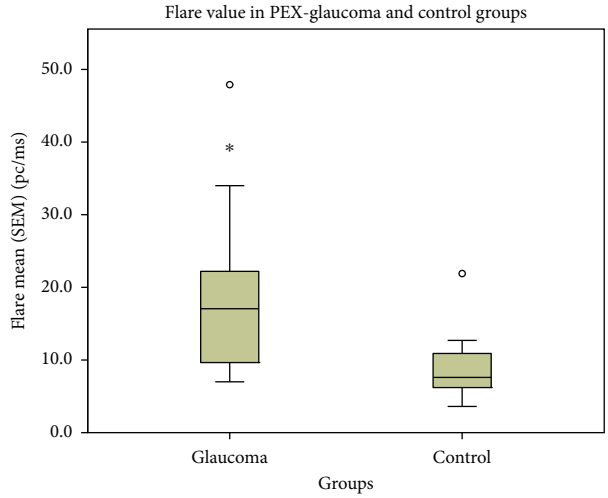


Figure 3: Flare value among PEX-control and glaucoma groups. The glaucoma group showed significantly higher aqueous humour flare mean values than the control group. Ocular surface's subjective and objective evaluation.

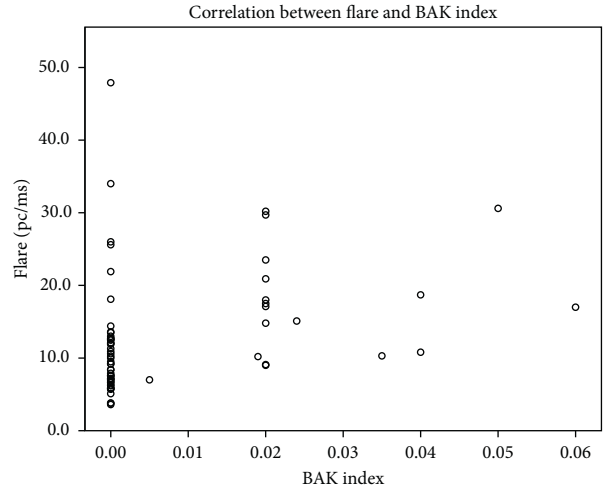


Figure 4: Correlation between flare and BAK index in the glaucoma group; a weak positive correlation (Spearman's rho = 0.390, $p = 0.001$).

[13–15, 20]. Arcieri et al. investigated the aqueous humour flare 4 weeks after prostaglandin analogues prescription but did not find significant aqueous humour flare increase [14]. We did not exclude participants with prostaglandins; however, our results did not differ much from Kahloun et al.'s findings [15]. The majority of our overall participants with glaucoma received treatment with the prostaglandin analogues. Most of the participants, who received antiglaucomatous monotherapy, received the prostaglandin analogue. We also found moderate positive correlation between the number of different antiglaucomatous medications and aqueous humour flare value. This would mean that, if prostaglandins were important in aqueous humour flare findings, the influence was not isolated.

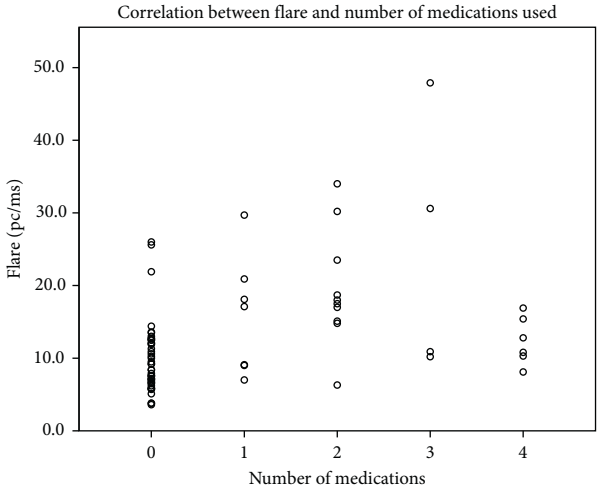


Figure 5: Correlation between flare and the number of different medications used in the glaucoma group; a moderate positive correlation (Spearman's rho = 0.495, $p < 0.001$).

We found that aqueous humour flare and BAK index had a weak positive correlation. Stevens et al. in their one-month long study observed that prescribing timolol *de novo* increased the aqueous humour flare; the BAK-preserved timolol increased the aqueous humous flare more than BAK-free timolol [16]. We also found a moderate positive correlation between aqueous humour flare and number of different medications prescribed. Our study presented long-term combined antiglaucomatous medications' relation with aqueous humour flare. It is obvious that there is no single causative factor for aqueous humour flare increase in long-term medical treatment perspective. Modification of medical substances is a difficult task; however, modifying the preservative is much more possible.

One of the advantages in our study was that we excluded patients with ocular hyperaemia and medication intolerance, which prevented significant inaccuracy in our findings. The OSDI© questionnaire, TBUT, and Schirmer's test results were similar among both groups, which allowed decreasing misinterpretation of our results due to dry eye disease. Aqueous humour flare photometry required clear media and no ocular surface inflammation for accurate flare measurement, and any ocular surface alterations could lead to false results [11].

The other advantage of our study was that participants with glaucoma had already received antiglaucomatous treatment for more than two years and were tolerating it well. This means that the participants, who received prostaglandin analogues, had already been past the transient ocular hyperaemia window [21, 22]. In contrast, Cellini et al. prescribed *de novo* treatment with prostaglandin analogues to their participants and found that after three months, the aqueous humour flare increased; however 6 months later, the flare values slightly decreased, except for the bimatoprost group [13].

As for shortcomings, due to relatively small study sample, we could not identify each antiglaucomatous medication's effect on aqueous humour flare separately, only the combined effect.

4. Conclusions

Numerous factors can affect aqueous humour flare increase, including PEX syndrome, medical substance used to treat glaucoma, number of different medications, and presence of BAK. The combination of these factors is of key importance to long-term glaucoma treatment. Further long-term studies are needed to evaluate the effect of flare increase and other causative factors impact on glaucoma treatment.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and supplementary materials.

Disclosure

This study will be partially presented at the Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting 2020, May 3–7.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

Provided Supplementary material is a table of all antiglaucomatous medications, used by patients in our study. Alongside, we provided brand names of medications and BAK concentrations in each medication, as provided in information leaflets. BAK concentrations were used to calculate the BAK index. (*Supplementary Materials*)

References

[1] P. Kalouda, C. Keskini, E. Anastasopoulos, and F. Topouzis, "Achievements and limits of current medical therapy of glaucoma," *Glaucoma Surgery*, vol. 59, pp. 1–14, 2017.

[2] H. Liang, F. Brignole-Baudouin, A. Pauly, L. Riancho, and C. Baudouin, "Polyquad-preserved travoprost/timolol, benzalkonium chloride (BAK)-preserved travoprost/timolol, and latanoprost/timolol in fixed combinations: a rabbit ocular surface study," *Advances in Therapy*, vol. 28, no. 4, pp. 311–325, 2011.

[3] C. Boimer and C. M. Birt, "Preservative exposure and surgical outcomes in glaucoma patients," *Journal of Glaucoma*, vol. 22, no. 9, pp. 730–735, 2013.

[4] D. W. Steven, P. Alaghband, and K. S. Lim, "Preservatives in glaucoma medication," *British Journal of Ophthalmology*, vol. 102, no. 11, pp. 1497–1503, 2018.

[5] L. Malvitte, T. Montange, A. Vejux et al., "Measurement of inflammatory cytokines by multicytokine assay in tears of patients with glaucoma topically treated with chronic drugs," *British Journal of Ophthalmology*, vol. 91, no. 1, pp. 29–32, 2007.

[6] A. Aguayo Bonniard, J. Y. Yeung, C. C. Chan, and C. M. Birt, "Ocular surface toxicity from glaucoma topical medications and associated preservatives such as benzalkonium chloride (BAK)," *Expert Opinion on Drug Metabolism & Toxicology*, vol. 12, no. 11, pp. 1279–1289, 2016.

[7] F. Brignole-Baudouin, N. Desbenoit, G. Hamm et al., "A new safety concern for glaucoma treatment demonstrated by mass spectrometry imaging of benzalkonium chloride distribution in the eye, an experimental study in rabbits," *PLoS One*, vol. 7, no. 11, Article ID e50180, 2012.

[8] M. T. Droy-Lefaix, L. Bueno, P. Caron, E. Belot, and O. Roche, "Ocular inflammation and corneal permeability alteration by benzalkonium chloride in rats: a protective effect of a myosin light chain kinase inhibitor," *Investigative Opthalmology & Visual Science*, vol. 54, no. 4, pp. 2705–2710, 2013.

[9] B. Brycki, I. Małecka, A. Koziróg et al., "Synthesis, structure and antimicrobial properties of novel benzalkonium chloride analogues with pyridine rings," *Molecules*, vol. 22, pp. 1–12, 2017.

[10] D. Lockington, E. C. A. Macdonald, P. Stewart, D. Young, M. Caslake, and K. Ramaesh, "Free radicals and the pH of topical glaucoma medications: a lifetime of ocular chemical injury?" *Eye*, vol. 26, no. 5, pp. 734–741, 2012.

[11] I. Tugal-Tutkun and C. P. Herbort, "Laser flare photometry: a noninvasive, objective, and quantitative method to measure intraocular inflammation," *International Ophthalmology*, vol. 30, no. 5, pp. 453–464, 2010.

[12] M. Küchle, N. X. Nguyen, F. Horn, and G. O. H. Naumann, "Quantitative assessment of aqueous flare and aqueous "cells" in pseudoexfoliation syndrome," *Acta Ophthalmologica*, vol. 70, pp. 201–208, 1992.

[13] M. Cellini, R. Caramazza, D. Bonsanto, B. Bernabini, and E. C. Campos, "Prostaglandin analogs and blood-aqueous barrier integrity: a flare cell meter study," *Ophthalmologica*, vol. 218, no. 5, pp. 312–317, 2004.

[14] E. S. Arcieri, P. T. P. Pierre Filho, T. H. Wakamatsu, and V. P. Costa, "The effects of prostaglandin analogues on the blood aqueous barrier and corneal thickness of phakic patients with primary open-angle glaucoma and ocular hypertension," *Eye*, vol. 22, no. 2, pp. 179–183, 2008.

[15] R. Kahloun, S. Attia, I. Ksiaa et al., "Anterior chamber aqueous flare, pseudoexfoliation syndrome, and glaucoma," *International Ophthalmology*, vol. 36, no. 5, pp. 671–674, 2016.

[16] A. M. Stevens, P. A. Kestelyn, D. De Bacquer, and P. G. Kestelyn, "Benzalkonium chloride induces anterior chamber inflammation in previously untreated patients with ocular hypertension as measured by flare meter: a randomized clinical trial," *Acta Ophthalmologica*, vol. 90, pp. 221–224, 2012.

[17] I. Karaca, S. Güven Yılmaz, M. Palamar, and H. Ateş, "Effect of tropicamide on laser flare meter measurements in patients with pseudoexfoliation," *Ocular Immunology and Inflammation*, vol. 0, pp. 1–5, 2019.

[18] S. M. El-Harazi, R. S. Ruiz, R. M. Feldman, A. Z. Chuang, and G. Villanueva, "Quantitative assessment of aqueous flare: the effect of age and pupillary dilation," *Ophthalmic Surgery Lasers*, vol. 33, no. 5, pp. 379–382, 2002.

[19] M. Küchle, N. X. Nguyen, E. Hannappel et al., "Tyndallometry with the laser flare cell meter and biochemical protein determination in the aqueous humor of eyes with pseudoexfoliation syndrome," *Ophthalmologe*, vol. 91, pp. 578–584, 1994.

[20] F. Selen, O. Tekeli, and Ö. Yanık, "Assessment of the anterior chamber flare and macular thickness in patients treated with topical antiglaucomatous drugs," *Journal of Ocular Pharmacology and Therapeutics*, vol. 33, no. 3, pp. 170–175, 2017.

[21] J. Chen, T. Dinh, D. F. Woodward et al., "Bimatoprost: mechanism of ocular surface hyperemia associated with topical therapy," *Cardiovasc Drug Reviews*, vol. 23, pp. 231–246, 2005.

[22] M. Yanagi, Y. Kiuchi, Y. Yuasa et al., "Association between glaucoma eye drops and hyperemia," *Japanese Journal of Ophthalmology*, vol. 60, no. 2, pp. 72–77, 2016.