AUCTORES
Globalize your Research

Experimental and Clinical Ophthalmology
Asaad A. Ghanem *

Open Access

Review Article

Biomarkers in Primary Open Angle Glaucoma, Part II: A Review

Asaad A. Ghanem [1*] Ahmed A. Ghanem [2] and Tharwat H. Mokbel [1]

[1] Mansoura Ophthalmic Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

[2] Faculty of medicine, Mansoura University, Egypt.

***Corresponding Author:** Asaad A. Ghanem, Mansoura Ophthalmic Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Received date: April 05, 2022; Accepted date: May 16, 2022; Published date: May 30, 2022

Citation: Asaad A. Ghanem, Ahmed A. Ghanem and Tharwat H. Mokbel (2022). Biomarkers in Primary Open Angle Glaucoma, Part II: A Review. *J. Experimental and Clinical Ophthalmology*. 2(1). DOI: 10.31579/eco.2022/007

Abstract

Primary open angle glaucoma (POAG) described as a multifactorial neurodegenerative optic neuropathy that is chronic and progressive. It manifests by cupping and atrophy of the optic disc, associated with deterioration of visual field in absence of other known causes of glaucomatous disease.

Glaucoma, is the second most common cause of blindness. Since early diagnosis facilitates timely treatment, it is therefore essential to identify appropriate markers.

In POAG, biomarkers could potentially aid in earlier diagnosis, grading, and/or progression risk. Biomarkers will offer a new clinical tool useful in POAG diagnosis, in prediction of disease prognosis, and in monitoring clinical responses to standard treatment.

In the future, so-called biomarkers could be helpful in early detection and follow-up. In glaucoma, these parameters could be obtained in the aqueous humor, and blood.

This review provides insight into possible changes in the aqueous humor regarding the non-genetic biochemical molecular markers of patients with primary open-angle glaucoma (POAG).

Keywords: aqueous humor; biomarker; intraocular pressure; primary open angle glaucoma; trabecular meshwork

Running title: *Biomarkers in primary open angle glaucoma*

Introduction

As part of neurodegenerative disease, glaucoma is defined by changes in the optic nerve and visual field defects, which is characterized by retinal ganglion cells (RGCs) loss. In primary open-angle glaucoma (POAG), intraocular pressure (IOP) is the most crucial risk factor, whereas in normal-tension glaucoma (NTG), both intraocular pressure and hemodynamic factors are considered important. The pathophysiology of neurodegenerative changes in glaucoma is not fully understood [1].

Glaucoma is one of the main causes of irreversible visual field loss and blindness worldwide. The incidence of glaucoma is one in 200 people aged 50 or younger, and one in 10 people aged 80 or older [2].

POAG is a complex heterogeneous disease and by the year 2020 is predicted to affect more than 50 million people worldwide [3]. It is the most common form of glaucoma, with reported prevalence rates ranging from 1.1% [4] to 3.8% [5].

In POAG, the levels of certain proteins, including interleukins and endothelin-1, were elevated. The vasoconstrictor endothelin-1 may play a role in regulating intraocular pressure. By contrast, proteins playing a role in the response to oxidative stress were downregulated. Levels of various cytokines have been found to correlate with the extent of IOP or glaucomatous disc damage in several independent studies [6].

TGFβ1, MMP-2, TIMP-2 levels in aqueous humor have been found to be elevated in PXG eyes as compared to POAG. Yet, the molecular mechanisms regulating clinical findings and those that cause transition from one phenotype to the other remain unclear. In addition, most studies demonstrate the complex changes in aqueous humor in glaucoma [6].

Biomarkers are biological markers defined as an objective and quantifiable characteristic of any biological processes. The National Institutes of Health Biomarkers Definitions Working Group defines a biomarker as “a characteristic that is objectively measured and evaluated

as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [7].

A biochemical marker is a biochemical variable associated with a disorder (directly or indirectly) and might be any biochemical compound (antigen, antibody, enzyme, hormone) that is sufficiently altered to provide diagnostic value. They may be present due to their causative role in glaucoma or as a result of the disease process [7].

This review provides insight into possible changes in the aqueous humor, blood (serum or plasma), tissues, alone or in combination with each other regarding the non-genetic biochemical molecular markers of patients with primary open-angle glaucoma (POAG).

Methods:

A literature conducted a detailed review using various electronic databases, including PubMed, Science Direct, Google scholar, Scopus, Web of science and Journals of Ophthalmology. For the PubMed search, Medical Subject Headings (MeSH) were used. The principal term used to dictate the MeSH search was "POAG biomarkers". It was connected to the following terms: glaucoma, intraocular pressure, trabecular meshwork, aqueous humor, biomarkers. Multiple clinical studies were identified and reviewed. Sources from these studies were identified, reviewed.

Biomarkers in POAG

Myocilin

Myocilin is a secreted glycoprotein with a molecular weight of approximately 55-57kDa which forms dimers and multimers. In the eye myocilin is highly expressed in the trabecular meshwork (TM), the sclera, the ciliary body, and the iris. In the chamber angle, myocilin is associated with fibrillar components of the extracellular matrix in the cribriform portion of the TM [8].

Mutations myocilin (MYOC), optineurin (OPTN), and TANK binding kinase 1 (TBK1), may cause primary open-angle glaucoma (POAG) that is inherited as a Mendelian trait [9]. Prevalence of Myocilin mutations in juvenile onset open angle glaucoma (JOAG) is higher among familial forms of JOAG and hence they need to be screened for Myocilin. Myocilin associated with JOAG [10].

Pathogenic MYOC variants appear to be population-associated in open-angle glaucoma. Many of the probable pathogenic variants are over-represented in some of the populations causing doubt of their status as monogenic disease-causing variants [11].

Myocilin, a glaucoma-associated protein with unknown function, has been found elevated in some glaucomatous ocular tissues. TM from high tension glaucoma (HTG), normal tension glaucoma (NTG), and exfoliative glaucoma (ExG) patients were shown to have increased myocilin immuno-reactivity when compared with normal tissue [12].

Increased extracellular matrix (ECM) deposition and endoplasmic reticulum stress induction was observed in the TM of mutant myocilin transduced quadrants. These findings suggest that the ex-vivo cultured human corneoscleral segment model is cost-effective and can be used as a pre-screening tool to study the effects of glaucoma factors and anti-glaucoma therapeutics on the TM [13].

Ghanem et al. [14] revealed a statistically significant higher myocilin level in HTG patients compared to control patients. Also, the study identified myocilin as a protein elevated in 37% of HTG aqueous humor samples in concentration per volume and as a percent of the total aqueous humor protein. These findings are supported by Jacobson et al [15], who found a trend toward elevated myocilin levels in human glaucomatous aqueous humor. Also, Konz et al [16], reported structural changes in the juxtacanalicular tissue including an increase in myocilin in the extracellular pathways of aqueous humor in human OAG eyes.

Tamm [17] considered that mutations in the MYOC gene that encodes for myocilin are causative for some forms of juvenile and adult-onset OAG. In the TM, myocilin is found within the cytoplasm of TM cells and in the juxtacanalicular region in association with fibrillar extracellular matrix components.

Ghanem et al. [14] concluded that the percentage of senile cataract patients who were positive for myocilin in the aqueous humor was as low as 2.3%. On the other hand, the percentage of glaucoma patients who were positive for myocilin was as high as 17.7%. This finding, revealed that myocilin concentration in the aqueous humor of normal eyes is below the threshold level of detection for the commercially available kits.

Both myocilin protein and MYOC gene has been reported in some form in human [18]. Possible functions for the myocilin protein include: modifying the expression of other protein products [19], misfolding of the mutant myocilin in the endoplasmic reticulum (ER) can lead to ER cytotoxicity and mechanical blockage of the angle [20].

Ghanem et al. [14] reported non-significant relationship between the IOP and the aqueous humor myocilin concentration. Although the average IOP in the HTG group was the highest observed (19.5 ± 2.2 mmHg), the correlation with the myocilin concentration was not statistically significant. Also, they reported non-significant correlation between mean deviation of the visual field and aqueous humor myocilin levels among the various subtypes of glaucoma.

The expression of human intraocular myocilin is considered to be the result from the secretion of ocular resident cells including TM, vitreous humor, lamina cribrosa, ciliary body, ciliary epithelium, stromal and smooth muscles of the iris, corneal endothelium and stroma, corneoscleral meshwork, surface of the rods and cones, neurons of the inner and outer nuclear layer, and optic nerve ganglion cells [21,22].

Topical therapy (beta blocker, prostaglandin analog, carbonic anhydrase inhibitor, and alpha agonist) was not associated with myoclin levels in aqueous humor. There was no association found between the myocilin levels in the aqueous humor and number of topical anti-glaucomatous drugs (r=0.546; F=0.121; r=0.452, F=0.152, respectively, One-way ANOVA) Ghanem et al. [14].

Ghanem et al. [14] revealed that aqueous humor myocilin levels were statistically significant in the glaucoma subgroups than in the cataract group, with a highly statistically significant difference observed for HTG patients among glaucoma subtypes (ExG and NTG) when compared with control patients. The elevation of intraocular expression of myocilin may be responsible for nerve damage. This finding, suggest targeting myocilin as a mean to inhibit the neurodegenerative mechanisms associated with glaucoma.

Ghrelin

Ghrelin is the endogenous ligand for the human G protein-coupled growth hormone secretagogue receptor-type 1a (GHS-R1a). It is a 28-amino-acid peptide hormone described in the rat's stomach oxyntic mucosa in 1999 by Kojima et al. [23]. Production of ghrelin has also been identified, in smaller amounts, in every tissue studied, including for example, the brain, [23] pituitary gland, small intestine, adrenal gland, kidney, liver, myocardium, and eye [24,25].

The acylated form of the ghrelin is a relatively unstable molecule and exerts mostly neuroendocrine effects after binding to the GHS-R1a [23]. The des-acylated form (des-acyl-ghrelin) of the peptide constitutes more

than 90% of the total circulating ghrelin, [5] does not bind to the GHS-R1a and exhibits important peripheral metabolic and cardiovascular effects [26,27].

Ghrelin is a peptide that exerts both endocrine and paracrine effects, as it is involved in the regulation of metabolic balance and energy homeostasis, cardiovascular function [26]. Ghrelin expression in vascular endothelial cells and its effects on vascular smooth muscle cells, including an endothelium-independent vasodilatory effect of similar potency and efficacy between both the acylated and des-acylated forms of ghrelin [25].

Ghrelin has emerged as the first identified circulating hormone. Ghrelin is also the only known circulating appetite enhancing hormone. Ghrelin plays a significant role in neurotrophy, particularly in the hippocampus, and is essential for cognitive adaptation to changing environments and the process of learning [28,29].

Study have shown that circulating ghrelin can pass the blood-brain barrier in a complex and highly regulated process [30]. In the eye, the role of the blood–aqueous barrier and the extent to which circulating ghrelin can affect its aqueous levels is not known.

Ghrelin's mRNA observed in the iris posterior segment and in the non-pigmented segment has been reported in glaucoma, an optic nerve damage which presents as the main cause an increase in IOP [31].

A statistically insignificant aqueous humor total ghrelin levels were lower than those of the control subjects but not parallel with the acylated ghrelin levels in glaucoma patients. The relative increase in the ghrelin/acylated ghrelin ratio in glaucoma cases supports the view that proportional increases of acylated ghrelin might play a role in the pathogenesis of glaucoma [32].

A statistically significant decrease in the ghrelin aqueous humor level in POAG patients compared with control patients was reported with Katsanos et al.[33] Ghanem et al. [34], found that plasma ghrelin level in POAG patients were lower 586.51±134.45 pg/ml than in the control group (621.36±123.61pg/ml) but non-statistically significant when compared with the controls (p=0.456). Katsanos et al. [33], found that plasma levels of ghrelin were higher 490.5 ±156.0 pg/ml, in the open angle glaucoma than in the control group (482.2 ±125.4 pg/ml) but non-statistically significant (Mann-Whitney test, p=0.897).

Ghanem et al. [34], revealed non-significant differences in aqueous levels of total ghrelin in patients with POAG compared with controls. These findings may indicate a role of ghrelin on the tissues that are anatomically and functionally related to the aqueous production and/or circulation. However, all glaucoma patients enrolled in this study were under topical treatment without glaucoma therapy drugs. Another potential explanation of our findings is that glaucoma medication causes the concentration of ghrelin to be lower. A third potential explanation for our findings may be related to the higher IOP levels that glaucoma patients generally have.

Ghanem et al. [34], reported a significant difference in aqueous levels of total ghrelin in POAG patients compared with controls. Others studies, found the same results on other ethnics. This revealed that there is no potential genetic difference between our study and other studies.

Ghanem et al. [34], found that there was non-significant correlation between either ghrelin levels with the POAG visual field loss at any stage which may indicate absence of potential secondary consequences such as ischemia, hypoxia, or reactive oxygen species caused by glaucomatous damage. Also, they reported a significant correlation between the aqueous humor and plasma levels of ghrelin results suggested that ghrelin in aqueous humor was related to breakdown of blood-retinal barrier.

Hepcidin Prohormone

The Hepcidin prohormone (Hep) is a small peptide produced in the liver. Human Hep is produced from an 84 amino acid precursor, including a putative single peptide. Hep is an important peptide hormone that plays a critical role in the regulation of iron efflux from numerous cell types, including intestinal cells, macrophages, and hepatocytes [36].

A study found that Hep is expressed in Müller cells, photoreceptor cells, and retinal pigmented epithelium. The expression of Hep in the retina points to the local intraocular regulation of iron metabolism, separate from a dependence on the circulating liver-derived hormone: circulating Hep would likely be inaccessible to intraocular tissues due to the presenceof blood-ocular barriers[37].

Transforming growth factors (TGF-β2) and hepcidin form a self-sustained feed-forward loop through iron-catalyzed reactive oxygen species (ROS). This loop is partially disrupted by a hepcidin antagonist and an anti-oxidant, implicating iron and ROS in TGF-β2-mediated POAG [38].

Hep is a small peptide produced in the liver. Human Hep is produced from an 84 amino acid precursor, including a putative single peptide. Hep binds to and induces the degradation of ferroportin, an iron exporter expressed in specific cell types, as the receptor for this iorn-regulatory hormone [39].

Hep is responsible for iron homeostasis, decreasing iron uptake from the intestine, and release from the liver in conditions of iron overload. Conversely, Hep syntheses are decreased in iron deficiency, resulting in increased iron uptake from the intestine and release from liver stores [40].

A study found that Hep is expressed in Muller cells, photoreceptor cells, and retinal pigmented epithelium in an expression pattern similar to that of ferroportin, suggesting that this transporter may play a role in the maintenance of iron homeostasis within the retina. The increase in Hep expression correlates with a decrease in ferroportin expression, as well as an increase in oxidative stress and apoptosis [41].

Iron metabolism in the eye include the regulation of glutamate production and secretion, glutathione synthesis, and the activity of hypoxia inducible factor-1 (HIF-1). HIF-1 has been shown to have either a clinically or experimentally mediating or contributing role in several oxygen-dependent retinal diseases such as glaucoma.[42-45] Gnana Parksam et al. [46] found that Hep is expressed in Müller cells, photoreceptors, and RPE in an expression pattern similar to that of ferroportins.

Ghanem et al. [47], found that concentration of Hep in the aqueous humor of the POAG group was statistically significantly lower than that of the control group (25.28±23.06 ng/m1 Vs 89.21±14.25 ng/m1). Sorkhabi et al. [48], revealed that the mean aqueous humor Hep concentration in eyes with POAG was significantly higher than those controls (34.55±23.01 ng/ml versus 20.82± 24.63 ng/ml).

Ghanem et al. [47] and Sorkhabi et al [48], found that plasma Hep level in POAG patients were higher but non-statistically significant when compared with the controls. These findings may indicate a role of Hep on the tissues that are anatomically and functionally related to the glaucomatous optic neuropathy, which consequently, induce iron metabolism de-regulation and additive deterioration. Another potential explanation is that glaucoma medication causes the concentration of Hep

to be lower. A third potential explanation may be secondary to a cascade of the higher IOP levels that glaucoma patients generally have.

Ghanem et al. [47], revealed a statistically significant decrease levels of Hep in aqueous humor associated with non-significant serum changes. It may be explained that the Hep levels reflect increased protein turnover, which might play a role in the pathophysiology of POAG or result from the disease process. Also, they reported a non-significant correlation between Hep levels with the POAG visual field loss at any stage may indicate absence of potential secondary consequences such as ischemia, hypoxia, or reactive oxygen species caused by glaucomatous damage.

There was significant correlation between the aqueous humor and plasma levels of Hep results suggested that levels Hep in aqueous humor were related to breakdown of blood-retinal barrier and/ or ocular blood. This correlation supported that Hep may be consequence with glaucomatous damage Ghanem et al. [47].

Transforming growth factors

Transforming growth factors (TGFs) constitute a family of multifunctional polypeptides of approximately 25 kDa, and exhibit pleiotropic regulatory actions upon most vertebral cell types [49,50]. Depending on the cell type, they regulate proliferation, migration, differentiation, cytokine production, synthesis of extracellular matrix, wound healing, immunosuppression, and *in vivo* angiogenesis [51].

TGF-β exists in at least five genetically distinct isoforms, β_1-β_5 [52]. Among these, only three isoforms, namely β_1, β_2, and β_3 are expressed in human ocular tissues [52]. TGF-β_2 is regarded as the major isoform in the eye [49,51]. Elevated levels of TGF-β_2 have been detected in the AH of glaucomatous eyes [53]. A distinct structural change in the TM of patients with POAG is the increase in fibrillar extracellular matrix in the juxtacanalicular region of the TM.

Suberoylanilide hydroxamic acid (SAHA) prevents TGF-β2-induced increases in outflow resistance and regulates the non-Smad pathway of TGF-β signaling in TM and Schlemm's canal cells [54]. The activation and proliferation of human Tenon's fibroblasts play a vital role in the fibrosis in the pathology of the scar formation after the glaucoma filtration surgery. Transforming growth factor β1 (TGFβ1)/Smads signaling has been reported to promote fibrosis [55].

TGFβ1, MMP-9 and fibronectin protein expression were upregulated in tears, tenon's capsule and aqueous humor samples in PXG compared to PXF, though the MMP-9 protein activity was downregulated in PXG compared with control or PXF [56].

TGF-β_2 signaling may be involved, as TGF-β_2 is significantly increased in the AH of patients with POAG. In cultured human TM cells, TGF-β_2 causes an increase in extracellular matrix deposition [57]. The concentration of TGF-β_2 has been previously measured in several studies [58]. The results have shown that the levels of total TGF-β_2 in the aqueous samples of POAG are elevated and have suggested that minimizing TGF-β_2 levels may help to prevent the ageing process in the TM as seen in POAG [58].

Ghanem et al. [59], revealed a significant increase in aqueous humor levels of total TGF-B2 and mature TGF-B2 in POAG eyes compared to the corresponding value of eyes with senile cataract and PACG (P, 0.001). Also, they reported an increased expression of TGF-B2 in POAG patients.

Matrix Metalloproteinases

Intraocular matrix metalloproteinase (MMP) activity and its relationship to the aqueous humor outflow pathway have been quantified in normal and glaucomatous canine eyes [60,61], and elevated levels of intraocular MMP-2 and MMP-9 have been documented with glaucoma [60].

Immunohistochemistry has been used to identify MMP-2 and MMP-9 in normal and glaucomatous eyes and specifically localized withintrabecular meshwork cells, the extracellular matrix, and inflammatory cells [61].

The *MMP9* gene polymorphisms are associated with POAG and intraocular pressure in POAG patients; rs1799750 of *MMP1* was associated with the earlier age of manifestation of the disease symptoms [62]. The aqueous humor of POAG and PEXG patients contained several miRNAs that have been linked to tissue development, neurological disease and cellular organization [63].

In addition, PEXS initiation is tightly connected with the dysregulation of ECM homeostasis since aberrant expression of ECM molecules is linked to both the accumulation and low degradation of pseudoexfoliation material [64].

Regulation of the extracellular matrix is carried out by proteolytic enzymes - metalloproteinases in particular - as well as specific inhibitors of their activity (tissue metalloproteinases inhibitors). Pathogenesis of various types of glaucoma, decrease in stability of the intraocular liquid outflow, proves the role of MMR in the ganglionic apoptosis, remodeling of the optic disk and change of lamina cribrosa in primary open-angle glaucoma [65].

Ghanem et al. [66], reported that a statistically significant increases in aqueous humor levels of TIMP-2 in XFG patients compared to the corresponding values of POAG patients or controls. Also, The MMP-2 aqueous humor level was significantly increased in the XFG patients when compared with controls ($P < 0.001$). In addition, the total protein level in the aqueous humor of eyes of the XFG patients was significantly higher than in POAG patients or controls ($P < 0.001$). A positive correlation was found between MMP-2 in aqueous humor samples of XFG patients ($P < 0.001$).

Autoantibodies

By enzyme-linked immunosorbent assay (ELISA), Tezel and coworkers [67] compared the serum immunoreactivity to glycosaminoglycans, and by immunohistochemistry they compared the distribution patterns of glycosaminoglycans in the optic nerve head of POAG eyes versus controls. Authors found that these autoantibodies may increase the susceptibility of the optic nerve head to damage in these patients by changing the functional properties of the lamina cribrosa, its vasculature, or both [67].

The glutathione S-transferase (GST) supergene family, which encodes detoxification enzymes, is widely expressed in mammalian tissue cytosols and membranes. GST is present in glial and neuronal cells of the central nervous system and in the retina [68]. Increased titers of autoantibodies to GST in some patients with POAG may represent a generalized response to tissue stress and/or damage as a consequence of the glaucomatous neurodegeneration process and thereby secondary production of serum antibodies to GST in the glaucomatous retina [68].

Approximately 20% of POAG patients possess a serum antibody against neuron specific enolase (NSE), and the maximum IOP levels in POAG patients with anti-NSE antibody are statistically lower than those without the antibody [69]. It has been suggested that the anti-NSE antibody can reach the retina through circulation and cause retinal ganglion cell damage and progression of visual field loss in addition to elevated IOP [70], and that the presence of serum autoantibody against NSE may be clinically

useful for predicting the progression of visual field loss in POAG patients [71].

Antibodies and proteins associated with glaucoma that can be detected for example by microarray and mass spectrometric analyzes, which (i) provide information about expression profiles and associated molecular signaling pathways, (ii) can possibly be used as a diagnostic tool in future and, (iii) can identify possible targets for therapeutic approaches [72].

Epitope analyses identified the third extracellular loop of the β_2-AR as the target of the inhibitory β_2-AAbs, being of IgG3 subtype in PEXS patients. In contrast, patients with PEXG showed β_2-agAAbs (5.6 ± 0.9 U), but no inhibitory ones. The β_2-agAAbs levels of patients with PEXG and primary OAG patients (3.9 ± 2.8 U; $p > 0.05$) were at a similar level [73].

Serum titers of antibodies against heat shock proteins [74], and the entire IgG autoantibody patterns against different retina, optic nerve, and optic nerve head antigens in sera have been investigated [75]. Another study [76] was carried out to investigate the levels of anti-Helicobacter pylori IgG antibodies in the AH and serum of POAG patients. A significant increase of Helicobacter pylori IgG antibody levels was demonstrated, suggesting that the titer of anti-Helicobacter pylori IgG antibody in the AH might reflect the severity of glaucomatous damage in POAG patients.

Yuki et al. [77], analyzed the serum of POAG patients for Chlamydia pneumoniae and Chlamydia trachomatis immunoglobulin G antibody titers by ELISA, and found significantly higher immunoglobulin G titers for Chlamydia pneumoniae in POAG patients than in controls. This may indicate either a common factor that causes susceptibilities to both glaucoma and Chlamydia pneumoniae infection, or that Chlamydia pneumoniae may be a causal factor for developing POAG [77].

Brain-derived neurotrophic factor

The brain-derived neurotrophic factor (BDNF) is important for RGC survival. It also maintains the function of RGCs and protects them from apoptosis. The eyes are similar to the brain regarding the anatomy and function. The blood-ocular barrier surrounds the eyes and shares features with the blood–brain barrier. The aqueous humor fills the anterior chamber and contains anti-inflammatory and other factors similar to those in cerebrospinal fluid [78].

Brain-derived neurotrophic factor is one of the polypeptide growth factors known to be vital components for building up and preserving of neurons. BDNF is transported to the retinal ganglion cell bodies through a retrograde axonal transportation system and the synaptic connections within [79].

BDNF crosses the blood–brain barrier and as a result, the level of this factor in the blood can relatively reflect its concentration in the brain. BDNF levels in serum were determined in POAG patients and controls by ELISA [79]. The authors concluded that BDNF in the serum might be a useful biochemical marker for early detection of POAG.

BDNF levels in serum and AH were markedly lower in the glaucoma groups (POAG and NTG) than in the control group. When comparing the NTG and POAG groups, the average serum BDNF level was significantly lower in the NTG group than in the POAG group. The difference in the mean BDNF levels in AH between the POAG and NTG groups was not statistically significant. Thus, serum BDNF levels were lower in patients with NTG than in those with POAG. BDNF could be a causative systemic biomarker in NTG [1].

Cystatin C

Cystatin C is a low molecular weight protein that is a member of the cystatin superfamily of cysteine protease inhibitors. It is filtered by the kidney and then metabolized by the tubules so that it cannot be collected in the urine and does not reappear in the blood [80].

It is produced by all nucleated cells in the body. Its production rate is not affected by the subject's diet, but its levels are affected by either hyper-or hypothyroidism and they fluctuate with other markers of inflammation such as C-reactive protein [80].

Cystatins, and in particular cystatin C, have been shown to be involved in many biological events and have not always been related to protease inhibition; examples include a neural stem cell factor, osteoclast differentiation, pathophysiological process in brain ischemia as well as in atherosclerotic plaque development [81].

A significant increase in protein levels of POAG (p =.0009); interestingly, a similar increase in PACG compared to cataract (p <.0001) and POAG (p =.02). Proteomics analysis identified 184, 190, and 299 proteins in control, POAG and PACG. OPN was increased in POAG and PACG compared to control. The precursor form of cathepsin D was increased in POAG and decreased in PACG, though not significant compared to control. Cystatin C was also increased in both POAG and PACG compared to control [82].

Duan and coworkers [83], reported a significant increase of cystatin C was observed in the AH of POAG patients. The increase was similar to the changes in cerebrospinal fluid of Alzheimer's disease, suggesting that POAG shares similar mechanisms with Alzheimer's disease.

Conclusion

Primary open-angle glaucoma is a neurodegenerative disease, *which is* characterized by retinal ganglion cells loss. In primary open-angle glaucoma, intraocular pressure is the most crucial risk factor, whereas in normal-tension glaucoma, both intraocular pressure and hemodynamic factors are considered important. Thus**, the pathophysiology of neurodegenerative changes in glaucoma sho**uld be fully understood

- A biomarker is a characteristic that is specifically with adequate accuracy and precision measured as an indicator of normal biological or pathogenic processes, or to monitor pharmacologic responses to a therapeutic intervention. Also, biomarkers can be useful tools to identify patients at risk and to measure the outcomes of therapies.
- Changes in aqueous humor, blood (serum or plasma), tissues, alone or in combination with each other regarding the non-genetic biochemical molecular markers of patients with primary open-angle glaucoma could be crucial for the early diagnosis
- The analytical value of the molecular tests for the detection of the discussed biomarkers will be guaranteed by the validation of relevant methods.

Declarations

Conflict of interest

All authors have no conflicts of interest that are directly relevant to the content of this review.

Funding: No sources of funding were used to conduct this review.

Reviewer disclosures: No relevant financial or other relationships to disclose.

Declaration of interest: No financial affiliations or financial involvement with any organization or entity with a financial competing with the subject matter or materials discussed in the review.

The authors have no propriety interest.

Consent for publication

Not applicable

Availability of data

All data generated during this review are included in this study

Standards of reporting

CONSORT guidelines were followed

Author's contributions: Authors interpreted and discussed the data, and wrote the first version of the manuscript. All authors read and approved the final manuscript.

Acknowledgement

The authors thank Taha Baker for his care and diligence during writing the paper.

Abbreviations: POAG: primary open angle glaucoma; IOP: intraocular pressure; TM: trabecular meshwork; AH: aqueous humor; ELISA: enzyme-linked immunosorbent assay; ECM: extracellular matrix

References

1. Cha YW, Kim ST. (2021). Serum and aqueous humor levels of brain-derived neurotrophic factor in patients with primary open-angle glaucoma and normal-tension glaucoma. Int Ophthalmol. 2021 Nov; 41(11):3869-3875.
2. Thomassen TL, Perkins ES, Dobree JH. (1950). Aqueous veins in glaucomatous eyes. Br J Ophthalmol 1950; 34:221-227.
3. Quigley HA, Broman AT. (2006). The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90:262-267.
4. Dielemans I, Vingerling JR, Wolfs RC, Hofman A, Grobbee DE, de Jong PT. (1994). The prevalence of primary open-angle glaucoma in a population-based study in The Netherlands. The Rotterdam Study. Ophthalmology 1994; 101:1851-1855.
5. Topouzis F, Wilson MR, Harris A, Anastasopoulos E, Yu F, Mavroudis L, et al. (2007). Prevalence of open-angle glaucoma in Greece: the Thessaloniki Eye Study. Am J Ophthalmol 2007; 144:511-519.
6. Eraslan N, Elgin U, Şen E, Kilic A, Yilmazbas P. (2018). Comparison of total/active ghrelin levels in primary open angle glaucoma, pseudoexfoliation glaucoma and pseudoexfoliation syndrome. Int J Ophthalmol. 2018 May 18; 11(5):823-827.
7. Grierson I, Calthorpe CM. (1988). Characteristics of meshwork cells and age changes in the outflow system of the eye: Their relevance to primary open-angle glaucoma. In: Mills KB, ed. Glaucoma. New York: Pergamon; 1988:12-31.
8. Ohlmann A, Tamm ER. (2002). The role of myocilin in the pathogenesis of primary open-angle glaucoma. Ophthalmologe. 2002; 99:672-677.
9. Sears NC, Boese EA, Miller MA, Fingert JH. (2019). Mendelian genes in primary open angle glaucoma. Exp Eye Res. 2019 Sep; 186:107702.
10. Gupta V, Somarajan BI, Gupta S, Walia GK, Singh A, Sofi R, Chaudhary RS, Sharma A. (2021). The mutational spectrum of Myocilin gene among familial versus sporadic cases of Juvenile onset open angle glaucoma. Eye (Lond). 2021 Feb; 35(2):400-408.
11. Liuska PJ, Harju M, Kivelä TT, Turunen JA. (2021).Prevalence of MYOC risk variants for glaucoma in different populations. Acta Ophthalmol. 2021 Nov; 99(7):e1090-e1097.
12. Lutjen-Drecoll E, May CA, Polansky JR, et al. (1998). Localization of the stress proteins alpha B-crystallin and trabecular meshwork inducible glucocorticoid response protein in normal and glaucomatous trabecular meshwork. Invest Ophthalmol Vis Sci. 1998; 39:517–525.
13. Kasetti RB, Patel PD, Maddineni P, Zode GS. (2020). Ex-vivo cultured human corneoscleral segment model to study the effects of glaucoma factors on trabecular meshwork. PLoS One. 2020 Jun 24; 15(6):e0232111.
14. Ghanem A A, El-Awady H E, Wolfs R C W, Arafa L F. (2012). Myocilin levels in the Aqueous Humor of Open-Angle Glaucoma Patients. Interventional Medicine & Applied Science, 2012; 4(1):42–47.
15. Jacobson N, Andrews M, Shepard AR, et al. (2001). Non-secretion of mutant proteins of the glaucoma gene myocilin in cultured trabecular meshwork cells and in aqueous humor. *Hum Mol Genet*. 2001; 10:117–125.
16. Konz DD, Flugel-Koch C, Ohlmann A, et al. (2009). Myocilin in the trabecular meshwork of eyes with primary open-angle glaucoma. Graefes Arch Clin Exp Ophthalmol. 2009; 247:1643-1649.
17. Tamm ER. (2002). Myocilin and glaucoma: facts and ideas. Prog Retin Eye Res. 2002; 21:395-428.
18. Adam MF, Belmouden A, Binisti P, et al. (1997). Recurrent mutations in a single exon encoding the evolutionarily conserved olfactomedin-homology domain of TIGR in familial open-angle glaucoma. Hum Mol Genet. 1997; 6:2091-2097.
19. Borras T, Morozova TV, Heinsohn SL, et al. (2003). Transcription profiling in Drosophila eyes that overexpress the human glaucoma-associated trabecular meshwork-inducible glucocorticoid response protein/myocilin (TIGR/MYOC). *Genetics.* 2003; 163: 637–645.
20. Joe MK, Sohn S, Hur W, et al. (2003). Accumulation of mutant myocilins in ER leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. *Biochemical and Biophysical Research Communications*. 2003; 312: 592–600.
21. Karali A, Russell P, Stefani FH, et al. (2000). Localization of myocilin/trabecular meshwork – inducible glucocorticoid response protein in the human eye. Inves Ophthalmol Vis Sci. 2000; 41:729-740.
22. Aung T, Okada K, Poinoosawmy D, et al. (2000). The phenotype of normal tension glaucoma patients with and without OPA1 polymorphisms. Br J Opththalmol. 2003; 87:149–152.
23. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402:656–660.
24. Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB, Korbonits M. (2002). The tissue distribution of the mRNAof ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002; 87:2988.
25. Rocha-Sousa A, Saraiva J, Henriques-Coelho T, Falcao-Reis F, Correia-Pinto J, Leite-Moreira AF. (2006). Ghrelin as a novel locally produced relaxing peptide of the iris sphincter and dilator muscles. Exp Eye Res 2006; 83:1179–1187.

26. Broglio F, Gottero C, Prodam F, Gauna C, Muccioli G, Papotti M, Abribat T, Van Der Lely AJ, Ghigo E. (2004). Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. J Clin Endocrinol Metab 2004; 89:3062–3065.
27. Baldanzi G, Filigheddu N, Cutrupi S, et al. (2002). Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. J Cell Biol 2002; 159:1029–1037.
28. Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B, Gaskin FS, Nonaka N, Jaeger LB. "Ghrelin controls hippocampal spine synapse density and memory performance". Nat Neurosci 2006; 9:381–388.
29. Atcha Z, Chen WS, Ong AB, Wong FK, Neo A, Browne ER, Witherington J, Pemberton DJ. (2009). "Cognitive enhancing effects of ghrelin receptor agonists". Psychopharmacology (Berl) 2009; 206:415–427.
30. Banks WA. (2008). The blood-brain barrier: connecting the gut and the brain. Regul Pept 2008; 149:11–14.
31. Azevedo-Pinto S, Tavares-Silva M, Pereira-Silva P, Leite-Moreira A, Rocha-Sousa A. (2002). The role of the Pro-Ghrelin derived peptides in the iris muscle regulation: Implications in glaucoma pathophysiology. In Ghrelin production action mechanism and physiological effects. Nova science publishers Chapter 2002; 2:33-56.
32. Eraslan N, Elgin U, Şen E, Kilic A, Yilmazbas P. (2018). Comparison of total/active ghrelin levels in primary open angle glaucoma, pseudoexfoliation glaucoma and pseudoexfoliation syndrome. Int J Ophthalmol. 2018 May 18; 11(5):823-827.
33. Katsanos A, Dastiridou A, Georgoulias P, Cholevas P, Kotoula M, Tsironi EE. (2011). Plasma and aqueous humour levels of ghrelin in open-angle glaucoma patients. Clin Experiment Ophthalmol 2011; 39:324-329.
34. Ghanem AA, Mady SE, Attia TN, Khattab AM, Arafa LF. (2014). Ghrelin Levels in Patients with Primary Open-Angle Glaucoma. Asia Pac J Ophthalmol (Phila). 2014 Mar-Apr; 3(2):126-129.
35. Rocha-Sousa A, Alves-Faria P, Falcão-Pires I, Falcão-Reis F, Leite-Moreira AF. (2009). Analyses of aqueous humor ghrelin levels of eyes with and without glaucoma. Br J Ophthalmol 2009; 93:131-132.
36. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004; 306:2090-2093.
37. Gnana-Prakasam JP, Martin PM, Mysona BA, Roon P, Smith SB, Ganapathy V. (2008). Hepcidin expression in mouse retina and its regulation via lipopolysaccharide/Toll-like receptor-4 pathway independent of Hfe. Bio chem J 2008; 411:79-88.
38. Ashok A, Chaudhary S, Kritikos AE, Kang MH, McDonald D, Rhee DJ, Singh N. (2020). TGFβ2-Hepcidin Feed-Forward Loop in the Trabecular Meshwork Implicates Iron in Glaucomatous Pathology. Invest Ophthalmol Vis Sci. 2020 Mar 9; 61(3):24.
39. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004; 306:2090-2093.
40. Nemeth E, Ganz T. (2006). Regulation of iron metabolism by hepcidin. Annu Rev Nutr 2006; 26:323-342.
41. Goralska M, Ferrell J, Harned J, Lall M, Nagar S, Fleisher LN, McGahan MC. (2009). Iron metabolism in the eye: a review. Exp Eye Res 2009; 88:204-215.
42. Narahari J, Ma R, Wang M, Walden WE. (2000). The aconitase function of iron regulatory protein 1. Genetic studies in yeast implicate its role in iron-mediated redox regulation. J Biol Chem 2000; 275:16227-16234.
43. McGahan MC, Harned J, Mukunnemkeril M, Goralska M, Fleisher L, Ferrell JB. (2005). Iron alters glutamate secretion by regulating cytosolic aconitase activity. Am J Physiol Cell Physiol 2005; 288:C1117-1124.
44. Zall MM, Ferrell J, Nagar S, Fleisher LN, McGahan MC. (2008). Iron regulates L-cystine uptake and glutathione levels in lens epithelial and retinal pigment epithelial cells by its effect on cytosolic aconitase. Invest Ophthalmol Vis Sci 2008; 49:310-319.
45. Goralska M, Ferrell J, Harned J, Lall M, Nagar S, Fleisher LN, McGahan MC. (2009). Iron metabolism in the eye: a review. Exp Eye Res 2009; 88:204-215.
46. Gnana-Prakasam JP, Martin PM, Mysona BA, Roon P, Smith SB, Ganapathy V. (2008). Hepcidin expression in mouse retina and its regulation via lipopolysaccharide/Toll-like receptor-4 pathway independent of Hfe. Biochem J 2008; 411:79-88.
47. Ghanem AA, Mady SE, Attia TN, Arafa LF. (2014). Hepcidin Prohormone Levels in Patients with Primary Open-Angle Glaucoma Open Journal of Ophthalmology, 2014, 4, 18-23.
48. Sorkhabi R, Ghorbanihaghjo A, Javadzadeh A, Motlagh BF, Ahari SS. (2010). Aqueous humor hepcidin prohormone levels in patients with primary open angle glaucoma. Molecular Vision 2010; 16:1832-1836.
49. Kokawa N, Sotozono C, Nishida K, Kinoshita S. (1996). High total TGF-beta 2 levels in normal human tears. Curr Eye Res 1996; 15:341-343.
50. Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C, Kinoshita S. (2000). Growth factors: importance in wound healing and maintenance of transparency of the cornea. Prog Retin Eye Res 2000; 19:113-129.
51. Roberts AB, Sporn MB. (1990). The transforming growth factor-ßs. In: Sporn MB, Roberts AB, editors. Handbook of Experimental Phamacology. Vol. 95. Heidelberg: Springer-Veriag; 1990. pp. 419–472.
52. Nishida K, Sotozono C, Adachi W, Yamamoto S, Yokoi N, Kinoshita S. (1995). Transforming growth factor-beta 1, -beta 2 and -beta 3 mRNA expression in human cornea. Curr Eye Res 1995;14: 235-241.
53. Inatani M, Tanihara H, Katsuta H, Honjo M, Kido N, Honda Y. (2001). Transforming growth factor-beta 2 levels in aqueous humor of glaucomatous eyes. Graefes Arch Clin Exp Ophthalmol 2001; 239:109-113.
54. Fujimoto T, Inoue-Mochita M, Iraha S, Tanihara H, Inoue T. (2021). Suberoylanilide hydroxamic acid (SAHA) inhibits transforming growth factor-beta 2-induced increases in aqueous humor outflow resistance. J Biol Chem. 2021 Sep; 297(3):101070.
55. Deng M, Hou SY, Tong BD, Yin JY, Xiong W. (2019). The Smad2/3/4 complex binds miR-139 promoter to modulate TGFβ-induced proliferation and activation of human Tenon's capsule fibroblasts through the Wnt pathway. J Cell Physiol. 2019 Aug; 234(8):13342-13352.
56. Sahay P, Reddy S, Prusty BK, Modak R, Rao A. (2021).TGFβ1, MMPs and cytokines profiles in ocular surface: Possible tear biomarkers for pseudoexfoliation. PLoS One. 2021 Apr 29; 16(4):e0249759.
57. Fuchshofer R, Stephan DA, Russell P, Tamm ER. (2009). Gene expression profiling of TGFbeta2- and/or BMP7-treated trabecular meshwork cells: Identification of Smad7 as a critical inhibitor of TGF-beta2 signaling. Exp Eye Res 2009; 88:1020-1032.

58. Yu AL, Birke K, Moriniere J, Welge-Lüssen U. (2010). TGF-{beta}2 induces senescence-associated changes in human trabecular meshwork cells. Invest Ophthalmol Vis Sci 2010; 51:5718-5723.
59. Ghanem AA, Moawad AI, Arafa LF, Elew A. (2009). Concentrations of transforming growth factor-Beta 2 and connective tissue growth factor in patients with primary open-angle glaucoma and angle-closure glaucoma. Delta Journal of Ophthalmology 2009; 10(1):54-60.
60. Chai F, Luo R, Li Y, Bai Y, He Y, Wei Y, et al. (2010). Down-regulation of GRP78 in human glaucomatous trabecular meshwork cells. Mol Vis 2010; 16:1122-1131.
61. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004; 306:2090-2093.
62. Ponomarenko I, Reshetnikov E, Dvornyk V, Churnosov M. (2022). Functionally significant polymorphisms of the *MMP9* gene are associated with primary open-angle glaucoma in the population of Russia. Eur J Ophthalmol. 2022Mar 7.
63. Reinehr S, Mueller-Buehl AM, Tsai T, Joachim SC. (2022). Specific Biomarkers in the Aqueous Humour of Glaucoma Patients. Klin Monbl Augenheilkd. 2022 Feb; 239(2):169-176. English, German.
64. Mastronikolis S, Pagkalou M, Baroutas G, Kyriakopoulou K, Makri OE, Georgakopoulos CD. (2022). Pseudoexfoliation syndrome: The critical role of the extracellular matrix in pathogenesis and treatment. IUBMB Life. 2022 Feb 24.
65. Svinareva DI, Churnosov MI. (2020). The role of metalloproteinases in the development of primary open-angle glaucoma. westn Ophthalmol. 2020; 136(4):146-150.
66. Ghanem AA**,** Arafa LF, El-Baz A. (2011). Connective Tissue Growth Factor and Tissue Inhibitor of Matrix Metalloproteinase-2 in Patients with Exfoliative Glaucoma Current Eye Research. 2011; 36:540-545
67. Tezel G, Edward DP, Wax MB. (1999). Serum autoantibodies to optic nerve head glycosaminoglycans in patients with glaucoma. Arch Ophthalmol 1999; 117:917-924.
68. Yang J, Tezel G, Patil RV, Romano C, Wax MB. (2001). Serum autoantibody against glutathione S-transferase in patients with glaucoma. Invest Ophthalmol Vis Sci 2001; 42:1273-1276.
69. Maruyama I, Ohguro H, Ikeda Y. (2000). Retinal ganglion cells recognized by serum autoantibody against gamma-enolase found in glaucoma patients. Invest Ophthalmol Vis Sci 2000; 41:1657-1665.
70. Maruyama I, Ikeda Y, Nakazawa M, Ohguro H. (2002). Clinical roles of serum autoantibody against neuron-specific enolase in glaucoma patients. Tohoku J Exp Med 2002; 197:125-132.
71. Ikeda Y, Maruyama I, Nakazawa M, Ohguro H. (2002). Clinical significance of serum antibody against neuron-specific enolase in glaucoma patients. Jpn J Ophthalmol 2002; 46:13-17.
72. Auler N, Tonner H, Pfeiffer N, Grus FH. Antibody and Protein Profiles in Glaucoma: Screening of Biomarkers and Identification of Signaling Pathways. Biology (Basel). 2021 Dec 8;10(12):1296.
73. Hohberger B, Schlötzer-Schrehard U, Mardin C, Lämmer R, Munoz L, Kunze R, Herrmann M, Wallukat G. (2021). Inhibitory and Agonistic Autoantibodies Directed Against the β_2-Adrenergic Receptor in Pseudoexfoliation Syndrome and Glaucoma. Front Neurosci. 2021 Aug 6; 15:676579.
74. Tezel G, Seigel GM, Wax MB. (1998). Autoantibodies to small heat shock proteins in glaucoma. Invest Ophthalmol Vis Sci 1998; 39:2277-2287.
75. Joachim SC, Reichelt J, Berneiser S, Pfeiffer N, Grus FH. (2008). Sera of glaucoma patients show autoantibodies against myelin basic protein and complex autoantibody profiles against human optic nerve antigens. Graefes Arch Clin Exp Ophthalmol 2008; 246:573-580.
76. Kountouras J, Mylopoulos N, Konstas AG, Zavos C, Chatzopoulos D, Boukla A. (2003). Increased levels of Helicobacter pylori IgG antibodies in aqueous humor of patients with primary open-angle and exfoliation glaucoma. Graefes Arch Clin Exp Ophthalmol 2003; 241:884-890.
77. Yuki K, Kimura I, Shiba D, Imamura Y, Tsubota K. (2010). Elevated serum immunoglobulin G titers against Chlamydia pneumoniae in primary open-angle glaucoma patients without systemic disease. J Glaucoma 2010; 19:535-539.
78. Perez-Garmendia R, Rodriguez A, Ramos-Martinez I et al. (2020). Interplay between oxidative stress, inflammation, and amyloidosis in the anterior segment of the eye; its pathological implications. Oxid Med Cell Longev 2020; 3:6286105
79. Ghaffariyeh A, Honarpisheh N, Heidari MH, Puyan S, Abasov F. (2011). Brain-derived neurotrophic factor as a biomarker in primary open-angle glaucoma. Optom Vis Sci 2011; 88:80-85.
80. Velez G, Roybal CN, Binkley E, Bassuk AG, Tsang SH, Mahajan VB. (2017). Proteomic Analysis of Elevated Intraocular Pressure with Retinal Detachment. Am J Ophthalmol Case Rep. 2017 Apr; 5:107-110.
81. Bäcklund A, Holmdahl M, Mattsson R, Håkansson K, Lindström V, Nandakumar KS, et al. (2011). Cystatin C influences the autoimmune but not inflammatory response to cartilage type II collagen leading to chronic arthritis development. Arthritis Res Ther 2011; 13:R54.
82. Nikhalashree S, George R, Shantha B, Lingam V, Vidya W, Panday M, Sulochana KN, Coral K. (2019). Detection of Proteins Associated with Extracellular Matrix Regulation in the Aqueous Humor of Patients with Primary Glaucoma. Curr Eye Res. 2019 Sep; 44(9):1018-1025.
83. Duan X, Xue P, Wang N, Dong Z, Lu Q, Yang F. (2010). Proteomic analysis of aqueous humor from patients with primary open angle glaucoma. Mol Vis 2010; 16:2839-2846.