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Research Article

Biomarkers in Primary Open Angle Glaucoma, Part 1: A Review

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Abstract

Primary open-angle glaucoma (POAG) is the second leading cause of irreversible blindness worldwide. Increasing evidence suggests oxidative damage and immune response defects are key factors contributing to glaucoma onset. Indeed, both the failure of the trabecular meshwork tissue in the conventional outflow pathway and the neuro inflammation process, which drives the neurodegeneration, seem to be linked to the age-related over-production of free radicals (i.e., mitochondrial dysfunction) and to oxidative stress-linked immune stimulatory signaling.

Glaucoma, a leading cause of blindness worldwide, is currently defined as a disturbance of the structural or functional integrity of the optic nerve that causes characteristic glaucomatous changes in the optic nerve, which may lead to specific visual field defects.

Primary open angle glaucoma (POAG) is described distinctly as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of optic nerve fibers. It manifests by cupping and atrophy of the optic disc, in the absence of other known causes of glaucomatous disease.

In POAG management, biomarkers could potentially aid in earlier diagnosis, grading, and/or progression risk, in research on potential new medical therapies, for better patient selection or outcome measures. Biomarkers will offer a new clinical tool useful in POAG diagnosis, in prediction of disease prognosis, and in monitoring clinical responses to standard treatments or investigational therapies.

Running title: Biomarkers in primary open angle glaucoma

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

Introduction

The number of people affected by glaucoma is estimated at 80 million in 2021, with 3.5% prevalence in people aged 40–80 while the number of people affected is projected to increase to 112 million by 2040 [1]. The incidence of glaucoma is one in 200 people aged 50 or younger, and one in 10 people aged 80 or older.

Glaucoma is one of the main causes of irreversible visual field loss and blindness worldwide [2]. Vision loss in this multifactorial neurodegenerative disease results from progressive degeneration of retinal ganglion cells (RGCs). Currently, there is no single gold standard test for glaucoma screening and diagnosis.

The mechanical theory argues that the IOP elevation, either at the lamina crib Rosa or the optic nerve head (ONH) level, lead initially to hypo perfusion and then reperfusion damage. Therefore, IOP elevation is considered a direct or indirect cause of RGC damage, which results in a retrograde transport blockade and the accumulation of neurotrophic factors at the lamina crib Rosa instead of reaching the RGC. In addition to growth factor starvation, mitochondrial damage and glial cell activation, as well as oxidative stress, play an important role in promoting RGC apoptosis [3].

The vascular theory is based on evidence of either primary (vasospastic syndrome) or secondary vascular dysregulation found in some glaucomatous patients. The chronic impairment of ONH blood flow, which may result from an imbalance in the ocular blood flow auto-regulation and oxidative stress (vasospastic syndrome) or from systemic levels of vasoconstrictive peptides (i.e., endothelin-1), seems to be responsible for ischemia-reperfusion nerve injury [3].

Glaucoma has been nicknamed the 'silent thief of sight' because the loss of vision normally occurs gradually over a long period of time and is often only recognized when the disease is quite advanced. Worldwide, it is the second leading cause of blindness. It is also the leading cause of blindness among Africans.

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

from 1.1% [5] to 3.8% [6]. About 5% of POAG is currently attributed to single-gene or Mendelian forms of glaucoma and many genes and loci have been found to play a role in the pathogenesis of the disease.

Biomarkers are biological markers defined as an objective and quantifiable characteristics 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 disease (directly or indirectly) and might be any biochemical compound (antigen, antibody, enzyme, hormone, etc) that is sufficiently altered to provide diagnostic or predictive value. Biochemical markers may differentiate specific diseases and guide therapy. Biomarkers obtained from biological samples (eg, blood) may indicate subclinical disease, stage, or severity of disease. They may be present due to their causative role in glaucoma or as a result of the disease process [8].

A biomarker is a characteristic that is specifically with adequate accuracy and precision measured and evaluated as an indicator of normal biological or pathogenic processes, or to monitor pharmacologic responses to a therapeutic intervention [9]. Thus, biomarkers might be invaluable tools to identify individuals at risk for disease and, depending on the approach, could serve to measure the outcomes of therapies.

The purpose of the present study is to summarize all the current information regarding POAG biomarker candidates. These markers, measured in blood (serum or plasma), AH, or tissues, alone or in combination with each other, could be crucial for the early diagnosis of POAG, or even for the prevention of the disease.

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

Erythropoietin

Erythropoietin (EPO) is an oxygen-regulated hormone produced in the fetal liver and the adult kidney and released in the blood-stream. The systemic function of EPO is to stimulate erythrocyte formation in the bone marrow in response to hypoxia [10]. EPO is a 165 amino acid sialo-glycoprotein that has demonstrated a remarkable tissue-protective ability in numerous animal and cell culture studies of ischemia and neuronal degeneration as well as stroke patients [11].

Erythropoietin is the first target gene for hypoxia inducible factor-1(HIF-1) to be identified and still one of the best-characterized genes activated by reduced oxygen levels. HIF-1 has been shown to have, either clinically or experimentally, a mediating or contributing role in several oxygen-dependent retinal diseases such as glaucoma [12].

Erythropoietin, characterized by hematopoietic, antigenic and neuroprotective properties. Its hematopoietic function is dependent on its interaction with the homodimer receptor on erythrocyte progenitor cells within the bone-marrow. While, its neuroprotective function is dependent on its ability to traverse the intact blood-brain and blood-retinal barrier in therapeutic amounts (13).

Mokbel et al [14] revealed a statistically significant increase in the aqueous humor EPO level in POAG patients compared with control patients but there was no significant increase in the serum level. Cumurch et al [15] who also found a statistically significant difference between EPO serum and EPO aqueous humor concentration in the POAG group and the control group. However, Mokbel et al [14].study revealed no significant correlation between EPO serum and EPO aqueous humor concentration.

The cause of the elevated aqueous humor EPO concentration in eyes with POAG may be related to the ischemia, hypoxia, or elevated reactive oxygen species caused by glaucomatous damage [12-16]. Also, EPO may increase with a compensatory mechanism owing to the increase in glutamate, nitric oxide, and the free radicals after the glaucomatous damage [17].

Some experimental studies showed that exogenous EPO protected retinal ganglion cells in glaucomatous eyes through an anti-apoptosis mechanism [11]. Auricchio et al [18] revealed that following a systemic administration of rapamycin in nude rats, EPO protein was detected in the baseline 2-3 weeks after withdrawal of the drug.

Brines et al [19]. Suggested that EPO is a multifunctional, pro-angiogenic, and pro-survival growth factor that does much more than stimulate erythrogenesis. Oxygen regulates EPO production not only in the kidney but also in the retina. Local retinal production of EPO was found to be as critical as vascular endothelial growth factor (VEGF). EPO like VEGF, is responsible for the regulation and activation of hypoxia-inducible factor- 1.

Mokbel et al [14] revealed no correlation between the aqueous humor and plasma levels of EPO. These results suggested that EPO levels in aqueous humor were not related to breakdown of blood-retinal barrier and/ or ocular blood. In addition, increased levels of aqueous humor EPO may be associated with POAG. In addition, EPO may be useful proteins levels in aqueous humor of POAG patients as a result of glaucoma damage and not a cause. EPO concentrations in aqueous humor are a possible biomarkers for visual field loss in patients with POAG.

Soluble CD44

Soluble CD44 (sCD44), the ectoderm fragment of transmembrane protein receptor CD44, is a cytotoxic protein to trabecular meshwork and retinal ganglion cells in vitro [20]. sCD44 toxicity was prevented by a pancaspase inhibitor, indicating that sCD44 is a proapoptotic factor. SCD44 toxicity was also prevented by co-administration of hyaluronic acid, indicating that sCD44 binding to hyaluronic acid blocks sCD44 activity. However, hypo-phosphorylated sCD44, which is present only in POAG and normal pressure glaucoma, was significantly more cytotoxic to trabecular meshwork and retinal ganglion cells than the standard sCD44 [21].

sCD44 functions as a part of the regulation of immune-mediated inflammation and is released from the cell surface by metalloproteases in response to metabolic stress and hypoxia (23).sCD44 is most likely shed from the ciliary epithelium into aqueous humor because the ciliary body has the highest concentrations of CD44. Increased sCD44 concentration in POAG aqueous is secondary to the underlying heterogeneous pathophysiology, such as, genetic anomaly aberrant protein, or metabolic stress, which may impact the severity and clinical course of the POAG disease process [22].

The bioavailability of sCD44 depends on its binding to hyaluronic acid, and the results of this binding may influenced by pressure. Hyaluronic acid has hydrophobic patches [23].That are affected by elevated pressure and disrupted by glycine, as demonstrated by electron microscopy and rotary shadowing.

The apparent change in the hyaluronic acid polymer with increased pressure and the decreased binding of sCD44 to hyaluronic acid with increased pressure may be a reason why increased IOP is a clinical risk factor in POAG. The high toxicity and low hyaluronic acid-binding affinity of hypophosphrylated sCD44 may represent specific pathophysiological features and a biochemical hallmark of the POAG disease process [20].

CD44 was identified as a primary phagocytic receptor that mediates the internalization of large particles. Because sCD44 binding to CD44 receptor inactivates the CD44 receptor, a consequence of increased sCD44 concentration is the accumulation of extracellualr matrix material which may increase the outflow resistance, and thus increase IOP [22].

Mokbel et al [14] revealed a statistically significant increase in the aqueous humor sCD44 level in POAG patients compared with control patients but there was no significant increase in the serum level. .Also, they did not found a significant correlation between aqueous humor sCD44 and severe visual field loss. Nolan et al [22] revealed a significant correlation between sCD44 severe visual field loss .

Miller et al [24] reported that metabolic stress using lactate administration to trabecular meshwork cells causes activation and the release of sCD44 in cultured trabecular meshwork cells. Moreover, VEGF secretion, COX-2 activation, and prostacyclin formation in endothelial cells, occurs by CD44 binding to hyaluronic acid [25].

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Mokbel et al. [14] reported no correlation between the aqueous humor and plasma levels of sCD44. These results suggested that sCD44 levels in aqueous humor were not related to breakdown of blood-retinal barrier and/ or ocular blood. A significant increase in aqueous humor sCD44 at the onset of the POAG visual field loss and insignificant increase at end stage of disease may indicate potential secondary consequence.

Oxidative Stress Markers

Oxidative stress can be defined as an increase over physiologic values in the intracellular concentrations of reactive oxygen species (ROS) that include superoxide anion ($O2^{-}$), hydrogen peroxide (H2O2), hydrogen radical (OH), peroxyl radical (ROO), and singlet oxygen ($^1O_2$). This situation is reflected by changes in the levels of antioxidant defenses that can be increased, as a protective response or depleted due to the ROS action.

Oxidative stress has been implicated in the possible pathophysiology of different ocular diseases such as retinopathy of prematurity, macular degeneration, and uveitis. The cell has antioxidant defense against oxidative damage. These antioxidants can be exogenous (vitamin E, C, or β-carotenes) or endogenous (enzymes, chemical substances, or DNA repair proteins). When levels of FR increase and antioxidant defense is not sufficient, then health problems may appear[26].

Antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase have been reported in aqueous humor. The formation of highly cytotoxic oxygen-derived free radicals is prevented by the enzyme copper-zinc SOD (Cu-Zn SOD) through the rapid conversion of the superoxide anion to hydrogen peroxide; SOD is present in all eye tissues, and three SOD isoenzymes (Cu-Zn SOD, Mn SOD, and EC-SOD) have been reported present in aqueous humor. High levels of extra-cellular SOD in cornea may be related to the exposure of the eye to visible and ultraviolet light [27].

It has been suggested that cataracts enhance oxidative stress, Benoist d'Azy C et al [29] demonstrated that the most important effect size for oxidative stress was in aqueous humor.

Dysbalance between prooxidative status and antioxidant defense activity has been suggested to play an essential role in early retinal ischemia injury[28] and in glaucoma pathogenesis [30].

Furthermore, the use of topical drugs with preservatives had an impact on the tear cytokine expression levels, with significantly higher levels of IL2, IL5, IL10, IL12, IL13, IL15, IL17, FGFβ, platelet derived growth factor BB and TNFα in patients receiving treatments with preservatives, compared to controls. Therefore, the topical use of glaucoma medications resulted in specific changes of inflammatory or oxidative stress markers in the tear film [1].

Some authors [31, 32]. Had described the presence of oxidative agents in the aqueous humor, such as super-oxide anion and hydrogen peroxide. It has also been proposed that oxidative stress can damage the cells of the trabecular meshwork. Moreover, SOD has been localized in these cells. In a designed study close to this, Ferreira et al [33] observed an increase in SOD and glutathione peroxidase activity in glaucoma patients when compared with those with cataracts, and no significant changes were found in CAT levels.

De La Paz and Epstein [34] suggested a possible role for the oxidative damage in the pathogenesis of POAG which involves chronic exposure to the harmful effects of ROS in the aqueous humour. As, they have demonstrated a decline in the specific activity of human trabecular superoxide dismutase ,but no CAT ,with increasing age, thus supporting the view that oxidative stress may be involved in POAG .However ,Yu et al [35] concluded that oxidative stress is able to induce characteristic glaucomatous TM changes in vitro ,and these oxidative stress-induced TM changes can be minimized by the use of prostaglandin analogues .Thus ,prevention of oxidative stress exposure to the TM may help to reduce the progression of POAG.

Hydrogen peroxide is removed by two enzymes: catalase and glutathione peroxidase. Catalase directly catalizes the decomposition of H_2O_2 to ground state oxygen and water. Glutathione peroxidase removes H_2O using glutathione as a cofactor. In this pathologic condition in the eye, there is an increase rate of superoxide production that leads to a depletion of low-molecular-weight antioxidants and an increase in H_2O_2 levels. According, to this, glutathione peroxidase activity in the aqueous humor of glaucoma patients was higher than in the cataract group.

Ghanem et al [36] found no significant difference in the activities of CAT between POAG and cataract patients (P=0.201). Als0, aqueous humor GPO, SOD, MDA levels were significantly higher in POAG patients (P<0.00). Ferreira et al [33] concluded that aqueous SOD and GPO activities increased by 57% and 300% respectively, in the glaucoma group compared with the cataract group. Thus, there is a good reason to suggest that raised IOP in some glaucoma patients originates from trabecular cell malfunction caused by oxidative stress as confirmed with Izzotti et al [37].

Ghanem et al [36] reported that GPO, SOD, MDA may be useful oxidative stress markers in the aqueous humor of POAG patients. Several antioxidants have been trialed as potential neuroprotective therapies. As mitochondria is a major site of ROS production, advances have been made in targeting antioxidants directly to within the mitochondria using techniques such as conjugating antioxidants to lipophilic cations. Thus, reducing oxidative stress by the use of antioxidants act as an adjunct neuroprotective therapy for glaucoma [38].

Homocysteine

Homocysteine (Hcy) is a neurotoxin amino acid that can induce

typical pathological changes seen in glaucomatous neuropathy such as apoptosis of RGCs [39] ECM alterations [40], oxidative stress [41], and ischemic vascular dysregulation [42]. Furthermore, hyperhomocysteinemia is suggested to be a risk factor for other ocular diseases including age-related macular degeneration [43]. Ischemic optic neuropathy [45]. Retinal vascular occlusive disease [45]. And secondary glaucoma due to pseudo-exfoliation syndrome [46].

Vitamin B12, vitamin B6, and folic acid act as cofactors in Hcy metabolism. These vitamins are the major environmental determinants of Hcy level. Low folic acid, vitamin-B12 status, or renal impairment account for the majority of cases where increased Hay is observed [47-49].

Homocysteine may induce vascular injury and alterations to the extracellular matrix, Hcy levels were quantified in the aqueous humor of PEXG patients and control subjects by ELISA, observing two-fold elevation in the glaucoma group. This upregulation may contribute to the abnormal accumulation of the extracellular matrix, reflecting the impairment of the blood–aqueous barrier in glaucoma [1].

A statistically significant increase in the Hcy aqueous humor level in POAG patients compared with control patients is in agreement with RoedI et al [50]. The results about plasma Hcy concentration in POAG are conflicting. Our study found that plasma Hcy level in POAG patients were not different significantly than in controls. This findings is in agreement with Wang et al [51], Altintas et al [52], Turgut et al [53], and Cumurcu et al [54]. However, Bleich et al [55], RoedI et al [50], and Clement et al [56] reported elevated plasma Hcy concentration in POAG patients and suggested that thermolabile methylene tetra hydrofolate reductase deficiency may be at least in part the cause of increased plasma Hcy concentration in patients with POAG.

The exact mechanism of action of Hcy remains to be elucidated. Current evidence suggests that an increase Hcy concentration may induce atherosclerosis by a combination of endothelial injury [57], smooth muscle proliferation [58], platelet activation and thrombogenesis [59]. These vascular effects could lead to changes in the optic nerve head microvasculature and consequent impairment of optic nerve head flow.

Accordingly, a major pathogenetic step in the development of POAG is the loss of retinal ganglion cells due to neurotoxic stimulation of the NMDA receptor [60]. The effect of Hcy on the NMDA receptor is several times greater than that of glutamate and simultaneous stimulation potentiates the excitoxic effect [39]. There is accumulating evidence that increased levels of matrix metalloproteinases [61], transforming growth factors and connective tissue components (collagen, elastin) [62] in POAG cause disorganization of the ECM architecture in the optic nerve head.

Ghanem et al [63] concluded that no significant association between aqueous humor Hcy levels and important systemic Hcy determinants, such as serum folate, was found in POAG. While, in the control group, a significant correlation between aqueous humor Hcy and serum folate could be observed and the association between aqueous humor and plasma Hcy was stronger than in POAG. This might imply an Hcy metabolism in the aqueous humor of POAG which is independent from the blood Hcy metabolism and its determinants. Also, this finding consistent with previous study showing an intact blood-aqueous barrier in POAG and an impaired barrier function in exfoliative glaucoma eyes [64].

Ghanem et al [63] revealed no significant correlation between the aqueous humor and plasma levels of Hcy suggested that Hcy levels in aqueous humor were not related to breakdown of blood-retinal barrier and/ or ocular blood. Also, they reported that elevation of intraocular expression of aqueous humour Hcy may be associated with optic nerve damage in POAG.

Hydroxyproline

Hydroxyproline (Hyp) is a post-translational product of proline hydroxylation by the enzyme prolyl 4-hydroxylase. During formation of collagen triple helices, prolyl 4-hydroxylase catalyzes the formation of 4-Hyp in collagen by the hydroxylation of proline residues. The reaction products, 4-Hyp residues, serve to stabilize the helices [65]. Thus, Hyp is a unique amino acid in collagenous molecules derived from connective tissue and increased in the serum of patients with primary glaucoma. Most of the endogenous Hyp present in biologic fluids derived from the degradation of various forms of collagen. Hyp levels were used to predict collagen turnover status [66].

Morrow and Roberts [67] reported that Hyp associated with higher 8-isoprostglandins F2 concentration in aqueous humor which may be attributed to higher oxidative stress. Furthermore, isoprostanes influence extracellular matrix synthesis and express growth factor activity in cultured rat aortic smooth muscle cells [68] and promotes the secretion of endothelin-1[69]. These metabolic abnormalities favor an abnormal and increased extracellular matrix turnover which in turn may cause elevated Hyp levels.

The significantly higher levels of Hyp in aqueous humor associated with non-significant serum changes could be attributed to enhanced intraocular synthesis. It may be explained that the increased Hyp levels reflect increased collagen turnover, which play a role in the pathophysiology of POAG or result from the disease process Ghanem et al [63].

Ghanem et al [63] found significantly higher aqueous humor Hyp levels that indicate increased collagen degradation in the aqueous humor of POAG patients. Disturbed basement membrane metabolism in POAG might cause increased degradation of the type 4 collagen, which contains significant amount of Hyp residues, leading to elevated Hyp levels.

The non-significant correlation between either Hcy or Hyp levels with the POAG visual field loss at any stage may indicate absence of potential secondary consequences such as ischaemia, hypoxia, or reactive oxygen species caused by glaucomatous damage Ghanem et al [63].

Ghanem et al [63] revealed no significant correlation between the aqueous humor and plasma levels of Hcy and Hyp results suggested that levels Hcy and Hyp in aqueous humor were not related to breakdown of blood-retinal barrier and/ or ocular blood. But, the Hcy level in aqueous humor was significantly correlated with aqueous humor Hyp. This correlation supported that both Hcy and Hyp levels may be consequence with glaucomatous damage. Thus, Hcy and Hyp may be useful proteins levels in aqueous humor of POAG patients as a consequence of glaucomatous damage.

Endothelin-1

ET-1 has been shown to be involved in the modulation of ocular blood flow and regulation of IOP by constricting the trabecular meshwork and the ciliary muscle via endothelin receptor (A) activation [70-72]. In healthy eyes, ET-1 is actively secreted by human non-pigmented ciliary epithelial cells into the aqueous humor [73]. Increased plasma ET-1 levels have been described in progressive open angle glaucoma [74]. Furthermore, prolonged administration of ET-1 has been shown to produce an optic neuropathy similar to that noted in glaucoma [75].

Ghanem et al [76] revealed that no significant difference in plasma ET-1 levels in the studied groups. This is consistent with the results of Tezel et al [77] who reported no significant difference between plasma ET-1 levels of 31 patients with POAG and 24 patients without glaucoma (P=0.07). While, Emre et al [74] reported a significantly higher

plasma levels of ET-1 in 16 POAG patients with progressive visual field damage compared with 15 POAG patients with stable visual field. However, plasma levels of ET-1 were significantly elevated in the aqueous humor of glaucoma groups compared to cataract group.

The chronic impairment of ONH blood flow, which may result from an imbalance in the ocular blood flow auto-regulation and oxidative stress (vasospastic syndrome) or from systemic levels of vasoconstrictive peptides (i.e., endothelin-1), seems to be responsible for ischaemia-reperfusion nerve injury [4].

Furthermore, endothelin (ET) levels, related to inflammation, were quantified in the aqueous humor and plasma of 40 POAG (n = 31) and control (n = 24) subjects by radioimmunoassay (RIA), observing limited significant upregulation of ET in the aqueous humor of POAG patients (44.26 + 2.6 pg·mL) when compared to controls (42.17 + 1.6 pg·mL), while no significant differences were observed in plasma [1].

Tezel et al [77] reported a significantly higher aqueous humor levels of ET-1 in 31 POAG patients compared with 24 normal subjects (P=0.001). Increased level of ET-1 in the aqueous humor but not in plasma of glaucomatous patients revealed that ET-1 appears to act mainly as a local agent and synthesized secondary to the highest IOP.

Ghanem et al [76] revealed no significant correlation between the POAG aqueous humor and plasma ET-1 levels suggested that levels ET-1 in POAG aqueous humor were not related to breakdown of blood-retinal barrier and/ or ocular blood. This supported that ET-1 may be associated with glaucoma. The elevation of intraocular expression of ET-1 may be responsible for causing nerve damage. This suggest targeting ET-1 as a mean to inhibit the neurodegenerative mechanisms associated with glaucoma.

Nitric Oxide

Nitric oxide (NO) is a noxious, unstable, free radical gas that promote angiogenesis in several tissue [78].

NO is a potent vasodilator and inflammatory factor [79,80]. It is an important mediator of the homeostatic functions of the eye including regulation of aqueous humor outflow by relaxing contractile elements of the trabecular meshwork [81], moduling local ocular blood flow, and control of retinal ganglion cell death by apoptosis [82]. It is believed that NO participates in the pathogenesis of glaucoma and may contribute to the pathological degeneration of eye tissues noted in glaucoma [83,84].

Both reactive oxygen species (ROS) and NOS trigger a cascade of events that can result in cellular damage. In particular, high levels of ROS may be responsible for increased levels of nitric oxide (NO) [4].

Resch et al [85] reported that a complex cascades result from decreased NO biovaliability in the vascular endothelium including: consolidated vasoconstriction by ET-1, oxidative stress caused by elevated superoxide level, activation of pro-inflammatory mediators, and platelet aggregation.

Ghanem et al [76] revealed no significant difference in between the studied groups as regards plasma nitrite levels. This findings consistent with results of Altinatas et al [86] who showed no significant increase in plasma NO marker levels between 19 patients with POAG and 20 control subjects (P=0.05). Also, aqueous humor total nitrite was significantly increased in glaucomatous patients than control group. This finding consistent with the results obtained by Tsai et al [87] who reported a significantly higher aqueous humor levels of NO in 31 patients with POAG, 76 patients with CCAG, and all of them were significantly higher than in cataract patients (P=0.05).

In the early 2000s, studies focused on the role of oxidative stress in glaucoma began to emerge, with the analysis of nitric oxides and cyclic guanosine monophosphate (cGMP). While the quantitation of nitrate, nitrite, and cGMP in the aqueous humor and serum of glaucomatous and control Finnish subjects by spectrophotometry, chemiluminescence, showed no statistically significant differences, the levels of cGMP and nitrite in Italian POAG patients were lower than in the control subjects [1].

While it is consistent with results of Kosior-Jarecka et al [88], who reported that there was no significant difference in aqueous humor NO levels between 14 patients with POAG, 12 patients with CCAG, and control subjects (P=0.535). However, the increased level of total nitrite in aqueous humor in the present study could be explained as a trial of the eye to increase the aqueous humor outflow facility through the IOP-lowering effect of the NO/cyclic GMP [89].

There was no significant correlation between the aqueous humor and plasma levels of ET-1 and NO results suggested that levels ET-1 and NO in aqueous humor were not related to breakdown of blood-retinal barrier. But, the ET-1 level in aqueous humor was significantly correlated with aqueous humor NO. This correlation supported that both ET-1 and NO may be associated with glaucoma Ghanem et al [76].

Increased concentrations of aqueous humor ET-1 and NO may be useful with POAG and CCAG. In addition, ET-1and NO may be a useful metabolite levels in the aqueous humor of POAG and CCAG patients as result of glaucoma damage and not a cause Ghanem et al., [76].

Serotonin

Oxidative stress is a result of imbalance between pro-oxidants and antioxidants. So in these conditions there are high levels of free radicals that act by damaging cells and can induce the retinal ganglion cell (RGC) death by apoptosis [90,91]. Glutamate toxicity has also been involved in RGC death, so even though IOP is efficient controlled, RGC death will continue if toxic effects of glutamate are not prevented [92,93]. Serotonin is another molecule that is involved in the pathogenesis of glaucoma [94].

Serotonin is an indolamine, which is a precursor of melatonin. The potential antioxidant capacity of melatonin is well known [95,96] and there is evidence to suggest that melatonin could decrease the intraocular pressure. Therefore, melatonin could be used in glaucoma therapy [97].

Serotonin is a neurotransmitter that is synthesized in neurons and stored into vesicles. It is present in the mammalian eye, and its levels are higher in the iris-ciliary body complex (ICBC). Seven types of serotonin receptors have been identified (5-HT1 to 5-HT7). The stimulation of 5-HT7 receptor causes an increase in IOP, and the stimulation of 5-HT1A receptor causes a decrease in IOP [98].

On the other hand, 5-HT is a precursor of melatonin, a hormone which has a cyclically variable concentration which plays a role in a variety of cellular processes such as oxidative stress [99]. Oxidative stress is an imbalance between pro-oxidant and anti-oxidant molecules and causes toxic effects that damage proteins, lipids, and DNA [100].

Ghanem et al [101] revealed statistically significant higher levels of serotonin and hydroxyindolacetic acid (HIAA) in aqueous humor of glaucoma patients compared to patients with cataract. In addition, 5-HT turnover was higher in glaucoma patients than in cataracts patients. Also, they reported that all POAG and cataract patients' plasma levels of serotonin and HIAA were significantly higher than aqueous humor levels.

Serotonin is a precursor of melatonin. The potential antioxidant capacity of melatonin is well known, and there is also evidence that melatonin could decrease IOP [102]. This evidence implies that melatonin may be useful in glaucomatous optic neuropathy [103]. For that reason, it might be supposed that in POAG patient's 5-HT is not turned over into

melatonin, but it is rather degraded by the monoamine oxidase enzyme (MAO) which would resultantly lead to an increase in 5-HIAA levels.

Ghanem et al [101] revealed a statistically significant increase in the aqueous humor level of both 5-HT and 5-HIAA in POAG patients compared with control patients but there was no significant increase in the serum level. This observation, according to the above mentioned proposed mechanisms, could be attributed to enhanced intraocular synthesis. However, increased 5-HT and 5-HIAA levels may attributed to intraocular metabolism of eye drops or any of their components. Also, they reported that 5-HT level in aqueous humor was significantly correlated with aqueous humor 5-HIAA. This correlation supported that both 5-HT and 5-HIAA may be associated with glaucoma.

The high concentration of serotonin implies that it has one or more functions: [104] it acts as neurotransmitter and precursor of melatonin; [105] conceivably it is related to aqueous humor dynamics; and [06] it affects the circadian rhythm of intraocular pressure.

Tumor Necrosis Factor-α and Interleukin-6

Cytokine secretions, such as interleukins (IL), interferons (IFN), colony-stimulating factors (CSF), chemokines, tumor necrosis factors (TNF), and growth factors, are implicated, either in local inflammatory processes or in non-immune functions (i.e., angiogenesis and development). However, POAG is characterized by immune activation with changes in cytokine profiles [4].

Cytokines are secreted proteins that play central roles in modulating immunity, but they can also perform non-immune functions in areas such as angiogenesis and development. If immune activation is associated with glaucoma, changes in cytokine secretion within the eye might be detectable as changes in the concentration of cytokines in the AH of glaucoma patients [107].

Cytokines include interleukins, interferons, colony-stimulating factors, chemokines, tumor necrosis factor, and growth factors. Tumor necrosis factor alpha (TNF-α), a macrophage/monocyte derived pluripotent cytokine, is associated with tissue ischemia, neuronal damage, and remodeling [108], and increased levels signify neuronal damage after brain trauma. In humans, the TNF-α expression is elevated in the optic nerve and the retina of glaucomatous eyes [108-110].

Tumor necrosis factors are pleiotropic cytokines that are considered to be the primary modifiers of inflammatory and immune reactions. Two forms of TNF, designated TNF-**α** and TNF-**β**, have been reported to compete for binding to the same receptors. Interestingly, recent studies have shown that ischemic or pressure-loaded glial cells produce TNF-α, which results in oligodendrocytes death and the subsequent apoptosis of retinal ganglion cells [111].

Proinflammatory cytokines such as TNF- α and IL-6 may play role in mediating inflammation associated with various ocular diseases [112]. TNF- α is known as a potent activator of neurotoxic substances such as nitric oxide, excitotoxins [113].

The proinflammatory cytokine tumor necrosis factor alpha (TNFα), analyzed by singleplex immunoassay, was found to be increased (1.7 fold) in the aqueous humor of 32 POAG patients when compared with the same number of controls, suggesting TNF-α to be a reliable biomarker in the progression of POAG [1].

TNF- α and IL-6 share many common characteristics with VEGF and are also reported to be hypoxia induced (114). TNF- α and IL-6 gene expression markedly increases in experimental rat retina after transient ischemia [115].

TNF-α is a pleiotropic inflammatory cytokine. Tissue ischemia or damage enhances the production of TNF-α, which contributes to the remodeling process during nerve degeneration (116). In addition to nitric oxide and excitotoxins, TNF-α has neurotoxic effects and functions as an activator. TNF-α concentrations in plasma, cerebrospinal fluid, and brain tissue are elevated in CNS disorders [117].

IL-6 is a multifunctional cytokine that act on a wide range of cells. Several reports have demonstrated its major role as a mediator of inflammatory and immune responses [118]. The pleiotropic effects of IL-6 include the stimulation and secretion of immunoglobulin, induction of neuronal differentiation, and activation of acute-phase protein synthesized by liver cells [119].

Ghanem et al [120] revealed a statistically significant increase in the aqueous humour level TNF-α in POAG patients compared with control patients but there was no significant increase in the serum level. The significantly higher levels of TNF-α and IL-6 in aqueous humor associated with non-significant serum changes could be attributed to enhanced intraocular synthesis.

Many studies have detailed the relationship between glaucomatous optic neuropathy and TNF-α. In an animal model with high intraocular pressure, elevation of TNF-α precedes the loss of retinal ganglion cells and oligodendrocytes. In addition, these cell losses are observed by administering of TNF-α without the elevated intraocular pressure [121]. TNF-α contributes to this process by adversely affecting oligodendrocytes [122] by increasing the susceptibility of axons to excitoxicity in the optic nerve head and retinal ganglion cell death [123].

Yan et al [124] and Tezel et al [125] found that the expression levels of TNF-α and its receptor TNF-R1 were higher in the inner retinal layers of glaucoma eyes than in control eyes. The increased expression of TNF-α in glaucoma eyes suggests that this cytokine is critically connected with the damaging processes in these tissues.

Intraocular TNF-α is likely secreted from activated macrophages, astrocytes, microglial cells [112], and retinal Müller glial cells [126]. These results suggest that intraocular stress, including high pressure and ischemia, stimulates TNF-α production, which may signal the progression of neuronal damage. Post-trauma increases in TNF-α levels are known to predict poor prognoses [116].

Ghanem et al [120] revealed a statistically significant increase in the aqueous humor level IL-6 in POAG patients compared with control patients but there was no siginificant increase in the serum level. However, the increased level of IL-6 in aqueous humor of POAG patients is a cytokine that is synthesized secondary to the higher intraocular pressure.

Optineurin gene is induced by TNF-α and interacts with several proteins to regulate apoptosis, inflammation, and vasoconstriction. For example, optineurin interacts with adenoviral E3-14.7k protein which protects cells from the cytolytic activity activity of TNF-α [127]. In glaucomatous eyes, the expression of TNF- α and TNF- α receptor-1 was upregulated in the retina and optic nerve head [128].

Ghanem et al [120] revealed significant increase in aqueous humor TNF-α and IL-6 at the moderate stage of the POAG visual field loss and insignificant increase at end stage of disease that indicate potential secondary consequence. As elevated levels of TNF- a and VIL-6 may be accompanied by programmed cell death.

Study concluded that no significant correlation between the aqueous humor and plasma levels of TNF-α and IL-6 results suggested that levels TNF-α and IL-6 in aqueous humor were not related to breakdown of blood-retinal barrier and/ or ocular blood. But, the TNF-α level in aqueous humor was significantly correlated with aqueous humor IL-6. This correlation supported that both TNF- α and IL-6 may be associated with glaucoma [120].

The elevation of intraocular expression of TNF-α and IL-6 may be responsible for causing nerve damage which suggest targeting TNF-α and IL-6 as a mean to inhibit the neurodegenerative mechanisms associated with glaucoma. TNF- α and IL-6 concentrations in aqueous humor are significant with visual field loss in patients with POAG. Thus, TNF- α and IL-6 may be useful pro-inflammatory cytokines levels in aqueous humor of POAG patients [120].

Highlights (Conclusion)

- Primary open-angle glaucoma is described distinctly as a multifactorial optic neuropathy that is chronic and progressive with a characteristic acquired loss of optic nerve fibers.
- A biomarker is a characteristic that is specifically with adequate accuracy and precision measured and evaluated as an indicator of normal biological or pathogenic processes, or to monitor pharmacologic responses to a therapeutic intervention.
- Biomarkers can be useful tools to identify individuals at risk and to measure the outcomes of therapies.
- Biomarkers measured in blood, aqueous humor, or tissues, alone or in combination with each other, could be crucial for the early diagnosis of primary open-angle glaucoma.
- The analytical value of the molecular tests for the detection of the discussed biomarkers will be guaranteed by the validation of relevant methods.
- No definitive molecular biomarker is established to diagnose POAG or predict progression, however clearly tremendous progress has been made. Thus, It will be important in future studies to understand whether a specific biomarker is sensitive not only to disease stage in cohort studies, but also to response to therapy, and/or helpful in progression prediction in longitudinal studies
- Potential uses of these biomarkers include identification of ‘‘at-risk’’ individuals or those in ‘‘preclinical’’ stages; reduction in disease heterogeneity in clinical trials or epidemiologic studies; monitoring the progression of disease, including induction, latency, and clinically detected phases; and as a direct target for therapeutic interventions.

Conflict of interest

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Availability of data

All data generated during this review are included in this study

Standards of reporting

CONSORT guidelines were followed

Authors contributions; Authors interpreted and discussed the data, and wrote the first version of the manuscript. All authors read and approved the final manuscript.

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

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