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ORIGINAL RESEARCH

Serum Alpha-1-Antitrypsin and Folic Acid as Biomarkers for Glaucoma Risk

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Introduction: Glaucoma remains a major cause of irreversible blindness, and many patients progress despite adequate intraocular pressure (IOP) control, suggesting contributions from systemic metabolic and inflammatory factors. This study investigated serum folic acid and α-1-antitrypsin (AAT) levels in glaucoma and non-glaucoma participants and examined their associations with global retinal nerve fiber layer (RNFL) integrity to clarify their potential relevance to glaucoma risk and pathophysiology.

Methods: In this case–control study, 31 open-angle glaucoma (OAG) patients and 26 controls underwent comprehensive ophthalmic assessment, venous blood sampling, and OCT-based global RNFL measurement. Serum folate and AAT were quantified using chemiluminescent immunoassay and ELISA, respectively. Data normality was evaluated with the Shapiro–Wilk test, and correlations were analyzed using Spearman's rank coefficient. Statistical analyses were conducted in Jamovi (version 2.5.5), with significance set at $p < 0.05$.

Results: Glaucoma patients showed significantly higher IOP ($p < 0.001$), lower serum folic acid ($p = 0.007$), and higher AAT levels ($p = 0.028$) compared to controls. In the RNFL subgroup ($n = 48$), glaucoma was associated with reduced global RNFL thickness ($p < 0.001$), lower folic acid ($p = 0.026$), and higher AAT levels ($p = 0.011$). AAT correlated inversely with both folic acid ($rs = –0.485$, $p < 0.001$) and global RNFL thickness ($rs = –0.386$, $p = 0.017$), while glaucoma status correlated positively with AAT and negatively with folic acid levels.

Conclusion: Glaucoma is associated with a distinct systemic biochemical pattern characterized by elevated AAT and reduced folate, alongside structural optic nerve damage. The relationship between AAT, folate and RNFL thickness suggests a potential interaction between inflammatory and metabolic pathways in glaucomatous neurodegeneration. These findings highlight AAT and folate as accessible systemic biomarkers warranting further longitudinal and mechanistic investigation.

Keywords: glaucoma, biomarkers, folic acid, alpha-1-antitrypsin

Introduction

The Global Burden and Pathophysiology of Glaucoma

Glaucoma is a leading cause of irreversible blindness globally and is characterized by progressive degeneration of the optic nerve and corresponding visual field loss. Its prevalence continues to increase with aging populations, and despite clinical heterogeneity across glaucoma subtypes—such as primary open-angle glaucoma (POAG), angle-closure glaucoma, and secondary glaucomas—all forms share the hallmark feature of retinal ganglion cell loss.[1] Reduction of intraocular pressure (IOP) remains the only proven intervention to slow disease progression; however, a substantial proportion of patients experience continued structural or functional deterioration despite achieving recommended IOP targets. These limitations suggest that additional pathogenic mechanisms beyond mechanical IOP-related stress contribute to optic nerve vulnerability. Identification of such mechanisms, including systemic biomarkers, may enhance risk stratification and inform new avenues for therapeutic intervention.[1]

Emerging evidence indicates that systemic metabolic and inflammatory alterations may influence glaucomatous neurodegeneration. Circulating biomarkers measurable in peripheral blood provide a non-invasive opportunity to investigate systemic processes that could modulate retinal ganglion cell survival, microvascular function, and

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extracellular matrix remodeling at the optic nerve head.[2] Among these, folic acid (vitamin B9) and alpha-1-antitrypsin (AAT) have attracted increasing scientific interest.

Folic Acid Metabolism, Homocysteine and Vascular Health

Folic acid is central to one-carbon metabolism, DNA methylation, and the regulation of oxidative stress.[3,4] Deficiency in folate can lead to elevated homocysteine levels, a metabolite implicated in neurotoxicity, endothelial dysfunction, and impaired ocular blood flow.[3,4] Several observational studies have suggested an association between altered folate–homocysteine metabolism and glaucoma, particularly pseudoexfoliation glaucoma, although the evidence remains inconsistent and causality has not yet been established.[5,6]

Multiple epidemiologic studies and meta-analyses have examined the relationship between folate, homocysteine, and glaucoma.[6] Elevated homocysteine—a functional marker of impaired folate-dependent remethylation—has demonstrated more consistent associations with glaucoma risk than serum folic acid levels alone.[7] Some population-based and prospective cohort studies suggest that higher circulating folate concentrations or increased folate intake may be associated with reduced glaucoma risk. However, substantial variability in biochemical measurement methods, residual confounding, and the absence of interventional trials limit causal inference regarding folate status and disease development.[6,7]

Neuroinflammation and Alpha-1-Antitrypsin (AAT)

Similarly, emerging clinical data suggest that elevated systemic AAT concentrations may be associated with glaucoma severity.[8] Recent studies have reported higher plasma AAT levels in glaucoma patients than in healthy controls, with concentrations correlating positively with disease stage.[3] Proposed mechanisms include dysregulated protease–antiprotease balance affecting optic nerve head, extracellular matrix remodeling, as well as systemic inflammatory environments that may propagate local neuroinflammatory processes within the eye.[6,8] However, existing evidence is predominantly cross-sectional, and it remains uncertain whether increased AAT reflects a pathogenic signal contributing to optic nerve damage or a compensatory response to neurodegeneration.

In light of these uncertainties, further investigation is required to elucidate the role of systemic metabolic and inflammatory markers in glaucoma. The present study assesses serum folate and AAT concentrations in patients with glaucoma, compared to control subjects, with the aim of determining their prognostic value and contribution to disease pathogenesis.

Materials and Methods

Study Population and Design

A total of 31 patients with open-angle glaucoma (OAG) and 26 control subjects without glaucoma were enrolled in this case–control study conducted at the Department of Ophthalmology, Pauls Stradins Clinical University Hospital (Riga, Latvia) between February 2024 and August 2025. The study protocol was approved by the Riga Stradins University Committee of Ethics on February 3, 2024, and was performed in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants after the nature and purpose of the study had been fully explained.

Ophthalmic Examination

All participants underwent a comprehensive ophthalmologic examination that comprised best-corrected visual acuity assessment, slit-lamp biomicroscopy, gonioscopy performed with a Volk goniolens, intraocular pressure measurement using an iCare 1000 tonometer, fundoscopic examination with a Volk 90D lens, automated visual field testing (program "Glaucoma extrapolated to HFA Central 30–2," Frey Model AP-300), and optical coherence tomography (OCT) using the Heidelberg Engineering Spectralis HRA+OCT system with the "Glaucoma Overview; RNFL and Asymmetry Analysis" protocol for evaluation of the global retinal nerve fiber layer (RNFL) thickness.

Inclusion Criteria

Glaucoma Group

The glaucoma group consisted of patients with a clinical diagnosis of primary open-angle glaucoma (POAG). Eligibility criteria included the presence of an open anterior chamber angle on gonioscopy (Shaffer grade III or IV), documented untreated intraocular pressure (IOP) greater than 22 mmHg measured by rebound tonometry for at least 3 times, and evidence of glaucomatous optic neuropathy characterized by typical optic nerve head morphological changes such as generalized or focal neuroretinal rim thinning, rim notching, or a vertical cup-to-disc ratio exceeding 0.6, or an interocular asymmetry greater than 0.2.

In addition, participants were required to demonstrate reproducible glaucomatous visual field defects on standard automated perimetry using the Frey Model AP-300 with the "Glaucoma extrapolated to HFA Central 30–2" program.

Patients with secondary forms of glaucoma, normal tension glaucoma, ocular inflammatory conditions, autoimmune diseases, or neurodegenerative disorders were excluded from the study.

Glaucoma RNFL Subgroup

Owing to incomplete data collection or missing data, only 38 of the 57 patients who underwent optical coherence tomography (OCT) using the Heidelberg Engineering Spectralis HRA+OCT system with the "Glaucoma Overview; RNFL and Asymmetry Analysis" protocol for measurement of global retinal nerve fiber layer (RNFL) thickness were eligible for inclusion in the RNFL thickness subgroup analysis.

Control Group

The control group comprised 26 age- and sex-matched healthy subjects with no history of elevated intraocular pressure (≥21 mmHg), absence of exfoliative material on the anterior lens capsule, normal optic disc appearance, normal visual field findings, and no ocular or systemic conditions that could affect the optic nerve.

A normal optic nerve head was defined by well-demarcated disc margins, a healthy pink neuroretinal rim following the ISNT rule (inferior ≥ superior ≥ nasal ≥ temporal), absence of focal or diffuse rim thinning, notching, or optic disc hemorrhages, and a physiologic cup-to-disc ratio appropriate for disc size, with interocular asymmetry not exceeding 0.2.

The retinal nerve fiber layer was required to be intact, without localized or diffuse defects. Normal visual fields on standard automated perimetry were characterized by acceptable reliability indices and the absence of reproducible glaucomatous defects, including arcuate scotomas, nasal steps, paracentral defects, or generalized depression, with visual field sensitivity and global indices within age-adjusted normative limits.

Exclusion Criteria (Both Groups)

Participants were excluded if they had retinal lesions unrelated to glaucoma that could affect visual field results, ocular conditions such as corneal opacities, inflammatory eye disease, or space-occupying lesions involving the optic nerve. Current use of medications including statins, antidepressants, dopaminergic agents, folic acid, or phenytoin also led to exclusion. Co-occurring medical conditions were allowed, except for pulmonary diseases such as chronic obstructive pulmonary disease or emphysema, provided these conditions were clinically stable and did not interfere with the study protocol.

Blood Collection and Biochemical Assays

Venous Blood Sample Collection

Venous blood samples were collected from each participant for the determination of serum AAT and folic acid levels. All samples were obtained from the antecubital vein using standard aseptic venipuncture procedures. Participants were seated comfortably with the arm extended and supported at heart level. After selecting a suitable vein in the antecubital fossa (typically the median cubital vein), the area was disinfected with a 70% isopropyl alcohol swab and allowed to air dry. A sterile tourniquet was applied approximately 5–10 cm above the puncture site to promote venous filling.

Using a sterile vacuum collection system (Vacusera/Becton Dickinson Vacutainer for serum) equipped with a 21-gauge needle, the vein was punctured at a 15–30° angle with the bevel facing upward. The required volume of blood (6 mL) was collected into the appropriate tubes: serum tubes with clot activator for folic acid determination and EDTA-anticoagulated Vacutainer CPT tubes for AAT analysis. Following collection, the tourniquet was released, the needle was

withdrawn, and sterile gauze was applied to the puncture site until hemostasis was achieved. All tubes were immediately labeled and transported for processing.

Folic Acid Biochemical Analysis

For folic acid analysis, samples were collected in the morning and allowed to clot at room temperature. Serum was separated by centrifugation and analyzed using a competitive chemiluminescent enzyme immunoassay (CLIA) on the Siemens Atellica Solution IM analyzer, following the manufacturer's instructions.

Serum Alpha-1-Antitrypsin Biochemical Analysis

For AAT measurement, blood samples were obtained in the morning after participants had fasted for at least 8 hours. Tubes were centrifuged at 3000 rpm for 10 minutes to separate plasma, which was then immediately frozen and stored at −80 °C until analysis. Plasma AAT concentrations were determined using the immunological agglutination test principle with commercially available α1-antitrypsin ELISA kit (Cobas Integra 400 Plus; Roche Diagnostics, Germany), according to the manufacturer's protocol.

The reference ranges provided by the manufacturers were **0.92–2.0 g/L** for serum alpha-1-antitrypsin and **4–26.8 ng/mL** for folic acid.

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. Based on the distribution of variables, appropriate statistical tests were selected: the Mann–Whitney *U*-test was applied for non-parametric data, and the independent samples *t*-test for parametric data in intergroup comparisons. Statistical analyses were performed using Jamovi software (version 2.5.5; The Jamovi Project), and a p-value < 0.05 was considered statistically significant.

To prevent non-independence of data arising from inclusion of both eyes from the same participant, global retinal nerve fiber layer (RNFL) thickness values from the right and left eyes were averaged to obtain a single mean global RNFL measurement per subject. Participants with unavailable global RNFL measurements in one eye—due to image quality issues, ocular pathology, or other technical factors—were excluded from the RNFL subgroup analysis.

Both the entire study cohort and the RNFL subgroup were evaluated for correlations between clinical and biochemical variables. Serum folic acid, AAT concentrations, IOP, and mean global RNFL thickness were each tested for normality using the Shapiro–Wilk test. Depending on the data distribution, correlations between variables were determined using Spearman's rank correlation coefficient for non-parametric data.

To determine the predictive value of glaucoma status on biochemical markers, linear regression analyses were performed using Jamovi (Version 2.5.5).

Results

Participant Characteristics

A total of 57 participants were enrolled, including 32 males and 25 females, with a mean age of 72.3 ± 9.86 years (range from 34 to 91 years). The total number of participants was divided into two major groups – a glaucoma group and a control group.

The glaucoma group consisted of 31 patients (22 males, 9 females; mean age 70.5 ± 8.90 years), while the control group was comprised of 26 participants (10 males, 16 females; mean age 74.3 ± 10.65 years).

Out of the total number of 57 patients, Global RNFL thickness data was available for 38 people (21 males, 17 females; mean age = 73.2 ± 8.02 years), therefore a further subdivision of groups was warranted. A separate analysis was performed between the subgroup of patients with glaucoma and RNFL data available (glaucoma subgroup), and the subgroup of patients with RNFL data available but no history of glaucoma (control subgroup).

The glaucoma subgroup (n = 20) had a mean age of 70.5 ± 8.09 years, while the control subgroup (n = 18) had a mean age of 70.3 ± 6.42 years.

Group Comparisons

IOP was significantly higher in the glaucoma group (median = 20.0, IQR = 17.0–33.0) than in controls (median = 15.0, IQR = 13.8–18.0; U = 698, p < 0.001, r = 0.567). Serum folic acid levels were significantly lower in glaucoma patients (median = 6.8, IQR = 5.1–8.72) compared to controls (median = 8.96, IQR = 6.95–10.77; U = 217, p = 0.007, r = 0.426). In contrast, AAT levels were significantly higher in the glaucoma group (1.55 ± 0.289) than in controls (1.38 ± 0.265), with a mean difference of 0.172 (95% CI [0.019–0.324]; t(52) = 2.26, p = 0.028, d = 0.617).

RNFL Thickness Subgroup Analysis

Within this subgroup, folic acid levels remained significantly lower in glaucoma patients (median = 6.64, IQR = 4.80–8.48) compared to controls (median = 8.09, IQR = 6.95–11.85; U = 104, p = 0.026, r = 0.425). Similarly, AAT concentrations were higher in glaucoma patients (median = 1.65, IQR = 1.30–1.79) than in controls (median = 1.37, IQR = 1.10–1.56; U = 93.0, p = 0.011, r = 0.483).

Global RNFL thickness was significantly reduced in the glaucoma group (73.1 ± 22.3 μm) relative to controls (96.0 ± 12.4 μm), with a mean difference of 22.9 μm (95% CI [10.8–34.9]; t(36) = 3.84, p < 0.001, d = 1.25).

Correlation Analyses

Spearman correlation analysis revealed several significant associations (Table 1). Glaucoma status correlated positively with IOP *(rs* = 0.396, p = 0.002) and AAT *(rs* = 0.310, p = 0.023), and negatively with folic acid *(rs* = –0.368, p = 0.006) and gender *(rs* = –0.326, p = 0.013). Folic acid levels were inversely correlated with AAT *(rs* = –0.485, p < 0.001). Age did correlate negatively with glaucoma status (rs= -0.277, p= 0.041).

In the RNFL subgroup (Table 2), glaucoma correlated positively with IOP *(rs* = 0.578, p < 0.001) and AAT *(rs* = 0.418, p = 0.009), and negatively with global RNFL thickness *(rs* = –0.553, p < 0.001) and folic acid *(rs* = –0.368, p = 0.023). AAT demonstrated inverse correlations with both folic acid *(rs* = –0.623, p < 0.001) and global RNFL thickness

Table 1 Heatmap Displaying the Correlation Matrix of the Analyzed Variables Including Glaucoma, Gender, Folic Acid, Alpha-1-Antitrypsin, IOP, and Age

	Glaucoma	p-value	Gender	p-value	Folic Acid	p-value	Alpha 1 Antitrypsin	p-value	IOP	p-value
Gender	−0,326	0,013			—					
Folic Acid	−0,368	**0,006**	0,128	0,351			—			
Alpha 1 antitrypsin	0,310	**0,023**	−0,075	0,588	−0,485	**<0.001**				
IOP	0,396	**0,002**	−0,251	0,06	0,003	0,984	−0,166	0,23		
Age	−0,277	**0,041**	0,221	0,105	0,077	0,574	0,180	0,194	−0,235	0,084

Notes: Spearman correlations were used to analyze the data with all corresponding p-values displayed. Statistically significant p-values are highlighted in bold.

Table 2 Heatmap Displaying the Correlation Matrix of the Analyzed Variables in the Global RNFL Thickness Subgroup Including Glaucoma, Gender, Folic Acid, Alpha-1-Antitrypsin, Global RNFL Thickness, and IOP

	Glaucoma	p-value	Gender	p-value	Folic Acid	p-value	Alpha 1 Antitrypsin	p-value	G RNFL	p-value
Gender	−0,206	0,214			—					
Folic Acid	−0,368	**0,023**	0,111	0,507			—			
Alpha 1 antitrypsin	0,418	**0,009**	−0,154	0,354	−0,623	**<0.001**				
Global RNFL thickness	−0,553	**<0.001**	0,316	0,053	0,307	0,06	−0,386	**0,017**		
IOP	0,578	**<0.001**	0,075	0,654	−0,249	0,131	0,521	**<0.001**	−0,267	0,105

Notes: Spearman correlations were used to analyze the data with all corresponding p-values displayed. Statistically significant p-values are highlighted in bold.

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Table 3 Linear Regression Analysis of Biochemical Markers for Glaucoma Status

Outcome Variable	Predictor (Glaucoma Status)	Estimate (B)	Std. Error	p-value	R2
A1-Antitrypsin (g/L)	YES vs NO	+0.172	0.076	0.028	0.089
Folic Acid (ng/mL)	YES vs NO	−5.29	2.37	0.030	0.086

(rs = –0.386, p = 0.017), and a positive correlation with IOP *(rs* = 0.521, p < 0.001). A weak positive correlation was observed between folic acid and global RNFL thickness (r = 0.307, p = 0.06), while gender showed a borderline association with global RNFL thickness *(rs* = 0.316, p = 0.053).

Linear Regression Analysis of Biochemical Markers

To further investigate the biochemical profile associated with Glaucoma, separate linear regression analyses were performed for AAT and Folic Acid levels (Table 3). Glaucoma status was a significant predictor for both markers. For AAT, the model was statistically significant (R^2 =0.089, p =0.028), with the Glaucoma group exhibiting significantly higher levels (B = 0.17, SE = 0.07, M = 1.55 g/L) compared to the control group (M = 1.38 g/L). Conversely, the regression model for Folic Acid (R^2 =0.086, p =0.030) revealed that Glaucoma status was associated with a significant reduction in levels (B = −5.29, SE = 2.37); the predicted mean for the Glaucoma group was substantially lower (6.94 ng/mL) than that of the control group (12.23 ng/mL).

Discussion

This study provides clinical evidence that primary open-angle glaucoma (POAG) is associated with systemic biochemical dysregulation, characterized by elevated serum α-1-antitrypsin (AAT) and reduced folic acid levels, alongside increased intraocular pressure (IOP) and marked thinning of the retinal nerve fiber layer (RNFL). These findings support the view that glaucomatous neurodegeneration reflects not only local mechanical stress but also systemic inflammatory and metabolic alterations influencing optic nerve vulnerability.

α-1-Antitrypsin as a Biomarker of Systemic Inflammation in Glaucoma

AAT is a serine protease inhibitor and acute-phase reactant that rises in response to inflammation to counter tissue-damaging enzymes such as neutrophil elastase.[3,4] The observed elevation of AAT in POAG patients likely reflects chronic low-grade systemic inflammation, a mechanism increasingly recognized in glaucoma pathogenesis, particularly through its contributions to vascular endothelial dysfunction and oxidative stress, which impair ocular blood flow and promote axonal injury.[8] In this context, AAT elevation may represent a systemic signature of inflammatory activity contributing to glaucomatous optic neuropathy.[8]

Emerging clinical evidence supports this interpretation. In a recent study involving 163 glaucoma patients and 111 controls, plasma AAT levels were significantly higher in glaucoma and correlated positively with disease severity across two standardized grading systems (HPA and AGIS), with the highest concentrations observed in advanced stages.[3] These findings are consistent with the current results and indicate that circulating AAT might reflect the burden of neural damage.

Historical immunoassay research found AAT elevations primarily in inflammatory ocular conditions such as uveitis and phacoallergic endophthalmitis, whereas phacolytic glaucoma did not show significant alterations.[9] This suggests that AAT expression may depend on the degree and chronicity of ocular inflammation.

Potential Mechanisms of AAT Elevation

The mechanistic basis for AAT elevation in glaucoma remains incompletely understood. One hypothesis is that elevated AAT reflects a protective response intended to counter proteolytic and inflammatory injury at the optic nerve head, limiting extracellular matrix degradation and oxidative tissue injury.[10,11] Experimental work supports a potential neuroprotective role- in diabetic mouse retina, AAT administration reduced microglia-mediated inflammation and neuronal loss,[12] and in experimental glaucoma, AAT attenuated microglial activation and oxidative stress, suggesting direct

cytoprotective effects on retinal ganglion cells (RGCs).[13] In human trabecular meshwork cells, AAT reduced extracellular matrix degradation and oxidative injury, indicating potential structural protection of outflow pathways.[14]

Taken together, these findings suggest that elevated AAT may represent a compensatory response to ongoing neuroinflammatory injury rather than a primary driver of glaucomatous degeneration.[8,12,13]

Correlation Between AAT Levels and RNFL Thickness

The observed inverse correlation in our study between circulating AAT and RNFL thickness may support the notion that systemic inflammation reflects the extent of structural damage. RNFL thickness measured by optical coherence tomography is a validated structural marker of RGC loss.[15] Consistent with our findings, proteomic studies have identified AAT as a candidate biomarker associated with glaucoma severity and neural injury.[3,16] Several mechanisms may underlie this correlation: (1) activated inflammation promotes microglial toxicity and axonal degeneration,[10] elevating AAT as a protective response; (2) oxidative stress may stimulate hepatic AAT production;[10,11] and (3) protease–antiprotease imbalance may facilitate extracellular matrix remodeling at the optic nerve head.[17]

Despite its promise, AAT is influenced by confounding factors including systemic inflammation, hepatic function, smoking, and SERPINA1 genotype.[18,19]

Alpha-1 Antitrypsin and Pulmonary Diseases

Although patients with pulmonary diseases were not included in the our study, it remains important to consider other potential interpretations of ATT levels, particularly those related to ATT deficiency.

Measurement of serum alpha-1 antitrypsin (AAT) is widely available in routine clinical laboratories and represents a reliable, cost-effective screening tool for identifying individuals at risk of alpha-1 antitrypsin deficiency (AATD).[19] Its assessment is clinically relevant not only in patients with established pulmonary disease but also in high-risk populations, particularly current and former heavy smokers.[19,20] Cigarette smoking is a well-established environmental factor that accelerates lung injury by increasing neutrophil elastase activity and oxidative inactivation of AAT, thereby exacerbating the protease–antiprotease imbalance central to the pathogenesis of COPD and emphysema.[20] In individuals with AATD, smoking markedly hastens the decline in lung function and leads to earlier and more severe disease expression.[21] Consequently, reduced serum AAT concentrations in smokers with airflow limitation or emphysema should prompt further diagnostic evaluation, including SERPINA1 genotyping or phenotyping.[20,22]

Early identification of low AAT levels has important implications for risk stratification, smoking cessation counseling, and disease management, as well as for family screening.[22]

Reduced Folic Acid Levels in Glaucoma

We observed significantly lower circulating folate levels in glaucoma patients. Although dietary differences may contribute, the consistent inverse association with glaucoma suggests a potential mechanistic link.[5,6] Folate is crucial for one-carbon metabolism, DNA methylation, and antioxidant defense.[3,4] Chronic inflammation and oxidative stress increase folate demand for DNA repair and glutathione regeneration, which can lead to functional folate depletion.[23–26] Low folate levels in glaucoma patients may therefore reflect increased consumption under oxidative stress, consistent with the concurrent elevation of AAT.

Supporting this, clinical studies report lower folate in glaucoma-related conditions. In a case–control study, serum folate was significantly lower in patients with pseudoexfoliative glaucoma (PEXG) compared with healthy controls, whereas other glaucoma subtypes (POAG, NTG) did not differ from controls.[27] Another study found reduced plasma folate and vitamin B12 in patients with pseudoexfoliation syndrome, with or without glaucoma, compared to healthy individuals.[28] Population-based studies further show that higher blood folate is associated with lower glaucoma prevalence,[6] and prospective cohort data suggest that greater folate intake modestly reduces the risk of exfoliation glaucoma.[7]

Together, these findings suggest that reduced folate may both reflect and contribute to glaucomatous vulnerability, linking systemic oxidative stress to neurodegeneration.

Homocysteine Dysregulation and Glaucoma

The main mechanistic link between folate deficiency and glaucoma involves homocysteine. Folate is required for the remethylation of homocysteine into methionine; therefore, low folate levels lead to increased homocysteine concentrations (hyperhomocysteinemia).[6,7,29] Elevated homocysteine is both neurotoxic and harmful to vascular endothelial cells,[30] which may contribute to glaucoma through endothelial dysfunction. Experimental glaucoma models have shown that higher homocysteine levels can worsen retinal ganglion cell (RGC) loss.[29] Although integrative analyses suggest that homocysteine has only modest predictive value in clinical populations, the biological mechanism linking it to glaucoma remains plausible.[29,31]

In addition, the enzymatic defects reported by Zemitis highlight the important interaction between B-vitamins in maintaining ocular metabolic balance. The production of neuroprotective kynurenine metabolites depends heavily on vitamin B6.[32] Because vitamins B9 (folate) and B6 work together in one-carbon metabolism to regulate homocysteine levels, deficiencies in either vitamin may disrupt both the methylation cycle and the kynurenine pathway.[33] This disruption could increase the risk of glaucomatous neurodegeneration.[33] Folate supplementation has been shown to lower homocysteine levels and improve endothelial function in humans.[34]

Since impaired optic nerve perfusion is a well-recognized factor in glaucoma—particularly in normal-tension and exfoliation glaucoma—a vascular mechanism linking folate deficiency to glaucomatous damage is therefore plausible.[29,35]

Correlation Between Folate and RNFL Thickness

In line with these mechanistic insights, we observed a positive trend between folic acid levels and retinal nerve fiber layer (RNFL) thickness in our cohort, although this did not reach statistical significance. This observation aligns with animal studies showing that vitamins involved in one-carbon metabolism can reduce RGC loss in experimental glaucoma.[31] Folate deficiency may increase neuronal vulnerability by promoting oxidative stress, reducing methylation capacity, and altering extracellular matrix remodeling.[24,27,29,36,37] Clinically, serum folate is an easily accessible biomarker. In patients with low folate or elevated homocysteine, nutritional optimization or supplementation could represent a low-risk strategy to support neuroprotection,[6,7,29,34] though clinical trials are required before formal recommendations can be made.

A Unifying Mechanism: Tetrahydrobiopterin (BH4)

The concomitant findings of elevated Alpha-1 Antitrypsin (AAT) levels and reduced folate concentrations may indicate a common upstream disturbance in redox metabolism. A recent study (2025) titled "Altered Biopterin-Related Cofactor Metabolism in the Aqueous Humor of Glaucoma Patients" highlights a potential association between glaucoma and impaired tetrahydrobiopterin (BH4) salvage pathways. This study suggests that disrupted BH4 regeneration may contribute to the progression of glaucoma.[38] Tetrahydrobiopterin (BH4) serves as a critical cofactor for endothelial nitric oxide synthase (eNOS).[38] A deficiency in BH4 can lead to eNOS "uncoupling," wherein eNOS produces superoxide instead of nitric oxide, thereby generating oxidative stress and impairing vascular function.[38–40]

In this context, the increased oxidative burden could stimulate hepatic AAT production as part of an acute-phase response, while simultaneously consuming folate through various antioxidant and repair pathways.[39,40] This framework positions the elevation of AAT and the depletion of folate as parallel downstream consequences of a shared oxidative pathology.

Implications

These findings support a multifactorial conceptual model of glaucoma in which mechanical IOP-related stress interacts with systemic inflammation and impaired one-carbon metabolism to influence optic nerve susceptibility. Future research should determine whether biochemical profiles involving AAT and folate can improve risk stratification, serve as progression biomarkers, or guide the development of adjunctive neuroprotective strategies.

Limitations of the Study and Future Directions

The observed associations, provide a strong rationale for prospective research. This study has several limitations that should be considered when interpreting the findings:

1. This study is cross-sectional and thus cannot establish causality; therefore, it remains unclear whether reduced folic acid levels contribute to global retinal nerve fiber layer (RNFL) thinning or simply reflect more advanced glaucomatous damage.
2. Folic acid status is influenced by dietary intake, systemic conditions, and medication use, and although major confounders were addressed, residual and unmeasured variables may have affected the associations.
3. Glaucoma is a heterogeneous disease, and the relationship between folic acid metabolism, AAT, and global RNFL integrity may vary across subtypes such as primary open-angle, normal-tension, and exfoliative glaucoma.

Future directions of research could include:

1. Longitudinal analyses to determine whether baseline folic acid and AAT levels predict rates of global RNFL thinning and glaucoma progression over time.
2. Intervention trials evaluating whether folic acid supplementation or modulation of one-carbon metabolism can influence structural or biochemical markers of disease.
3. Mechanistic studies investigating how folic acid availability and AAT activity affect retinal ganglion cell metabolism, mitochondrial resilience, and optic nerve microcirculation.
4. Expanded metabolic profiling including oxidative stress markers, BH4 bioavailability, homocysteine, and other folate-cycle intermediates to clarify underlying pathways.
5. Subtype-specific evaluations to determine whether associations differ across primary open-angle, normal-tension, and pseudoexfoliative glaucoma.
6. Genetic analyses examining whether SERPINA1 variants modify vulnerability or progression patterns.

Together, these lines of inquiry would be essential to determine whether folate metabolism and AAT represent modifiable biomarkers or therapeutic targets in the preservation of optic nerve structure.

Conclusions

In conclusion, this study provides evidence that glaucoma is associated with a systemic biochemical profile involving serum alpha-1 antitrypsin (AAT) and folate levels. Our results indicate that in glaucoma patients, elevated serum AAT and reduced folate levels are associated with greater disease risk and severity.

These findings highlight the potential value of AAT and folate as systemic biomarkers linked to glaucoma severity and neurostructural loss. Future research should investigate whether these biochemical changes actively contribute to disease progression or reflect a compensatory response. Additionally, exploring whether interventions that modulate folate status or inflammatory pathways could provide therapeutic benefits is warranted.

Abbreviations

AGIS, Advanced Glaucoma Intervention Study; AUC, area under the curve; ATT, α-1-antitrypsin; AATD, alpha-1 antitrypsin deficiency; BH4, tetrahydrobiopterin; CLIA, chemiluminescent enzyme immunoassay; CPT, cell preparation tubes; DNA, deoxyribonucleic acid; EDTA, Ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; HPA, Hodapp, Parrish, and Anderson classification; IOP, intraocular pressure; NO, nitric oxide; OCT, optical coherence tomography; POAG, primary open angle glaucoma; RGC, retinal ganglion cell; ROC, Receiver operating characteristic; RNFL, retinal nerve fibre layer.

Data Sharing Statement

All data generated or analysed during this study are presented within this article. Additional information is available upon request by contacting the corresponding author.

Ethics Approval and Informed Consent

The research adhered to the ethical principles delineated in the Declaration of Helsinki. Stringent ethical scrutiny was applied to the study's considerations, resulting in approval from the Medical Ethics Committee of Rigas Stradins University, denoted by decision number 2-PĒK-4/225/2025. Furthermore, the requisite approval was obtained from the Pauls Stradins Clinical University Hospital. Patients provided written informed consent to participate in the study.

Author Contributions

Liva Caikovska is the first author. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare no conflicts of interest in relation to this work.

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