• Clinical Research •

Aqueous humor adipokine profile of primary open angle glaucoma patients and cataract patients with or without metabolic disorders

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Received: 2024-08-13 Accepted: 2025-02-25

Abstract

• **AIM:** To investigate the role of adipokines in primary open angle glaucoma (POAG) by comparing the levels of these molecules in the aqueous humor among POAG patients and cataract patients with or without metabolic disorders.

• **METHODS:** In this cross-sectional study, aqueous humor samples of 22 eyes of POAG patients (POAG group), 24 eyes of cataract patients without metabolic disorders (cataract group), and 24 eyes of cataract patients with metabolic disorders (cataract+metabolic disorders group) were assessed for 15 adipokines by Luminex bead-based multiplex array. The correlation between aqueous humor adipokines and clinical indicators of POAG was analyzed and compared across the groups.

• **RESULTS:** The analysis revealed that the levels of adiponectin, leptin, adipsin, retinol-binding protein 4 (RBP4), angiopoietin-2, angiopoietin-like protein 4 (ANGPTL4), chemokine (C-C motif) ligand 2 (CCL2), interleukin-8 (IL-8), and interleukin-18 (IL-18) in the aqueous humor of the POAG group were significantly higher than those in the cataract group. Additionally, the level of angiopoietin-2 in the POAG group was higher than in the cataract+metabolic disorders group. However, no significant correlation was found between the levels of adipokines in the POAG group and intraocular pressure (IOP), severity of POAG, or the use of glaucoma medications.

• **CONCLUSION:** This study demonstrates significant differences in aqueous humor adipokine levels between POAG and cataract patients. The findings suggest that the levels of aqueous humor adipokines may reflect the inflammatory states in POAG and systemic metabolic abnormalities.

• **KEYWORDS:** glaucoma biomarkers; adipokine; primary open angle glaucoma; aqueous humor; metabolic disorder
DOI:10.18240/ijo.2025.04.07

Citation: Xia ZY, Xu K, Zhang WJ, Wu DF, Pan Z, Xian HC, Li XM, Wang HZ, Zhang C. Aqueous humor adipokine profile of primary open angle glaucoma patients and cataract patients with or without metabolic disorders. *Int J Ophthalmol* 2025;18(4):615-626

INTRODUCTION

Glaucoma is a leading cause of irreversible blindness globally, affecting an estimated 76 million individuals. This number is projected to rise to approximately 111.8 million by 2040[1]. The main lesion of primary open angle glaucoma (POAG) is irreversible damage to the optic nerve, which seriously threatens vision. POAG includes normal-tension glaucoma, in which patients may experience progressive visual field damage despite having intraocular pressure (IOP) controlled within the normal range or even lower. Recent research has expanded beyond the traditional hypotheses of mechanical pressure and microvascular abnormalities to include excessive inflammation as a potential pathogenic mechanism of glaucoma[2-3]. While the retina typically maintains a state of para-inflammatory homeostasis, excessive

inflammatory responses can result in optic nerve damage[2]. In recent years, numerous studies have reported significant differences in the levels of cytokines in the aqueous humor between patients with POAG and healthy controls. Cytokines such as interleukin (IL)-5, IL-8, IL-10, IL-12, IL-15, interferon-gamma (IFN-γ), macrophage inflammatory protein-1alpha (MIP-1α), and tumor necrosis factor alpha (TNF-α) were found to be significantly elevated in the aqueous humor of POAG patients[4-6], while levels of IL-6 and chemokine (C-C motif) ligand 2 (CCL2) were significantly reduced[7]. Additionally, some studies have found an association between POAG and metabolic abnormalities, with evidence indicating that a higher proportion of POAG patients have dyslipidemia[8]. Other research indicated that high triglyceride levels or metabolic syndrome is an independent risk factor for glaucoma[9-10]. Some studies also suggested that metabolic syndrome as a risk factor for POAG may be associated with the genotypes and allele frequencies of related genes[11]. However, the role of metabolic abnormalities as a pathogenic factor in POAG remains unclear. Adipokines primarily consist of hormones, growth factors, angiogenic factors and cytokines[12]. These active proteins are secreted by various tissues, including adipose tissue, vascular stromal cells, and macrophages. Adipokines exert significant effects on the brain, liver, pancreas, immune system, vascular system, and muscles, playing crucial roles in glucose metabolism, lipid metabolism, and inflammation regulation[13-14]. Besides metabolic diseases, serum adipokine levels have been increasingly linked to a variety of other conditions. For example, pro-inflammatory adipokines such as leptin and resistin have been associated with dementia, cognitive disorders, and Alzheimer's disease in cerebrospinal fluid and serum[15]. Through the increased permeability of the blood-brain barrier, accumulation of chronic systemic inflammation may lead to neuroinflammation. Adiponectin levels in cerebrospinal fluid have also been found to be independent of plasma adiponectin levels, suggesting potential intrathecal synthesis of adipokines[16]. POAG is also considered as a neurodegenerative disease. We hypothesize that the levels of adipokines may reflect the imbalance of the intraocular immune microenvironment in POAG. Moreover, the expression of adipokines in the aqueous humor of POAG patients may reflect the state of optic nerve injury in glaucoma, providing insights into the pathogenesis of POAG.

However, there are currently few studies examining the expression of adipokines in aqueous humor among glaucoma patients. This study aims to detect the levels of aqueous humor adipokines in POAG patients and to explore the relationship between adipokines and the disease status of POAG by comparing these levels with those in cataract patients with or without metabolic disorders.

PARTICIPANTS AND METHODS

Ethical Approval This study was approved by the Ethics Committee of Peking University Third Hospital (No.M2023196) and registered with ClinicalTrials.gov (NCT06190119). The experiment was conducted in accordance with the principles outlined in the Declaration of Helsinki. All patients were informed about the procedure and signed the consent forms.

Participants and Inclusion Criteria A total of 22 eyes of POAG patients (POAG group), 24 eyes of cataract patients without metabolic disorders (cataract group, serving as healthy controls), and 24 eyes of cataract patients with metabolic disorders (cataract+metabolic disorders group) scheduled for trabeculectomy or cataract surgery from May to October 2023 were included in this cross-sectional study. The diagnostic criteria for POAG followed the European Glaucoma Society Terminology and Guidelines for Glaucoma[17]. Exclusion criteria were as follows: previous intraocular surgery such as anti-glaucoma surgery, cataract surgery, or pars plana vitrectomy (PPV); the presence of uveitis, age-related macular degeneration (AMD), diabetic retinopathy (DR), retinal vascular obstruction, or secondary glaucoma; systemic autoimmune or inflammatory diseases; use of anti-inflammatory eye drops within 6mo prior to surgery. The POAG group was further divided into two subgroups based on the severity of visual field defects, using a mean deviation cutoff point of -12 dB[18]. POAG patients with a mean deviation <-12 dB were classified as having severe glaucoma, while those with a mean deviation ≥-12 dB were classified as having mild to moderate glaucoma. All patients underwent comprehensive ophthalmic examinations before surgery, including best-corrected visual acuity (BCVA), IOP measurement, slit-lamp examination, fundus photography, and optical coherence tomography (OCT; Cirrus HD OCT; Carl Zeiss Meditec). A 30-2 visual field examination was also performed on POAG patients using the Humphrey Field Analyzer (Carl Zeiss Meditec, USA). IOP was measured with the applanation tonometer at the last visit before surgery. Demographic data (age, sex, height, weight) were collected for all 70 patients, along with fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels. Additionally, each patient's history of systemic diseases, ocular diseases, and glaucoma medications was recorded.

Metabolic disorders in this study were defined as the presence of hyperlipidemia and/or diabetes and/or metabolic syndrome and/or obesity. According to the "Guideline for the prevention and treatment of type 2 diabetes mellitus in China (2016)"[19], hyperlipidemia is defined as TC≥6.2 mmol/L (240 mg/dL),

or LDL-C≥4.1 mmol/L (160 mg/dL), or TG≥2.3 mmol/L (200 mg/dL) in the absence of lipid-lowering agents, meeting the above criteria. And repeated examination results did not change the diagnosis of hyperlipidemia. Alternatively, a patient with a history of hyperlipidemia who is currently on lipid-lowering agents and has serum lipid levels below these thresholds is also diagnosed with hyperlipidemia. Diabetes was defined as fasting plasma glucose >7.0 mmol/L, self-reported diabetes, or currently taking hypoglycemic medications. According to the "Guideline for the prevention and treatment of type 2 diabetes mellitus in China (CDS 2020)"[20], metabolic syndrome is diagnosed if three or more of the following criteria are met: 1) central obesity: waist circumference ≥90 cm (male) or ≥85 cm (female); 2) blood pressure ≥130/85 mm Hg and/or have been diagnosed with hypertension and treated; 3) FPG≥6.1 mmol/L or ≥7.8 mmol/L 2h after fasting plasma glucose loading and/or diagnosed diabetes mellitus and/or treated; 4) fasting TG≥1.7 mmol/L 5) fasting HDL-C <1.04 mmol/L. Obesity is classified according to body mass index (BMI) as follows: normal body weight is 18.5≤BMI<24 kg/m^2; overweight is 24≤BMI<28 kg/m^2, obesity is BMI ≥28 kg/m^2[21].

Sample Collection A clear corneal puncture was performed with a 30G needle at the beginning of trabeculectomy or cataract surgery. A sample of aqueous humor (100 μL) was aspirated, taking care to prevent contamination from blood and intraocular tissues. After obtaining the sample, the anterior chamber was reconstructed with balanced salt solution, and the surgery proceeded as usual. Aqueous humor was taken from only one eye of each patient during the first operation. All samples are immediately frozen and stored at -80°C until analysis. All patients were given 0.5% levofloxacin eye drops 4 times a day for 3d before the surgery. Patients undergoing cataract surgery were administered topical mydriatic consisting of 1% tropicamide and 5% norepinephrine every 15min for 1h before the surgery. Anesthetics (0.4% oxybuprocaine hydrochloride) were instilled into the surgical eye every 5min for 20min before the surgery.

Multiplex Analysis of Adipokines in Aqueous Humour Samples The adipokines adiponectin, chemerin, leptin, resistin, adipsin, visfatin, lipocalin-2, retinol-binding protein 4 (RBP4), angiopoietin-2, angiopoietin-like protein 4 (ANGPTL4), CCL2, C-X-C motif chemokine ligand 5 (CXCL5), IL-1β, IL-6, IL-8, and IL-18 were analyzed using a multiplex immunobead system based on xMAP technology (Luminex, Austin, TX, USA), according to the manufacturer's instructions. Data were acquired using a Luminex-compatible workstation and its manager software (Bio-Plex workstation and version 6.0 software; Bio-Rad, Tokyo, Japan), according to the manufacturer's instructions. Undiluted aqueous humor samples were analyzed simultaneously for the adipokines.

Table 1 Detection range of adipokines

Adipokines	Limit of detection (pg/mL)
Adiponectin	823-200000
Chemerin	432-105000
Leptin	494-120000
Resistin	53.5-13000
Adipsin	724-175000
Lipocalin-2	1.23-30000
RBP4	609-150000
Angiopoietin-2	94.7-23000
ANGPTL4	1850-450000
Visfatin	12200-2975000
CCL2	30.9-7500
CXCL5	53.3-12500
IL-1β	17.7-4300
IL-6	4.53-1100
IL-8	4.12-1000
IL-18	7.12-1730

RBP4: Retinol-binding protein 4; ANGPTL4: Angiopoietin-like protein 4; CCL2: Chemokine (C-C motif) ligand 2; CXCL5: C-X-C motif chemokine ligand 5; IL: Interleukin.

The multiplex assay kit quantitatively measures multiple adipokines from minimal bodily fluids [for the limit of detection (LOD) range; Table 1]. Each sample was run as a single measurement due to the limited quantity of collected aqueous humor. When detected concentrations were outside the LOD range, they were calculated using the assignment method. Values below the lower LOD were counted as LOD/2 for analysis, while values exceeding the upper LOD were counted as LOD×2 for analysis. The standard of reliability for adipokine detection in the aqueous humor is that the adipokine level of the sample above 50% is not lower than the minimum standard concentration obtained from the standard curve.

Statistical Analysis Data are presented as mean±standard deviation (SD). All statistical analyses were performed using SPSS v27.0 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to assess the normality of data distribution. For the comparison of continuous variables, such as adipokine concentrations, age, FPG, or TG among groups, the Kruskal-Wallis test was conducted with post hoc analysis using Dunn's test. One-way analysis of variance (ANOVA) was used to examine differences in BMI, TC, HDL-C, and LDL-C among groups; if significant, Bonferroni post hoc analysis was used for pairwise comparisons. Fisher's exact test was employed to analyze differences in sex and eye laterality between groups. Mann-Whitney *U* test was used to compare adipokine levels between POAG subgroups. Spearman's correlation test calculated the correlations between the concentration of each adipokine, IOP, mean deviation, and the number of glaucoma

Table 2 Patient characteristics in each group mean±SD

Characteristics	POAG (*n*=22)	Cataract (*n*=24)	Cataract+metabolic disorders (*n*=24)	*P*
Sex, male/female	12/10	13/11	13/11	1.000
Age, y	61.05±10.83	62.92±12.11	61.16±6.28	0.565
Eye, OD/OS	14/8	11/13	11/13	0.384
BMI, kg/m^2	23.27±2.53	23.22±2.66	24.62±3.49	0.280
FPG, mmol/L	5.77±1.01	5.32±0.51	7.07±2.45	0.041[a]
TC, mmol/L	4.55±1.03	4.45±0.74	5.39±1.13	0.025[a]
TG, mmol/L	1.04±0.41	1.20±0.30	2.15±1.24	0.001[a]
HDL-C, mmol/L	1.40±0.31	1.24±0.23	1.31±0.31	0.265
LDL-C, mmol/L	2.74±0.83	2.70±0.63	3.06±1.03	0.614

POAG: Primary open angle glaucoma; SD: Standard deviation; OD: Right eye; OS: Left eye; BMI: Body mass index; FPG: Fasting plasma glucose; TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol. [a]*P*<0.05.

medications. Point-biserial correlation was used to analyze the correlation between each adipokine and the use of specific glaucoma medications. Statistical significance was set at *P*<0.05.

RESULTS

Patient Characteristics Table 2 presented the characteristics of all subjects in this study. No significant difference was observed in age, gender, eye laterality, HDL-C, or LDL-C among the groups (all *P*>0.05). FPG, TC, and TG were significantly elevated in the cataract+metabolic disorders group compared to the cataract group and POAG group (*P*=0.041, *P*=0.025, *P*=0.001, respectively). Within the POAG subgroups, no significant differences were noted in age, gender, eye laterality, FPG, TC, TG, HDL-C, or LDL-C (all *P*>0.05; Table 3).

Table 3 Patient characteristics in subgroups of POAG group mean±SD

Characteristics	Mild & moderate POAG (*n*=8)	Severe POAG (*n*=14)	*P*
Sex, male/female	4/4	8/6	1.000
Age, y	62.38±6.78	60.29±12.77	0.494
Eye, OD/OS	6/2	8/6	0.649
BMI, kg/m^2	24.16±2.82	22.75±2.30	0.133
FPG, mmol/L	5.40±0.42	6.10±1.27	0.175
TC, mmol/L	4.68±1.07	4.45±1.04	0.791
TG, mmol/L	0.82±0.36	1.20±0.40	0.064
HDL-C, mmol/L	1.55±0.30	1.29±0.28	0.152
LDL-C, mmol/L	2.81±0.91	2.69±0.82	0.711

POAG: Primary open angle glaucoma; SD: Standard deviation; OD: Right eye; OS: Left eye; BMI: Body mass index; FPG: Fasting plasma glucose; TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol.

Adipokine Levels of Aqueous Humor in Each Group While visfatin was below the lower LOD in most samples, the concentrations of the remaining 15 adipokines were measurable. Table 4 and Figure 1 showed the levels of adipokines in aqueous humor samples from the POAG group, cataract group, and cataract+metabolic disorders group. The concentration of adiponectin, leptin, adipsin, RBP4, angiopoietin-2, ANGPTL4, CCL2, IL-8 and IL-18 were significantly elevated in the POAG group than in the cataract group (*P*=0.010, *P*=0.012, *P*=0.004, *P*=0.000, *P*=0.001, *P*=0.021, *P*=0.027, *P*=0.025, *P*=0.035 respectively). Angiopoietin-2 was significantly higher in the POAG group than in the cataract+metabolic disorders group (*P*=0.002). There were no significant differences in adipokine concentrations between the POAG subgroups (*P*>0.05; Table 5).

Associations Between Aqueous Adipokines Levels and Clinical Indicators in POAG group We examined the associations between aqueous adipokine levels and clinical indicators in the POAG group, including preoperative IOP, mean deviation values, the number of preoperative glaucoma medications, and the use of specific glaucoma medications such as prostaglandin analogues, carteolol, brinzolamide, or brimonidine. Apart from angiopoietin-2, which exhibited a moderate correlation with the use of brimonidine (*r*=0.499, *P*=0.036), no other clinical indicators were significantly correlated with adipokine levels (Table 6).

DISCUSSION

This study investigated the levels of adipokines in the aqueous humor of patients with POAG compared to cataract patients with or without metabolic disorders. To our knowledge, this is the first study to assess aqueous humor adipokine levels specifically in POAG patients. The inclusion criteria were designed with the consideration that plasma adipokine levels in patients with metabolic disorders differ from those in healthy controls, which may also affect aqueous humor adipokine levels; hence, the cataract+metabolic disorders group was included. Our findings reveal that levels of adiponectin, leptin, adipsin, RBP4, angiopoietin-2, ANGPTL4, CCL2, IL-8, and IL-18 were significantly elevated in the aqueous humor of POAG patients compared to those in the cataract group. Notably, the concentration of angiopoietin-2 was also higher in

Table 4 Quantification and comparison of the concentrations of adipokines in the aqueous humor by group mean±SD, pg/mL

Characteristics	POAG	Cataract	Cataract+metabolic disorders	*P*	*P*		
					Cataract *vs* cataract+metabolic disorders	POAG *vs* cataract	POAG *vs* cataract +metabolic disorders
Adiponectin	57422.69±38778.69	28316.35±22948.85	53010.43±67789.51	0.013	0.700	0.010[a]	0.231
Chemerin	10666.38±4568.35	7887.73±2917.27	9160.02±4338.18	0.202			
Leptin	140.45±103.89	80.09±92.22	121.03±126.95	0.015	0.646	0.012[a]	0.280
Resistin	68.69±60.96	41.56±20.01	70.85±97.92	0.400			
Adipsin	110462.49±61691.1	62434.17±25549.81	97256.82±106070.42	0.004	1.000	0.004[a]	0.060
Lipocalin-2	7384.34±4243.49	5288.88±2228.25	7104.53±9604.85	0.337			
RBP4	2538856.15±1414748.02	567528.22±1118866.93	1504139.55±1612176.37	<0.001	0.064	0.000[a]	0.058
Angiopoietin-2	154.49±67.35	92.43±55.86	125.67±154.89	<0.001	1.000	0.001[a]	0.002[a]
ANGPTL4	3386.55±1911.28	1932.21±937.81	9140.11±21136.43	0.015	0.072	0.021[a]	1.000
CCL2	854.16±432.18	573.03±207.65	673.86±351.89	0.027	1.000	0.027[a]	0.173
CXCL5	22.2±12.48	15.39±8.46	18.69±14.42	0.166			
IL-1β	0.83±0.55	0.76±0.57	0.75±0.49	0.72			
IL-6	13.23±36.48	3.62±3.15	25.4±90.94	0.119			
IL-8	11.26±9.05	5.88±3.72	9.19±10.78	0.026	1.000	0.025[a]	0.176
IL-18	14.44±5.51	10.12±4.95	11.1±7.69	0.021	1.000	0.035[a]	0.062

POAG: Primary open angle glaucoma; SD: Standard deviation; RBP4: Retinol-binding protein 4; ANGPTL4: Angiopoietin-like protein 4; CCL2: Chemokine (C–C motif) ligand 2; CXCL5: C-X-C motif chemokine ligand 5; IL: Interleukin. [a]*P*<0.05.

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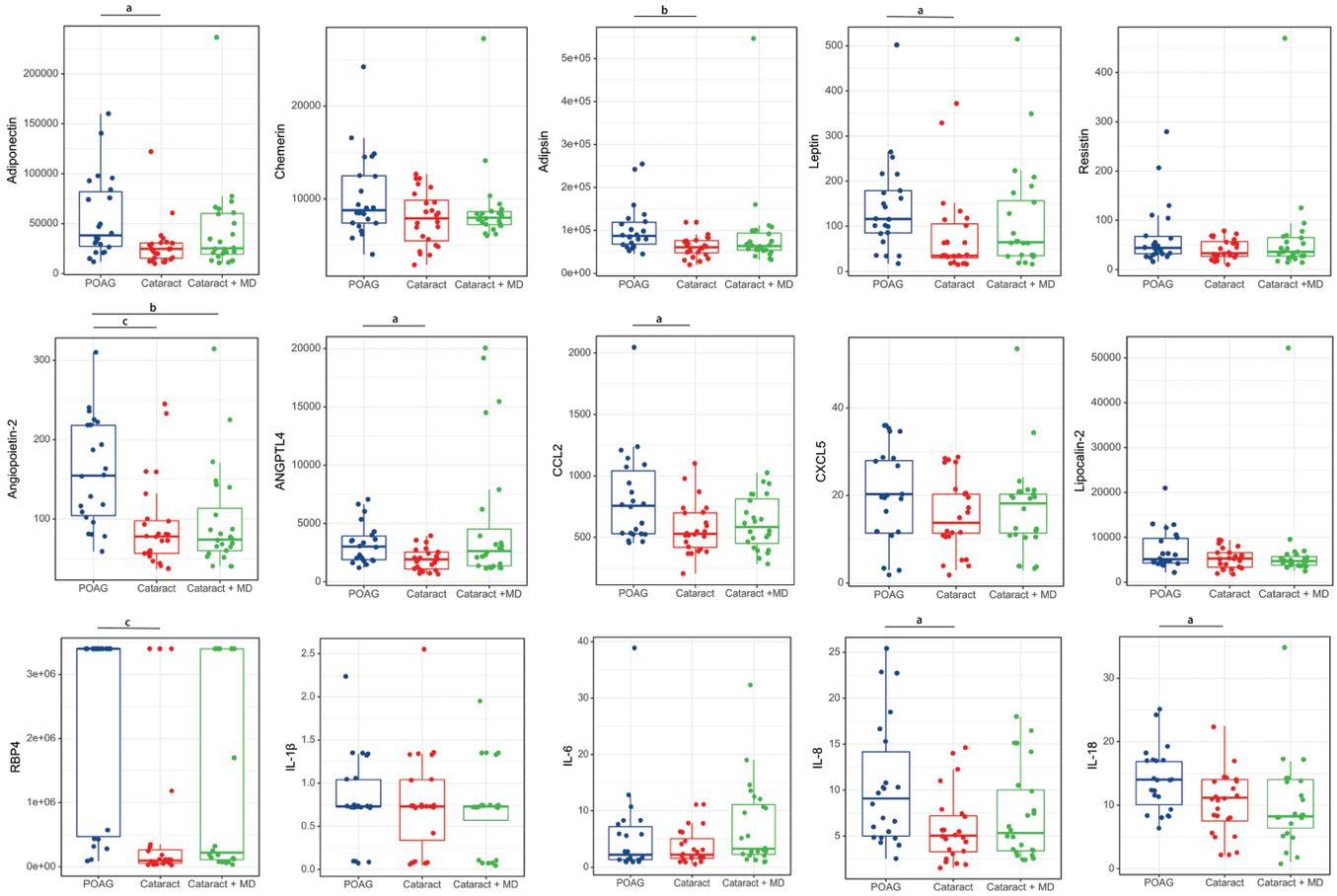

Figure 1 Levels of adipokines in aqeous humor samples collected from three groups [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001. MD: Metabolic disorders; POAG: Primary open angle glaucoma; ANGPTL4: Angiopoietin-like protein 4; CCL2: Chemokine (C-C motif) ligand 2; CXCL5: C-X-C motif chemokine ligand 5; RBP4: Retinol-binding protein 4; IL: Interleukin.

Table 5 Quantification and comparison of the concentrations of adipokines in the aqueous humor by POAG subgroup mean±SD, pg/mL

Characteristics	Mild & moderate POAG	Severe POAG	*P*
Adiponectin	60183.32±33293.68	54061.76±54061.76	0.413
Chemerin	9666.25±4148.97	10587.08±10587.08	0.682
Leptin	165.04±50.11	130.84±130.84	0.075
Resistin	75.48±59.55	64.81±64.81	0.219
Adipsin	89291.24±23690.76	111839.62±111839.62	0.785
Lipocalin-2	6777.52±3621.21	7483.07±7483.07	0.946
RBP4	2644744.82±1401730.74	2293474.65±2293474.65	0.563
Angiopoietin-2	156.5±68	160.17±160.17	0.918
ANGPTL4	3377.45±1646.21	3189.68±3189.68	0.453
CCL2	805.05±326.11	820.52±820.52	0.891
CXCL5	21.81±8.2	20.26±20.26	0.835
IL-1β	1.07±0.52	0.68±0.68	0.098
IL-6	7.9±12.79	4.31±4.31	1.000
IL-8	8.41±4.77	11.75±11.75	0.357
IL-18	15.06±5.02	13.38±13.38	0.389

POAG: Primary open angle glaucoma; SD: Standard deviation; RBP4: Retinol-binding protein 4; ANGPTL4: Angiopoietin-like protein 4; CCL2: Chemokine (C–C motif) ligand 2; CXCL5: C-X-C motif chemokine ligand 5; IL: Interleukin.

the POAG group compared to the cataract+metabolic disorders group. Although most adipokine levels were higher in the cataract+metabolic disorders group relative to the cataract group, these differences did not reach statistical significance. This suggests that while systemic metabolic abnormalities might influence aqueous humor adipokine levels, the impact

Table 6 Correlation of aqueous humor adipokines level with IOP, mean deviation, number of glaucoma medications, or whether using specific eye drops in POAG Patients

Items	IOP		Mean deviation		Number of glaucoma medications		Topical prostaglandin analogues or not		Topical carteolol or not		Topical brinzolamide or not		Topical brimonidine or not	
	Correlation coefficient	*P*	Correlation coefficient	*P*	Correlation coefficient	*P*	Correlation coefficient	*P*	Correlation coefficient	*P*	Correlation coefficient	*P*	Correlation coefficient	*P*
Adiponectin	-0.071	0.754	0.072	0.751	-0.036	0.873	-0.134	0.552	-0.110	0.627	-0.057	0.803	0.009	0.967
Chemerin	-0.414	0.056	0.301	0.174	0.042	0.853	0.255	0.252	0.163	0.468	0.279	0.208	-0.109	0.629
Leptin	-0.131	0.560	-0.288	0.193	0.402	0.064	-0.144	0.522	0.193	0.388	0.147	0.513	0.417	0.053
Resistin	-0.175	0.436	0.249	0.265	-0.112	0.619	0.073	0.746	-0.062	0.784	-0.073	0.746	0.257	0.249
Adipsin	-0.278	0.211	0.259	0.244	0.023	0.920	0.129	0.567	0.047	0.835	0.161	0.475	0.147	0.513
Lipocalin-2	-0.308	0.163	0.276	0.214	0.040	0.861	0.200	0.371	0.185	0.411	0.307	0.165	-0.133	0.556
RBP4	-0.186	0.408	0.043	0.849	0.145	0.519	-0.271	0.223	0.099	0.663	0.161	0.474	0.039	0.864
Angiopoietin-2	-0.325	0.141	0.061	0.787	0.066	0.771	-0.304	0.170	0.101	0.655	0.209	0.350	0.449	0.036[a]
ANGPTL4	0.037	0.869	-0.018	0.938	0.136	0.545	0.140	0.533	-0.163	0.469	-0.021	0.926	0.269	0.227
CCL2	-0.074	0.745	0.075	0.740	0.058	0.799	0.211	0.347	-0.230	0.303	0.090	0.691	0.000	1.000
CXCL5	-0.388	0.075	0.178	0.427	0.219	0.327	0.239	0.283	0.097	0.667	0.151	0.502	0.152	0.499
IL-1β	-0.216	0.333	0.001	0.996	0.032	0.886	-0.010	0.966	0.330	0.134	-0.049	0.828	-0.300	0.176
IL-6	0.057	0.800	0.061	0.786	-0.199	0.375	0.137	0.544	-0.370	0.090	0.122	0.589	0.048	0.833
IL-8	-0.111	0.624	0.203	0.365	0.194	0.386	0.321	0.145	-0.246	0.271	0.107	0.635	0.325	0.140
IL-18	-0.362	0.098	0.021	0.926	0.098	0.665	0.076	0.738	-0.165	0.463	0.139	0.538	0.239	0.285

IOP: Intraocular pressure; POAG: Primary open angle glaucoma; RBP4: Retinol-binding protein 4; ANGPTL4: Angiopoietin-like protein 4; CCL2: Chemokine (C-C motif) ligand 2; CXCL5: C-X-C motif chemokine ligand 5; IL: Interleukin. [a]*P*<0.05.

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of POAG is more pronounced, possibly due to the excessive inflammatory activation state in the eye of POAG.

Recent studies have increasingly indicated that inflammatory responses play a critical role in optic nerve injury associated with glaucoma. Typically, mild para-inflammation plays a role in maintaining retina or trabecular meshwork tissue homeostasis and restoring its functionality. However, excessive inflammation can result in retinal ganglion cell (RGC) death, optic nerve injury, and impaired trabecular outflow function. Proteins secreted by the retina enter the vitreous body, with most diffusing into the aqueous humor in the anterior chamber and subsequently exiting the eye through the trabecular meshwork. The protein concentration in the aqueous humor reflects not only the secretion of proteins from various tissues adjacent to the anterior chamber but also their levels in the vitreous body and retina. When the blood-aqueous barrier is broken down, the protein concentration in the aqueous humor is also influenced by the protein concentration in the blood. The varied expression profiles of inflammatory markers have confirmed the involvement of inflammatory activation in the pathogenesis of POAG, suggesting that some of these inflammation-related cytokines could serve as potential biomarkers for the disease. Previous research has demonstrated altered cytokine levels in the aqueous humor of glaucoma patients compared to healthy individuals. The sources of these cytokines within the anterior chamber remain unclear, though they may be secreted by resident cells in the iris, trabecular meshwork, and ciliary body, and subsequently diffuse through the vitreous fluid or corneal surface[22]. Our prior research indicated a Th1/Th2 cytokine imbalance in glaucomatous eyes[23]. Freedman and Iserovich[24] reported elevated levels of chemokines (CCL2/MCP-1, CXCL1) and cytokines (IL-6, IL-8) in the aqueous humor of open-angle glaucoma patients. Consistent with these findings, multiple studies have documented elevated IL-8 levels in the aqueous humor of glaucoma patients[7,25-26], aligning with the results observed in this study.

Adipokines, a diverse group of bioactive molecules, have been implicated in the inflammatory processes associated with various diseases, including multiple sclerosis, Alzheimer's disease, systemic lupus erythematosus, and rheumatoid arthritis. The proinflammatory adipokines that have been discovered so far include leptin, resistin, lipocalin 2, RBP4, ANGPTL2, IL-6, IL-18, CCL2, CXCL5, tumor necrosis factor (TNF), and nicotinamide phosphoribosyl transferase (NAMPT). Conversely, anti-inflammatory adipokines include adiponectin and secreted frizzled-related protein 5 (SFRP5)[27]. Research on the role of adipokines in ocular diseases has primarily focused on retinal conditions such as DR, AMD, and retinopathy of prematurity (ROP). For example, serum leptin levels have been inversely associated with AMD[28]. Leptin has been shown to exacerbate oxidative stress in vascular endothelial cells, contributing to endothelial dysfunction in retinopathy[29]. Elevated levels of adiponectin have been observed in the aqueous humor and vitreous fluid of patients with microangiopathy and chronic inflammation, as well as in subretinal fluid, vitreous body, and preretinal membranes of those with AMD, proliferative vitreoretinopathy (PVR), and proliferative diabetic retinopathy (PDR)[30]. Adiponectin mitigates inflammatory damage to vascular endothelial cells and thus inhibits the formation of retinal and choroidal neovascularization[31-32]. Other research suggests that adiponectin may promote fibrosis in the posterior segment of the eye[30].

However, the role of adipokines in glaucoma remains unclear. Huang *et al*[33] conducted a Meta-analysis indicating that dyslipidemia increases the risk of POAG. Specifically, TG and TC were not correlated with the prevalence of POAG, but HDL-C was significantly negatively correlated with the risk of POAG. Zhao *et al*[34] identified a significant association between diabetes, its duration, and FPG with an elevated risk of glaucoma, as well as observed that both diabetes and FPG correlated with increased IOP. Stewart and Clearkin[35] suggested that insulin-resistant microvascular and hemodynamic abnormalities may contribute to optic nerve injury in glaucoma. These studies primarily analyzed the association between blood markers reflecting systemic metabolic status and glaucoma. However, our study differs from these studies by measuring adipokine levels in the aqueous humor, which can directly reflect the local disease status within the eye in glaucoma. This approach rendered our research on the relationship between the two more focused. Given the strong correlation between plasma adipokine levels and metabolic abnormalities and the potential relationship between metabolic factors and glaucoma, metabolic factors were taken into account in our grouping. Our findings indicate that most aqueous humor adipokines were elevated in the cataract+metabolic disorders group compared to the cataract group, suggesting that metabolic disorders may influence intraocular adipokine levels, though the differences were not statistically significant.

In our study, the POAG group exhibited significantly higher levels of pro-inflammatory adipokines (leptin, adipsin, RBP4, angiopoietin-2, CCL2, IL-8, and IL-18) compared to the cataract group. Adiponectin, an anti-inflammatory adipokine, was also elevated in the POAG group. Elevated ANGPTL4 in the POAG group is thought to have both pro-inflammatory and anti-inflammatory properties[36]. However, we have yet to determine whether elevated adipokine levels are a pathogenic factor of POAG or whether POAG leads to increased

intraocular adipokine levels. The gene encoding adiponectin, ADIPOQ, was found with predisposing genotype and the allele frequencies in POAG[10]. Adiponectin protects against oxidative stress in vascular endothelium by promoting nitric oxide (NO) synthesis via AMPK-mediated phosphorylation of endothelial nitric oxide synthase (eNOS)[37]. Low levels NO exhibit neuroprotective effects and high levels mediate neurodegenerative effects. Adiponectin is a marker for early screening of diabetes, with its reduction occurring prior to the elevation of FPG, which is currently used in clinical practice. Our study found that POAG patients without metabolic abnormalities exhibited elevated levels of adipokines in their aqueous humor, suggesting that the risk of metabolic disorders in patients with POAG may be higher than that in individuals without glaucoma. In addition, it remains to be experimentally validated whether various cells within the eye can secrete adipokines.

Adiponectin, the most abundant adipokine in the blood, was first described by Scherer *et al*[38] in 1995 as Acrp30 (adipocyte complement-related protein 30 kDa) or adiponectin. It is thought to have neuroprotective effects and is expressed in the human retina[39]. Yucel Gencoglu *et al*[40] detected plasma adiponectin levels in patients with POAG, exfoliative glaucoma, and healthy controls. They found that there was no significant difference in plasma adiponectin levels between the three groups under investigation. Our study observed elevated adiponectin levels in the aqueous humor of POAG patients, suggesting increased retinal secretion or possible disruption of the blood-aqueous barrier. Further experimental research is warranted to clarify these observations.

Leptin, which comes from the human obese (OB) gene, was discovered and characterized in 1994[41]. Its mRNA expression was later found to be upregulated in human Th-1 lymphocytes, Th-1 clones, and active lesions of multiple sclerosis. Leptin is known to impair the function of CD4+ CD25+ regulatory T cells, thereby facilitating autoimmune processes[28]. Recent studies proposed that leptin might serve as a neuroprotective agent in glaucoma, potentially preventing RGC death from oxidative stress, apoptosis, and excitotoxicity through various molecular pathways[42].

Adipsin, a 24 kDa serine protease initially described in 3T3 fat cells and later identified as complement factor D[43], is secreted by adipocytes, macrophages, and monocytes[44]. Research has indicated that adipsin levels in the aqueous humor of DR patients are higher compared to controls, while plasma adipsin levels are lower[45].

RBP4, a member of the lipocalin family, plays a key role in transporting retinol from the liver to peripheral tissues, with its receptor, stimulated by retinoic acid 6 (STRA6), being notably expressed in the retina[46]. *In vitro* studies have demonstrated that RBP4 stimulates basal lipolysis in human adipocytes and activates macrophages, leading to the release of pro-inflammatory cytokines such as TNF-α[47]. The level of RBP4 in aqueous humor is elevated in the advanced stage of DR[48], and is specifically regulated by anti-vascular endothelial growth factor (VEGF) therapy[49].

Angiopoietin-2 is a ligand for the tyrosine kinase receptor Tie2 and is predominantly expressed by endothelial cells and monocyte-macrophages. It plays a crucial role in vascular development, stability, and monocyte-macrophage activation[50-51]. Angiopoietin-2 induces IL-10, which inhibits T cell proliferation and promotes the expansion of regulatory T cells[52]. Additionally, angiopoietin-2 and VEGF-A act synergistically to drive vascular leakage, neovascularization, and inflammation, making them key components in retinal vascular diseases[53]. Gharahkhani *et al*[54] reported that *ANGPT2* gene variants were associated with increased IOP and glaucoma. Furthermore, *ANGPT2* deletion in mice has been linked to moderate Schlemm's canal deficiency and impaired limbal vasculature development[55].

ANGPTL4, a member of the angiopoietin superfamily, is expressed in human adult retinal epithelial cells (ARPE-19) cells[56]. Studies have shown that ANGPTL4 can exhibit both pro-inflammatory and anti-inflammatory effects in different disease states. Jung *et al*[57] demonstrated that ANGPTL4 exacerbates acute pancreatitis by promoting alveolar cell damage and releasing inflammatory cytokines. Conversely, in acute myocardial infarction and peritonitis, ANGPTL4 mediates anti-inflammatory effects by regulating the expression of inflammatory genes (*Arg1*, *CD206*, *IL-10*, *iNOS*, *IL-6*, *IL-1β*, *CCL2/MCP-1*) in macrophages[36]. Increased ANGPTL4 expression has been observed in hypoxic retinal Müller glial cells, ischemic retinal cells in animal models, and diabetic eye disease[58]. The concentration of ANGPTL4 is increased in the vitreous and aqueous humor of diabetic patients, and levels of ANGPTL4 expression correlate with macular edema, retinal ischemia, and the level of DR[59-60]. ANGPTL4 is also overexpressed in the extracellular matrix following TGFβ3 treatment of human trabecular meshwork cells[61].

CCL2, a well-studied member of the CC chemokine family, primarily facilitates the recruitment of mononuclear cells, thereby amplifying the inflammatory cascade. Elevated CCL2/MCP-1 levels in the aqueous humor of POAG patients have been reported[24], consistent with our findings. However, others found that the level of CCL2/MCP-1 in aqueous humor of POAG patients is lower[7], which remains controversial.

IL-8, also known as CXCL8, belongs to the large family of C-X-C chemokines. Monocytes, lymphocytes, granulocytes, and fibroblasts are the main tissue-derived chemotactic agents of neutrophils under inflammatory conditions. IL-18, a member

of the IL-1 family of proteins, acts as an agonist. IL-18 can be produced by a variety of immune and non-immune cells, such as macrophages, monocytes, osteoblasts, chondrocytes, dendritic cells, keratinocytes, epithelial cells, and synovial fibroblasts. Monocyte-macrophages are the primary source of IL-18 during inflammatory responses. Our study supports previous findings, suggesting that inflammation and monocyte recruitment are involved in the pathology of POAG.

We also investigated the impact of glaucoma medications on adipokine concentrations in aqueous humor. Previous studies have produced conflicting results regarding the effect of glaucoma medications on aqueous humor cytokine levels. Engel *et al*[7] suggested that IOP-lowering eyedrops had minimal impact on cytokine expression in POAG eyes without a history of surgery. However, the study of Takai *et al*[26] reported a positive correlation between the number of glaucoma medications and levels of IL-8 and TGF-β1 in the anterior chamber, indicating an effect of these medications on aqueous humor cytokines. In our study, no significant effect of prostaglandin analogues, carteolol, brinzolamide, or brimonidine on adipokine concentrations in aqueous humor was observed. Furthermore, we did not find a correlation between adipokine levels in aqueous humor and the severity of glaucoma in POAG patients, which may be attributable to the limited sample size.

A limitation of this study is the small sample size of POAG patients. While our study identified an association between abnormal adipokine levels and POAG, the causal relationship remains unclear. Further experimental research is required to elucidate the source of elevated adipokines.

In conclusion, our analysis of 15 adipokines in the aqueous humor of POAG patients and cataract patients with or without metabolic disorders revealed significantly higher levels of adiponectin, leptin, adipsin, RBP4, angiopoietin-2, ANGPTL4, CCL2, IL-8, and IL-18 in the POAG group compared to the cataract group. Elevated adipokine levels may reflect an inflammatory state in glaucoma. The causal relationship between POAG and altered adipokine levels in the aqueous humor requires further investigation.

ACKNOWLEDGEMENTS

We would like to thank Wayen Biotechnologies, Shanghai for their technical support with Luminex bead-based multiplex array. We also thank Shanghai Tengyun Biotechnology Co., Ltd. for developing Hiplot Pro platform (https://hiplot.com.cn/) and providing valuable tools for data analysis and visualization.

Authors' contributions: Xia ZY: investigation, data curation, formal analysis, methodology, writing–original draft, writing–review & editing. Xu K: investigation, formal analysis. zhang WJ: investigation, methodology. Wu DF: data curation, investigation. Pan Z: investigation, supervision. Xian HC: conceptualization, software, visualization. Li XM: investigation. Wang HZ: conceptualization, investigation. Zhang C: funding acquisition, investigation, writing–review & editing.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Foundations: Supported by National Key R&D Program of China (No.2023YFC2506100); the Changping Key Research Project of the Beijing Natural Science Foundation Program (No.L234016).

Conflicts of Interest: Xia ZY, None; **Xu K,** None; **Zhang WJ,** None; **Wu DF,** None; **Pan Z,** None; **Xian HC,** None; **Li XM,** None; **Wang HZ,** None; **Zhang C,** None.

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