Proinflammatory Cytokine Profile Differences between Primary Open-Angle and Pseudoexfoliative Glaucoma

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Keywords

Glaucoma biomarkers · Proinflammatory cytokines · Primary open-angle glaucoma · Pseudoexfoliative glaucoma · Aqueous humor · Tear · Ocular hypotensive medication

Abstract

Introduction: Few studies have investigated glaucoma biomarkers in aqueous humor and tear and have found elevations of proinflammatory cytokines in patients with primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (PXG). In this study, we investigate differences in inflammatory cytokines between POAG and PXG patients to find specific disease biomarkers. **Methods:** For this purpose, tear and aqueous humor samples of 14 eyes with POAG and 15 eyes with PXG undergoing cataract surgery were immunoassayed for 27 proinflammatory cytokines. The concentrations of cytokines in tear and aqueous humor and their association with clinical variables were analyzed, correlated, and compared between the groups. **Results:** We found that the levels of three cytokines differed significantly in the aqueous humor of POAG and PXG patients: IL-12 and IL-13 were higher in the POAG group, while monocyte chemoattractant

protein-1 (monocyte chemotactic and activating factor) was higher in the PXG group. The number of topical hypotensive medications was correlated with diminished levels of two cytokines (IL-7 and basic fibroblast growth factor) in aqueous humor in the POAG group and with diminished levels of IL-12 in tear in the PXG group. **Conclusion:** We conclude that both POAG and PXG show elevated concentrations of proinflammatory cytokines in tear and aqueous humor that could be used as biomarkers for these types of glaucoma and that the concentrations in aqueous humor of three cytokines, IL-12, IL-13, and monocyte chemoattractant protein-1 (monocyte chemotactic and activating factor), could be used to differentiate POAG and PXG.

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Introduction

There are various types of glaucoma that affect predominantly certain age groups, but the most common form in adults is the open-angle glaucoma, which may be primary or secondary. Primary open-angle glaucoma (POAG) represents 75% of all glaucoma and, although various risk

factors for this type of glaucoma have been identified, its exact cause remains unknown [1, 2]. The most common form of secondary open-angle glaucoma is the pseudoexfoliative glaucoma (PXG) [1]. This occurs in the context of pseudoexfoliation syndrome (PXS), which is characterized by the deposition of fine fibrillary material in the anterior segment of the eye, along with other organs [1, 3].

Because POAG and PXG often course without symptoms, its diagnosis is often made when there is already extensive irreversible damage to the optic nerve. Therefore, it is important to find biomarkers of the disease that could allow an early diagnosis and deepen our knowledge of glaucoma pathogenesis. Recent studies have suggested that ischemia, oxidative stress, and, especially, inflammation may be involved in PXS and POAG [4, 5].

In this regard, recent studies have documented an elevation of inflammatory cytokines in POAG and PXG, with increased cytokine levels in tear of patients with POAG [6]. In addition, two earlier studies of our group revealed higher cytokine levels in tear and aqueous humor of POAG patients and specifically in the tears of patients treated with topical drugs containing preservatives [7, 8]. Other authors have also found increased cytokine levels in the aqueous humor of patients with POAG [9] or in both PXG and POAG [10, 11]. Finally, various studies have found differences in aqueous humor growth factors or cytokine levels, but only in patients with PXG [12–17]. Thus, although previous studies investigating cytokine levels have reported variable results in POAG and PXG, they all suggest that inflammation involved in their pathophysiology could thus serve as a biomarker for these types of glaucoma.

The main objective of the present study was to investigate differences in inflammation between POAG and PXG (the most common forms of the disease) that could give insight into their pathogenesis. For this purpose, we determined the levels of 27 inflammatory cytokines in tear and aqueous humor of patients with POAG and PXG and analyzed the differences between these types of glaucoma.

Methods

Patients

This cross-sectional observational study was carried out in patients with POAG or PXG of the Glaucoma Unit of the Hospital Clínico San Carlos in Madrid (Spain) who were controlled with topical hypotensive treatment and underwent cataract surgery. The study was approved by the Clinical Research Ethics Committee of the hospital and followed the Helsinki Declaration. All the patients were informed about the procedure and signed the consent form.

Eighteen patients with POAG and 17 patients with PXG who were scheduled for cataract surgery between January and April of

2019 were included. The inclusion criteria were: age older than 40 years, diagnosis of POAG or PXG at least 2 years before, and topical hypotensive treatment maintained for the last 6 months. The exclusion criteria were previous ocular surgery, YAG iridotomy, intraocular or intravitreal injections, or other ocular pathology.

From the medical records of the patients, the following data were obtained: age, sex, previous diseases, surgeries, and treatments, including the number and type of ocular hypotensive drugs. From the data of the visit prior to surgery, we also annotated the best corrected visual acuity (BCVA), the intraocular pressure, the mean deviation, and corrected loss of variance of the 24-2 visual field (Octopus, Haag Streit), and the peripapillary nerve fiber layer thickness (Optic Coherence Tomography, Spectralis, Heidelberg). In the 3-month follow-up, the BCVA and number of topical medications were included.

The patients were informed of the study on their arrival to the hospital for the cataract operation (between 8:30 and 12:30 a.m.). Before initiating the procedure for anesthesia or dilation of the pupil, a tear sample (3–5 μ L) was obtained without anesthesia from the inferior fornix with a glass capillary tube (5 μ L; Drummond Microcaps; Broomall, PA, USA). The aqueous humor (40–50 μ L) sample was obtained through paracentesis with a 30G needle on a syringe as the first step of the surgery. Both samples were immediately transported to the Immunology Department, where tear samples were diluted with buffered saline to give a final volume of 50 μ L and then immediately frozen at -80°C until processing.

Cytokine Determination

Cytokine levels in tear and aqueous humor were analyzed using the Bio-Plex Pro™ Human Cytokine 27-Plex Immunoassay kit (Laboratorios Bio-Rad SA, Madrid, Spain) according to the manufacturer's instructions. This kit uses fluorescent magnetic surfaces and antibodies and allows the quantitation of 27 proinflammatory cytokines: Interleukin (IL)-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17A, eotaxin, basic fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon gamma (IFN- γ), interferon gamma-induced protein 10, monocyte chemoattractant protein-1 (monocyte chemotactic and activating factor) (MCP-1 [MCAF]), macrophage inflammatory proteins (MIP) forms 1 α and 1 β (MIP-1 α and MIP-1 β), platelet-derived growth factor BB dimer, Regulated on Activation, Normal T Cell Expressed and Secreted factor, tumor necrosis factor- α , and vascular endothelial growth factor (VEGF). For this assay, 50 μ L of each sample was placed in a well and the platform was read using Luminex MAGPIX™ (Luminex Corporation, Austin, TX, USA). Cytokine concentrations were determined by interpolation of the fluorescence measurements of each sample and adjusting it to their standard curves. Also, for each sample a correction factor according to the dilution was used. Finally, the software Bio-Plex Manager™ (Bio-Rad, Hercules, CA, USA) calculated the concentration of the 27 cytokines.

Data Analysis

The data from the medical records and cytokine concentrations were introduced in Microsoft Excel sheets and analyzed with the software SPSS (IBM SPSS Statistics for Mac 22.0 (IBM Corp., Armonk, NY, USA)). Data are expressed as means $\pm$ standard deviation (SD). Quantitative variables were compared using the *t* test or the Mann-Whitney test depending on the normality of the data. The correlation between cytokine levels in tear and

Table 1. Demographic and clinical variables and comparisons between groups

Variable	Diagnosis		Statistical comparison ($p = \text{test}$)
	POAG	PXG	
Age, years	76.14±6.89	77.40±9.45	0.652 Mann-Whitney
Baseline BCVA	0.43±0.13	0.29±0.15	0.019 Mann-Whitney
Postoperative BCVA	0.85±0.12	0.61±0.32	0.019 Mann-Whitney
Topical hypotensive drugs (baseline), n	1.69±0.63	1.64±0.84	0.864 t test
Topical hypotensive drugs (postoperative), n	0.69±0.75	0.79±0.80	0.757 t test
IOP (baseline)	19.08±3.45	19.79±2.75	0.488 Mann-Whitney
IOP (postoperative)	16.54±1.81	16.71±4.08	0.756 Mann-Whitney
MD of the VF	6.49±6.28	10.45±6.35	0.020 Mann-Whitney
CLV of the VF	3.98±1.41	4.93±1.41	0.102 Mann-Whitney
pRNFL thickness			
GLOBAL	83.43±20.88	70.93±18.56	0.085 Mann-Whitney
Nasal	59.07±19.29	57.73±18.51	0.813 Mann-Whitney
Nasal superior	93.64±25.32	78.20±29.96	0.158 Mann-Whitney
Temporal superior	116.50±32.38	86.53±32.52	0.023 Mann-Whitney
Temporal	66±17.66	58.07±13.35	0.158 Mann-Whitney
Temporal inferior	119.29±45.80	91.87±32.08	0.057 Mann-Whitney
Nasal inferior	87.36±27.99	79.80±22.89	0.146 Mann-Whitney

Values are the mean $\pm$ SD. Postoperative values were obtained 3 months after cataract surgery. The value in bold indicates a significant difference between the groups. $n = \text{number}$

IOP, intraocular pressure; MD, mean deviation; CLV, corrected loss of variance; VF, visual field; BCVA, best corrected visual acuity; POAG, primary open-angle glaucoma; PXG, pseudoexfoliative glaucoma.

aqueous humor and number of treatments was analyzed using the Spearman's Rho correlation coefficient. A p value <0.05 was considered statistically significant.

Results

Patients

Although 18 eyes of patients with POAG and 17 eyes of patients with PXG were initially included in the study, some samples had to be discarded because of insufficient quantity and finally only 14 eyes with POAG and 15 eyes with PXG could be included. Table 1 depicts the characteristics of both study groups. Mean age of the patients was similar in both groups. BCVA increased after cataract surgery in both groups, and was significantly higher in the POAG than in the PXG group both at baseline and postoperatively. The number of topical hypotensive drugs used preoperatively and postoperatively was similar in both groups and all patients had been on that treatment for at least 6 months. Preoperative and postoperative intraocular pressure was also similar in both groups.

The mean deviation of the visual field was -6.49 ± 6.28 and -10.45 ± 6.35 dB in the POAG and PXG group,

respectively, with significant differences between them (Table 1). The global and sectorial pRNFL thicknesses were similar between groups, except for the superotemporal sector that was significantly thinner in the PXG group (Table 1).

Cytokine Concentrations in Tear and Aqueous Humor

Cytokine concentrations (pg/mL) can be observed in Figures 1 and 2 and Table 2. Cytokine concentrations varied greatly in aqueous humor and tear in both experimental groups. However, when cytokine concentrations in tear or aqueous humor were compared between groups, three cytokines showed significant differences: IL-12 and IL-13 were significantly higher in the POAG group and MCP-1 (MCAF) was significantly increased in PXG patients (Fig. 1, 2; Table 2).

Correlation between Cytokine Concentrations in Tear and Aqueous Humor

There were no significant correlations between the cytokine concentrations in tear and aqueous humor in the POAG group (Table 2), but there were significant correlations in the concentrations of three cytokines in the PXG group: IFN- γ , MIP-1 β , and VEGF (Table 2).

Fig. 1. Cytokine concentrations (pg/mL $\pm$ SD) in tear in POAG and PXG. *x*-axis: logarithmic scale. POAG, primary open-angle glaucoma; PXG, pseudoexfoliative glaucoma.

Correlation between Cytokine Concentration and the Number of Topical Treatments

There was a significant negative correlation between the number of topical treatments used at baseline and the concentrations of two cytokines in POAG patients:

IL-7 and basic fibroblast growth factor in aqueous humor (Table 2). There was also a significant negative correlation between the number of topical treatments used at baseline and the concentration of IL-12 (Table 2) in tear in the PXG group.

Fig. 2. Cytokine concentrations (pg/mL $\pm$ SD) in aqueous humor in POAG and PXG. *x*-axis: logarithmic scale. POAG, primary open-angle glaucoma; PXG, pseudoexfoliative glaucoma.

Discussion

In the present study, we have used a sensitive immunoassay to analyze the concentrations of 27 inflammatory cytokines in tear and aqueous humor of patients with POAG or PXG who were undergoing cataract surgery to

discern whether these cytokines were involved differently in these types of glaucoma and could, therefore, be used as biomarkers.

We documented great variations of cytokine concentrations in both groups of patients, both in tear and aqueous humor. This indicates a great variability of cytokine

Table 2. Cytokine concentrations (pg/mL) in tear and aqueous humor, comparison and correlations between them and with number of topical treatments

Cytokine	Diagnosis	Tear		Comparison of POAG/PXG (tear)		Aqueous humor		Comparison of POAG/PXG (aqueous humor)		Correlation with tear/aqueous		Correlation with number of treatments (Spearman, <i>p</i>)	
		mean	SD	Mann-Whitney <i>p</i>		mean	SD	Mann-Whitney <i>p</i>		Pearson	tear	tear	aqueous humor
IL-1β	POAG PXG	2.66	3.87	1		1.23	1.90	0.425		0.522	0.159	-0.151	-0.083
		14.95	39.43			0.86	2.22						
IL-1ra	POAG PXG	8,005.29	10,535.04	0.847		2,425.90	3,085.20	0.78		0.481	0.021	-0.206	-0.166
		3,148.25	4,993.16			7,506.10	19,693.08						
IL-2	POAG PXG	8.73	9.52	0.425		8.96	12.86	0.715		0.325	-0.243	-0.475	-0.347
		2.75	2.01			7.16	13.93						
IL-4	POAG PXG	0.15	0.15	0.123		0.14	0.12	0.715		0.134	-0.188	0.092	-0.010
		0.22	0.15			0.12	0.09						
IL-5	POAG PXG	43.41	68.39	0.683		45.00	56.29	0.505		0.522	0.216	-0.235	0.023
		32.44	68.14			42.33	62.52						
IL-6	POAG PXG	8.52	10.31	0.377		6.54	6.91	1		0.484	-0.009	-0.141	0.022
		10.68	12.10			13.13	23.28						
IL-7	POAG PXG	25.67	41.08	0.88		14.30	16.63	0.78		0.317	0.101	-0.659 <i>p</i> = 0.014	-0.123
		17.52	19.76			24.50	54.87						
IL-8	POAG PXG	53.97	91.34	0.747		40.73	66.66	1		0.163	-0.343	-0.446	-0.202
		36.25	46.18			228.86	801.84						
IL-9	POAG PXG	81.29	86.32	0.561		58.53	76.23	0.813		0.089	-0.040	0.028	-0.198
		106.63	157.65			75.42	101.62						
IL-10	POAG PXG	39.86	45.95	0.451		38.18	38.33	0.186		0.353	-0.117	-0.223	-0.059
		27.43	42.48			24.26	37.05						
IL-12 (p70)	POAG PXG	7.03	10.64	0.172		12.52	10.07	0.000		0.293	-0.167	-0.249	-0.214
		1.75	3.62			0.63	0						
IL-13	POAG PXG	4.10	4.74	0.621		5.37	4.73	0.037		-0.44	0.008	-0.452	0.09
		6.29	6.83			2.89	3.99						
IL-15	POAG PXG	970.05	966.32	0.847		1,115.45	1,041.91	0.252		-0.279	-0.125	-0.159	0.054
		1,058.39	1,199.51			697.87	1,041.52						
IL-17	POAG PXG	53.63	77.02	0.134		31.48	62.03	0.425		-0.267	0.194	-0.483	0.205
		17.76	40.26			29.42	29.60						
Eotaxin	POAG PXG	9.80	8.30	0.4		7.20	6.37	0.201		-0.077	-0.017	-0.156	-0.166
		7.19	3.71			14.28	19.68						

Table 2 (continued)

Cytokine	Diagnosis	Tear		Comparison of POAG/PXG (tear)		Aqueous humor		Comparison of POAG/PXG (aqueous humor)	Correlation with tear/aqueous		Correlation with number of treatments (Spearman, <i>p</i>)	
		mean	SD	Mann-Whitney <i>p</i>		mean	SD		Pearson	tear	aqueous humor	
FGF basic	POAG	71.38	103.32			85.91	123.26		–0.149	0.209	–0.661 <i>p</i> = 0.014	–0.146
	PXG	66.56	53.55	0.477		59.65	83.98	0.715	0.25	–0.322		
G-CSF	POAG	215.21	300.05			388.29	471.57		–0.444	0.041	–0.023	–0.201
	PXG	184.83	435.76	0.477		207.27	341.44	0.29	0.449	–0.286		
GM-CSF	POAG	16.41	18.15			6.57	7.28		–0.487	–0.028	–0.075	0.207
	PXG	7.63	8.98	0.377		5.46	12.88	0.252	–0.079	–0.432		
IFN- γ	POAG	119.38	159.26			37.87	49.23		–0.195	–0.052	–0.295	–0.182
	PXG	39.50	51.02	0.270		31.58	52.63	0.621	0.792 <i>p</i> < 0.001	–0.355		
IP-10	POAG	12,235.07	19,101.59			10,045.68	13,887.78		–0.268	–0.141	–0.490	–0.143
	PXG	8,429.80	13,399.64	0.451		10,981.94	24,934.31	0.914	–0.296	–0.284		
MCP-1 (MCAF)	POAG	324.43	274.71			144.12	122.24		–0.137	–0.253	0.058	–0.239
	PXG	251.16	184.59	0.561		345.76	407.48	0.014	0.255	0.061		
MIP-1 α	POAG	3.55	3.24			2.96	4.12		0.423	–0.160	–0.435	0.016
	PXG	2.79	2.63	0.591		2.50	3.00	0.88	0.005	–0.434		
PDGF-BB	POAG	127.72	148.31			129.87	118.31		–0.502	–0.125	–0.163	–0.040
	PXG	75.50	122.13	0.561		121.57	195.56	0.290	0.269	–0.274		
MIP-1 β	POAG	22.53	19.45			28.39	37.07		0.061	–0.064	–0.291	–0.311
	PXG	18.48	25.47	0.4		19.99	21.38	0.949	0.750 <i>p</i> < 0.001	–0.282		
RANTES	POAG	58.31	67.40			42.74	52.28		–0.483	–0.067	–0.243	–0.018
	PXG	51.03	59.56	0.88		38.70	64.28	0.331	0.356	–0.219		
TNF- α	POAG	76.93	93.50			59.33	76.93		–0.463	–0.189	–0.441	–0.129
	PXG	49.66	52.29	0.813		42.53	71.63	0.505	0.168	–0.097		
VEGF	POAG	1,681.76	3,448.88			2,713.41	4,551.83		–0.462	0.151	–0.160	–0.083
	PXG	2,390.89	6,381.50	1		1,900.25	3,338.63	0.813	0.621 <i>p</i> = 0.014	–0.152		

Values in bold indicate a significant difference or a significant correlation between the groups.

POAG, primary open-angle glaucoma; PXG, pseudoexfoliative glaucoma; VEGF, vascular endothelial growth factor; TNF- α , tumor necrosis factor- α ; RANTES, Regulated on Activation, Normal T Cell Expressed and Secreted; MIP-1 β , macrophage inflammatory protein form 1 β ; MIP-1 α , macrophage inflammatory protein form 1 α ; PDGF-BB, platelet-derived growth factor BB dimer; MCP-1 (MCAF), monocyte chemoattractant protein-1 (monocyte chemotactic and activating factor); IP-10, interferon gamma-induced protein 10; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; FGF, fibroblast growth factor; IL, interleukin.

expression between patients even within a diagnostic group, and it has been documented previously in normal patients and in patients with different pathologies such as Sjögren syndrome or glaucoma [6–8, 18–20].

Our results document a tendency for the cytokines to show a high or low concentration both in tear and in aqueous humor. However, we could not document a significant correlation between the cytokine concentrations in tear and aqueous humor in the POAG group and we could only document a significant correlation in the concentrations of three cytokines in the PXG group: IFN Gamma, MIP-1 β , and VEGF. When comparing cytokine concentrations in tear and aqueous humor, other authors have found higher levels of cytokines in tears than in the aqueous humor [20], but in this study and in a previous study of our group we have failed to do so [8].

Our main objective was to add insight into the pathogenesis of POAG and PXG and for this purpose we have analyzed the levels of inflammatory cytokines in these two types of glaucoma and the differences between them. We have found high levels of four cytokines, IL-1RA, IL-15, interferon gamma-induced protein 10, and VEGF, both in tear and aqueous humor of POAG and PXG patients. Elevated levels of some of these cytokines in open-angle glaucoma patients when compared to the control group have also been observed in previous works of our group [7, 8], and increased levels of VEGF, tumor necrosis factor- α , and IL-6 have also been previously documented in tear of POAG patients [6]. It has been proposed that elevated levels of VEGF and TGF- β in the aqueous humor could be responsible for trabeculectomy failure in POAG patients [21], but other authors have argued that only diminished levels of IFN- γ , GM-CSF, and IL-5 in tear are associated with trabeculectomy complications [20].

When we compared cytokine concentrations in tear and aqueous humor of POAG and PXG patients, we found statistically significant differences only in the aqueous humor. IL-12 and IL-13 were significantly higher in the POAG group, while MCP-1 (MCAF) was significantly increased in the PXG group. Thus, these cytokines could be used as aqueous humor biomarkers for these types of glaucoma and may be involved in the different pathogenesis of each type of glaucoma. IL-12 is secreted by macrophages and dendritic cells. It acts by activating NK cells and promoting Th1 differentiation, as well as stimulating IFN- γ synthesis. Therefore, despite not being a commonly tested cytokine, IL-12 could serve as a marker for diagnosis and be specifically involved in POAG pathogenesis. However, the pleiotropic characteristics of cytokines render an exact assignment difficult. In accordance

with this, previous studies of our group have also found increased levels of IL-12 in tear and aqueous humor of POAG patients and increased levels of IL-12 and IL-13 in patients with glaucoma treated with preserved latanoprost [7, 8]. Cytokine elevations in the aqueous humor may depend on the type of glaucoma. In this regard, Duvess et al. [22] found that IL-8, eotaxin, and interferon gamma-induced protein 10 are significantly increased in closed-angle glaucoma, others revealing differences in IL-6, IL-8, granulocyte colony-stimulating factor, MCP-1, MCP-3, and VEGF [23, 24].

Our results document increased MCP-1 (MCAF) concentrations in aqueous humor of the PXG group when compared to the POAG group and may reflect an increased inflammation in the PXG group. MCP-1 (MCAF), also known as CCL2, is a potent chemotactic factor for monocytes and macrophages and plays a role in various inflammatory diseases and probably in conjunctival scarring [25, 26]. Notwithstanding, the role of this cytokine in PXG pathogenesis is still unclear. Given that the blood-aqueous barrier breakdown in PXG patients results in the entry of inflammatory cytokines and extracellular matrix material into the anterior chamber, the differences in cytokine levels could be due to this blood-aqueous breakdown rather than inflammation [27]. Although in our work MCP-1 (MCAF) is differentially elevated in PXG, it has been reported to be elevated in the aqueous humor in both POAG and PXG [11]. PXG is characterized by rapid evolution and severe prognosis and other cytokines, including IL-6, IL-17 [14], tumor necrosis factor- α [17], TGF- β , PDGF, and IL-8 [15], have been described to be elevated in the aqueous humor of PXG.

All the patients included in our POAG and PXG groups had cataracts and glaucoma and were receiving topical hypotensive medication (with and without preservatives) and this may have influenced the cytokine profiles. It is not known how cataracts affect cytokine concentrations in the aqueous humor, but it has been documented that ocular hypotensive treatments with preservatives influence the tear cytokine profile [6, 7]. As most patients in both groups were using >1 drug, it was not possible to correlate cytokine levels and the type of drug. However, all patients using one drug were on prostaglandins, and patients with two or more drugs were also on β -blockers and/or carbonic anhydrase inhibitors. We analyzed if cytokine concentrations were correlated to the number of ocular hypotensive drug medications that the patients were receiving. We found that the number of ocular hypotensive medications produced a decrease in the IL-7 and basic fibroblast growth factor concentrations in aqueous humor of the POAG group, and

of the IL-12 concentrations in tear of the PXG group. Thus, the topical treatment may have decreased inflammation and influenced in part the results.

Although more patients were initially included in the study, the samples of only 14 eyes with POAG and 15 eyes with PXG could be analyzed. The composition of the groups was generally comparable: at the baseline visit, age, intraocular pressure, global thickness of the pRNFL, and the number of topical hypotensive medications was similar in both groups. Nevertheless, the eyes included in the PXG group in this study could have more advanced cataracts and increased functional and structural glaucoma damage, or different disease timeline and/or different previous treatments. Indeed, the mean deviation of the visual field was significantly more negative in the PXG group, the BCVA was lower in the PXG, both at baseline and postoperatively, and the thickness of the superotemporal sector of the pRNFL was also significantly thinner in this group. However, we believe that the elevations in cytokine concentration in the tear aqueous humor both in POAG and PXG and the differences in cytokine concentrations that we find between the groups cannot be solely explained on the basis of different severity of glaucoma damage. Finally, although further studies are needed to define the concentrations of cytokines in tear and aqueous humor of POAG and PXG patients and their variations with the clinical course and severity of the disease, it is possible that these could be used in the future as non-invasive (in the case of tears) and rapid methods to diagnose and grade these diseases.

Conclusions

The analysis of 27 inflammatory cytokines in tear and aqueous humor of POAG and PXG patients has allowed us to document that the levels of three cytokines in the aqueous humor differed between the groups: IL-12 and IL-13 were significantly higher in the POAG group and MCP-1(MCAF) was significantly higher in the PXG group. Thus, although further studies are needed, these cytokines could be particularly involved in the pathogenesis of these forms of glaucoma.

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Statement of Ethics

The study was conducted according to the guidelines of the World Medical Association Declaration of Helsinki, and approved by the Ethics Committee of Hospital Clinico San Carlos (protocol code 18/255-E in December 2018).

Written informed consent was obtained from all subjects involved in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J.M.M.-C. and J.G.-F. contributed to conceptualization; J.M.M.-C., J.L.S.A., and L.E.-P. contributed to methodology; J.L.S. and L.E.-P. contributed to software; J.M.M.-C., J.G.-F., and J.F.-V. contributed to validation; B.V.-V. and B.B.-B. performed the formal analysis; B.V.-V., B.B.-B., and V.A.-G. carried out the investigation; V.A.-G., J.L.S.A., and L.E.-P. contributed to the resources; B.V.-V., B.B.-B., J.M.M.-C., J.L.S.A., and L.E.-P. involved in data curation; B.V.-V. and B.B.-B. involved in writing – original draft preparation; B.V.-V., B.B.-B., and J.M.M.-C. involved in writing – review and editing; J.F.-V. and J.G.-F. contributed to visualization; J.F.-V., J.M.M.-C., and J.G.-F. supervised the study; J.M.M.-C. and J.G.-F. contributed to project administration; J.M.M.-C. and J.G.-F. contributed to funding acquisition. All authors have read and approved the final manuscript and agreed to publish the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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