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Original Research

Aqueous Humor Cytokine Response in the Contralateral Eye after First-Eye Cataract Surgery in Patients with Primary Angle-Closure Glaucoma, High Myopia or Type 2 Diabetes Mellitus

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Abstract

Background: Bilateral sequential cataract surgery within a short period is becoming more prevalent because of the efficiency and safety of modern cataract surgery. It has been reported that the first surgical eye might affect the contralateral eye. This study investigated the cytokines involved in the immunopathogenesis of pre-existing ocular or systemic conditions, as well as the inflammatory biomarkers in response to topical stimuli, by analyzing the cytokine profile of aqueous humor (AH) from cataract patients without these morbidities as control and with type 2 diabetes mellitus (DM), primary angle-closure glaucoma (PACG) or high myopia (HM) in each eye at the beginning of first (defined as baseline) and second eye cataract surgery. **Methods**: Forty patients were recruited in this cohort study (10/group). Bilateral sequential cataract surgeries were conducted at intervals of 12.08 $\pm$ 1.2 days. Aqueous humor samples (100–200 μL/eye) were separately collected from 40 first-eyes and 40 second-eyes at the beginning of the cataract surgeries. Twenty-seven selected cytokines were detected with Luminex-multiplex immunoassay. The concentrations of cytokines in the aqueous humor and their association with pre-existing ocular or systemic conditions were analyzed and compared between and within the groups. **Results**: Before first-eye surgery (baseline), the levels of interleukin (IL)-1ra, IL-13 and tumor necrosis factor (TNF)-alpha were significantly increased in PACG compared with controls. The levels of IL-13 were increased while that of IL-15 were decreased in HM. Compared with controls, 11 cytokines were significantly increased in DM. In the AH of the contralateral eye after first-eye cataract surgery, basic fibroblast growth factor (bFGF) was significantly more abundant in PACG and HM, while the levels of monocyte chemoattractant protein-1 (MCP-1) and interferon gamma-induced protein 10 (IP-10) were decreased in PACG. We also identified 6 significantly upregulated cytokines in DM compared with controls. Compared with baseline, there was an overlap of 5 altered cytokines in the AH of contralateral eyes after first-eye surgery between the four groups. Some were exclusively altered in each subgroup, with 1 in the control group, 4 cytokines in the PACG and HM groups, and none in the DM group. **Conclusions**: From the initial profile, it is observed that patients with pre-existing ocular or systemic conditions have some degree of inflammation in their eyes before surgery and in the contralateral eye after the first eye cataract surgery, which could be peculiar of the morbid conditions of the patients. Inflammation was more detectable in patients with type 2 DM before surgery. PACG and HM patients showed stronger intraocular inflammatory reactions to topical stimuli compared with controls and DM patients. Our data suggest that ophthalmologists should pay closer attention to inflammatory responses, especially in cataract patients with pre-existing conditions, although the clinical significance of these changes following surgery remains to be further investigated.

Keywords: aqueous humor; cytokine; contralateral eye; cataract surgery; primary angle-closure glaucoma; high myopia; type 2 diabetes mellitus

1. Introduction

Phacoemulsification surgery (PHACO) is the most technically advanced and commonly performed cataract surgery, which includes the fragmentation and removal of the opacified crystalline lens and replacement with an intraocular lens (IOL) [1]. PHACO + IOL is a minimally invasive surgical procedure, often performed under topical or local anesthesia, and the patients can be discharged on the same day [1]. The most common postoperative complications are posterior capsule opacification and elevated intraocular pressure, macular edema, fibrinous reaction, vitreous hemorrhage, posterior synechiae, and recurrent retinal detachment [2]. It has been considered that postoperative complications are correlated with patients' pre-existing ocular diseases or co-morbidities. For instance, diabetes mellitus (DM) was considered a risk factor for macular edema [2,3], and it was reported that posterior vitreous detachment (PVD) progressed significantly faster in eyes with high myopia (HM) than in eyes without HM [4].

However, the exact correlation and the underlying

mechanism of postoperative complications and ocular or system conditions are still largely unclear. Postsurgical intraocular inflammation plays a central role in the development of postoperative complications, while the exact association between inflammatory factors, pre-existing ocular or systemic conditions and postoperative complications remains unknown. Common complications of ocular inflammation such as glaucoma, keratic precipitates, retinal edema and neovascularization may be mediated by cytokines [5]. For instance, choroidal neovascularization (CNV) was reported to develop more frequently in patients with HM after cataract surgeries [6]. Quality human eye tissue is difficult to obtain for research purposes [7]. Cytokines in the intraocular fluid can indicate the pathogenesis and progression of ocular inflammation [5].

Aqueous humor (AH) is a low viscosity fluid secreted from the ciliary body and circulates into the anterior chamber. It can exit the globe through the trabecular meshwork and move into a typical vortex due to the natural convection [8]. AH is a complex mixture of electrolytes, organic solutes, growth factors, cytokines and additional proteins that provide metabolic nutrients to avascular tissues of the anterior segment [9,10]. Although AH contains minimal amounts of proteins [11], these proteins may have a significant role in the pathogenesis and prognosis of eye disorders [12–14]. Several studies have measured cytokine concentration in aqueous humor samples of patients with several co-morbidities, including primary angle-closure glaucoma (PACG), myopia and diabetes. The cytokines have been reported to be involved in immune reactions, inflammation, ischemia, hypoxia and oxidative stress. A major limitation of testing aqueous humor in one specific patient group is the difficulty in identifying the unique cytokine changes to the existing co-morbidity. Moreover, the exact roles of these cytokine changes in the existing co-morbidities are not well understood.

Characterizing the AH cytokines at the beginning of first and second eye cataract surgery in patients with different ocular or systemic conditions could provide a foundation for biomarker discovery in various eye diseases, guide optimal operative time, and provide new insights into factors involved in postsurgical inflammation. By comparing the cytokine changes between the groups, we can evaluate the existence of comorbidity-specific cytokine changes. Based on these considerations, the aims of this study are: (1) to identify cytokine changes in AH of patients with different systemic (DM) or topical conditions (HM, PACG); (2) determine whether PACG, HM and DM might lead to different cytokine response to cataract surgery, and; (3) investigate different cytokine network patterns in PACG, HM and DM before cataract surgery and in response to cataract surgery.

2. Materials and Methods

2.1 Patients' Recruitment

This study was approved by the research ethics boards of Shanghai East Hospital of Tongji University and adhered to the Declaration of Helsinki. All patients provided written informed consent to participate in the study.

The data of 40 adult patients diagnosed with age-related cataract with an intention for bilateral cataract surgeries within a short period and underwent cataract surgery between September 2018 and March 2019 at the Ophthalmology Department of Shanghai East Hospital were investigated. The participants were further classified into four sub-groups (10 patients/group) based on their co-morbidities, such as type 2 DM, PACG, or HM. The 10 patients without comorbidities were considered as controls.

Type 2 DM was diagnosed according to a reported reference definition [15]. PACG was diagnosed based on the criteria of the International Society for Geographical and Epidemiological Ophthalmology (ISGEO) [16]. Briefly, PACG diagnosis was defined by a primary anatomic narrow-angle (180° or more of iridotrabecular contact assessed by indentation gonioscopy and ultrasound biomicroscopy (SW-3200L, Suoer Electronic Technology Co., Ltd., Tianjin, China)), with glaucomatous optic neuropathy (a vertical cup/disc [C/D] ratio $>$0.7 and/or C/D asymmetry $>$0.2 and/or focal notching of the neuroretinal rim), and compatible visual field defects measured using the Octopus 900 perimeter (Haag-Streit Inc., Köniz, Switzerland).

Patients with histories of acute glaucoma attack, ocular surgery, an advanced visual field defect and uncontrolled intraocular pressure (IOP) were excluded. All the PACG patients enrolled in the present study had controlled IOP under a maximum of one anti-glaucoma eyedrop (assessed by 24 h IOP profile). Patients with spherical equivalent $\leq$–6.00 D and axial length $>$26 mm of the eyes were diagnosed with HM and were included with an axial length $>$26 mm but $<$28 mm. The control group consisted of age-related cataract patients undergoing routine cataract surgeries without histories of other eye diseases or IOP over 21 mmHg.

The exclusion criteria for all groups were (1) signs of diabetic retinopathy; (2) history or onset of retinal diseases; (3) uveitis or iris neovascularization; (4) trauma; (5) previous history of eye surgery; (6) other ocular or immune diseases; (7) presence of co-morbidities other than type 2 DM, PACG, or HM, and; (8) presence of two or more of the described co-morbidities.

2.2 Humor Sample Collection

Eighty AH samples (100–200 μL/eye) from both eyes of 40 patients were collected at the beginning of cataract surgeries. Bilateral sequential cataract surgeries were conducted at intervals of 12.08 $\pm$ 1.2 days. The cytokines from AH of the first eye before first-eye surgery were considered the baseline, while that of the second eye after the first-

eye surgery were defined as post-operation. All the cataract surgeries and the withdrawal of AH were performed by the same surgeon (HP.C.) under a surgical microscope (Leica M844 F40/F20) in sterile conditions.

After topical anesthesia with Alcaine solution (0.5% proparacaine hydrochloride ophthalmic solution, S.A. Alcon-Couvreur N.V. Puurs, Belgium), a clear corneal incision was made at the 2 o'clock position with a 15-degree angle blade (Model: 8065921501, Alcon, Geneva, Switzerland). Approximately 150 μL of AH was withdrawn through the cornea incision using a 1 mL syringe attached to a 30-gauge needle. Each AH sample was centrifuged, and the supernatant was subsequently transferred into a sterile eppendorf tube, snap-frozen in liquid nitrogen, and stored at –80 °C.

2.3 Cytokine Analyses

Twenty-seven cytokines from the supernatant of the AH samples were measured using Luminex cytokine polystyrene color bead-based multiplex assay (Universal Biotech, Shanghai, China) in a duplicated manner. The cytokines measured were: interleukin (IL)-10, IL-13, IL-12p70, IL-15, IL-17, IL-1ra, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α). The chemokines determined were: eotaxin (CCL11), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), normal T cell expressed and presumably secreted (RANTES). The growth factors measured were: granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF-BB) and vascular endothelial growth factor (VEGF).

2.4 Bioinformatics, Functional Annotation and Pathways Analyses and Statistics

The data are presented as means $\pm$ standard deviations. All data obtained were checked for Gaussian distribution. Categorical variables were analyzed using the Chi-square test, and the Student's *t*-test was used for numerical variables comparing the differences within and between groups, at baseline and post-operation. Statistical analyses were performed using the GraphPad Prism software (Version 9.0.2 (134), GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < 0.05$ to identify significantly differentially expressed cytokines. Unsupervised hierarchical clustering analysis was done with the z-scores of the concentrations following the Euclidean distance (linkage = average; preprocess with k-means), and a heat map was generated.

To better understand the distinct inflammatory reaction in the second operated eye of the HM, PACG and DM patients in response to first-eye cataract surgery, the genes of significantly differentially expressed cytokines ($p < 0.05$) in each group were subjected to functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Top canonical pathways of the differentially expressed proteins were presented with their corresponding *p*-value.

3. Results

3.1 Patients' Demography

In total, the data of 17 males (34 eyes) and 23 females (46 eyes) were used for this study. The demographics of each sub-group, i.e., the controls (CTRL) and patients with DM, PACG or HM, are shown in Table 1. We observed no significant difference in age, gender, operation interval and operative time between the groups. All patients had good recovery without intraoperative and postoperative complications.

3.2 Cytokine Profiling of AH from the Controls and Patients with HM, PACG, and DM before and after First-Eye Surgery

Before first-eye surgery, the HM group had the most similar cytokine profile to the controls, with only the level of IL-13 and IL-15 being significantly differentially expressed, whereas the level of 11 among 27 cytokines in the DM group was significantly higher than those in the CTRL, with the 5 most significantly increased ones being IL-5, TNF-alpha, IL-2, bFGF and IL-4. In the PACG group, compared with the CTRL group, we observed higher levels of IL-1ra, TNF-alpha and IL-13 ($p < 0.05$, Fig. 1, Table 2).

At second-eye surgery, only bFGF was significantly upregulated in the HM group compared with controls, while 3 cytokines in the PACG group and 6 cytokines in the DM group were significantly different from the CTRL group (Fig. 1, Table 3).

3.3 Pathway Analysis of the Differentially Expressed AH Cytokines in the HM, PACG and DM Groups at the Baseline and Post-Operation

To better understand the intraocular conditions of the patients with HM, PACG and DM, the KEGG pathway analysis for the differentially expressed cytokines was performed. At baseline, the most significantly affected pathways in HM patients were the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathways. The IL-17 signaling pathway was the commonly affected pathway in PACG and DM patients at baseline and post-operation (Tables 4,5).

3.4 The Different Alterations in Cytokines in the AH after First-Eye Cataract Operation

In the control group, the levels of 3 cytokines, GM-CSF, IL-2 and IL-13, in the AH of the second eye were found to be significantly increased, while that of TNF-alpha

Table 1. Patients' demographics.

Demographics	CTRL group	HM group	PACG group	DM group	*p*-value
Age (years)	70.3 ± 9.74	66.0 ± 7.71	66.5 ± 8.07	67.9 ± 11.1	0.731
Male	4 (40%)	4 (40%)	3 (30%)	6 (60%)	0.439
Female	6 (60%)	6 (60%)	7 (70%)	4 (40%)	
Interval between operations (days)	10.9 ± 6.28	13.3 ± 5.48	12.9 ± 8.02	11.2 ± 4.42	0.771
Operation time (minutes)	12.7 ± 2.76	13.4 ± 2.71	12.95 ± 1.83	12.95 ± 2.04	0.357

Data are Mean ± SD (n = 10) or patient number (%); CTRL, control; HM, high myopia; PACG, primary angle-closure glaucoma; DM, diabetes mellitus.

Table 2. List of significantly differentially expressed cytokines in the HM, PACG and DM group, compared with the CTRL group at baseline.

HM group		
Cytokines	*p*-value	Level
IL-13	0.024424	upregulated
IL-15	0.015989	downregulated
PACG group		
Cytokines	*p*-value	Level
IL-1ra	0.015512	upregulated
TNF-alpha	0.001126	upregulated
IL-13	0.045	upregulated
DM group		
Cytokines	*p*-value	Level
IL-5	0.045685	upregulated
TNF-alpha	0.000143	upregulated
IL-2	0.038603	upregulated
bFGF	0.042124	upregulated
IL-4	0.002399	upregulated
MCP-1	0.040726	upregulated
IL-8	0.031556	upregulated
IL-10	0.008823	upregulated
IL-7	0.002665	upregulated
IL-17a	0.025998	upregulated
IL-9	0.00996	upregulated

Table 3. List of significantly differentially expressed cytokines in the HM, PACG, and DM group, compared with the CTRL group postoperatively.

HM group		
Cytokines	*p*-value	Level
bFGF	0.002994	upregulated
PACG group		
Cytokines	*p*-value	Level
bFGF	0.010821	upregulated
IP-10	0.047833	downregulated
MCP-1	0.026588	downregulated
DM group		
Cytokines	*p*-value	Level
TNF-alpha	0.048030	upregulated
RANTES	0.037963	upregulated
IL-2	0.046829	upregulated
IL-4	0.029184	upregulated
IL-10	0.022205	upregulated
IL-7	0.028212	upregulated

was significantly reduced after the first-eye cataract operation. The cytokine levels of DM patients showed little alteration, and the 3 cytokines IL-2, VEGF and PDGF-BB were significantly upregulated. Comparatively, in PACG and HM patients, 9 and 8 cytokines levels were found to be significantly altered, respectively (Fig. 2).

3.5 Pathway Analysis of Differentially Expressed AH Cytokines in the HM, PACG and DM Groups at Baseline and Post-Operation

KEGG pathway analysis of the differentially expressed cytokines was performed to better understand the distinct inflammatory reaction in AH of patients with HM, PACG and DM in response to the first-eye cataract surgery. The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance and regulation of actin cytoskeleton pathways were the most altered pathways in HM patients. In PACG patients, the IL-17 signaling pathway and the TNF-alpha signaling pathway were the most altered pathways. In DM patients, the most altered pathways were the T cell receptor signaling pathway, Fc epsilon RI signaling pathway and Jak-STAT signaling pathway (Table 6).

4. Discussion

In this study, the cytokine profile of aqueous humor in the first eye of cataract controls and patients with DM, PACG or HM were analyzed at baseline and in the contralateral eye after their first-eye cataract surgery. By comparing the cytokine profile of the first-eye operation and the second-eye operation, we found that all groups with pre-existing ocular or systemic conditions had signs of inflammation at both time points. PHACO + IOL was associated with inflammatory reactions in the AH of the contralateral eye. Strikingly, pre-existing ocular or systemic conditions were also associated with distinguishing between inflammatory reactions and topical stimuli, namely, PHACO + IOL.

At baseline, the HM group had the most similar cytokine profile to the controls and only the level of IL-13 and IL-15 was significantly differentially expressed, whereas

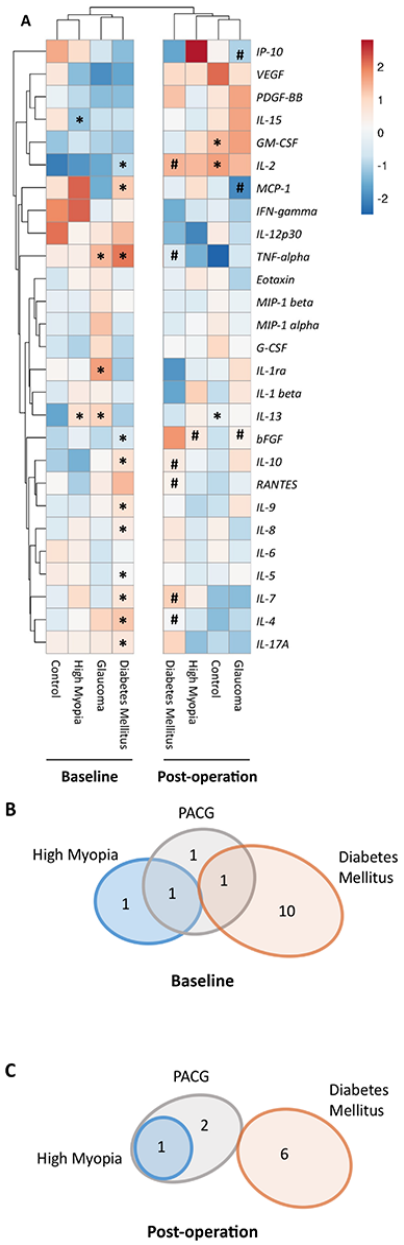

Fig. 1. Cytokine profile of AH of patients before and after the first-eye operation. (A) Heat map depicting the hierarchical clustering of the differentially expressed cytokines in the different groups compared to controls at baseline and post-operation. Each cluster represents the relative abundance of the cytokines on the heat map. The HM group demonstrated the most similar cytokine profile to the CTRL group at baseline and post-operation. Compared to the CTRL group at baseline, significantly differentially expressed cytokines are marked with the star symbol *. Compared with the CTRL group post-operation, the significantly differentially expressed cytokines are marked with the hash symbol #. (B,C) Venn diagram of the differentially expressed cytokines in different groups compared to controls at baseline and post-operation.

Table 4. List of significantly differentially regulated pathways in the HM, PACG and DM group, compared with CTRL group, at baseline.

HM group		
KEGG pathways	Strength	FDR (false discovery rate)
Jak-STAT signaling pathway	2.09	0.00095
Cytokine-cytokine receptor interaction	1.87	0.0013
PACG group		
KEGG pathways	Strength	FDR
Fc epsilon RI signaling pathway	2.29	0.00090
IL-17 signaling pathway	2.15	0.00097
Cytokine-cytokine receptor interaction	1.7	0.0062
DM group		
KEGG pathways	Strength	FDR
IL-17 signaling pathway	2.06	1.24e-10
T cell receptor signaling pathway	1.95	2.00e-08
Type I diabetes mellitus	1.95	0.00073
Fc epsilon RI signaling pathway	1.9	3.82e-05
Cytokine-cytokine receptor interaction	1.83	2.08e-16
Jak-STAT signaling pathway	1.82	2.04e-09
NF-kappa B signaling pathway	1.58	0.0034
Toll-like receptor signaling pathway	1.54	0.0038

Table 5. List of significantly differentially regulated pathways in the HM, PACG and DM group, compared with the CTRL group postoperatively.

HM group		
KEGG pathways	Strength	FDR (false discovery rate)
No significant enrichment detected	-	-
PACG group		
KEGG pathways	Strength	FDR
IL-17 signaling pathway	2.15	0.0019
TNF signaling pathway	2.08	0.0019
Chemokine signaling pathway	1.86	0.0021
Cytokine-cytokine receptor interaction	1.7	0.0030
DM group		
KEGG pathways	Strength	FDR
Allograft rejection	2.57	6.84e-09
Type I diabetes mellitus	2.21	0.00033
T cell receptor signaling pathway	2.12	1.83e-07
Fc epsilon RI signaling pathway	1.99	0.00073
Jak-STAT signaling pathway	1.91	7.97e-07
Cytokine-cytokine receptor interaction	1.85	4.34e-10
IL-17 signaling pathway	1.82	0.0011
Toll-like receptor signaling pathway	1.81	0.0011
TNF signaling pathway	1.78	0.0012

the level of 11 cytokines in the DM group was significantly different from the control group. Although the exact roles of IL-13 and IL-15 in HM are unclear, our findings align with a previous report in which the level of IL-13 and IL-

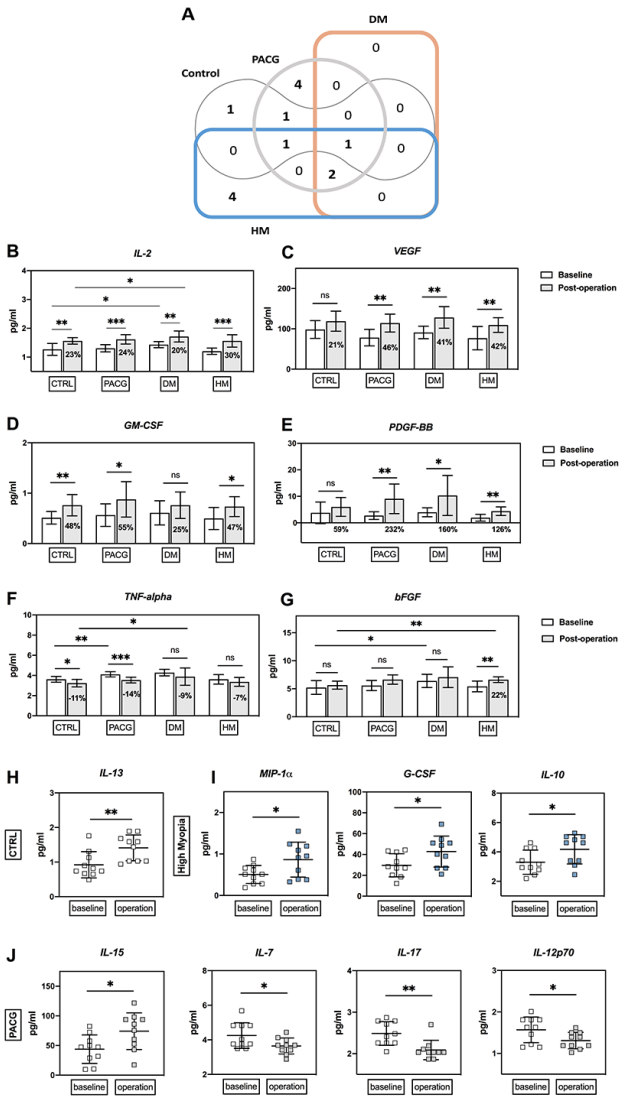

Fig. 2. Different alterations in cytokines in AH of patients after the first-eye cataract operation. (A) Venn diagram depicting overlaps of significantly altered cytokines in the HM, DM, PACG groups and the control group before and after the first-eye operation. (B–F) An overlap of 5 altered cytokines between the 4 groups. IL-2 is significantly upregulated in all groups. VEGF and PDGF-BB are exclusively altered in the HM, DM and PACG groups, and GM-CSF is exclusively altered in the HM, PACG and control groups, while TNF-alpha is exclusively altered in the PACG and control groups. The HM group shows an overlap of 2 cytokines with the control (25%), 3 cytokines with the DM group (37.5%) and 4 cytokines with the PACG group (50%). (G–J) Several cytokines are exclusively altered in each subgroup, with one in the control group, 4 cytokines in the PACG group and HM group, and none in the DM group.

15 was increased in AH of patients with myopic choroidal neovascularization [17,18].

The IL-13, TNF-alpha and IL-1ra level was found to be increased in cataract patients with PACG at baseline. Du-

Table 6. List of significantly differentially regulated pathways in HM, PACG, DM and CTRL group in the contralateral eye, in response to the first-eye operation.

HM group		
KEGG pathways	Strength	FDR (false discovery rate)
EGFR tyrosine kinase inhibitor resistance	1.96	4.61e-07
Regulation of actin cytoskeleton	1.72	1.08e-08
Ras signaling pathway	1.67	1.08e-08
PACG group		
KEGG pathways	Strength	FDR
IL-17 signaling pathway	2.15	0.0019
TNF signaling pathway	2.08	0.0019
Chemokine signaling pathway	1.86	0.0021
Cytokine-cytokine receptor interaction	1.7	0.0030
DM group		
KEGG pathways	Strength	FDR
Type I diabetes mellitus	2.21	0.00033
T cell receptor signaling pathway	2.12	1.83e-07
Fc epsilon RI signaling pathway	1.99	0.00073
Jak-STAT signaling pathway	1.91	7.97e-07
Cytokine-cytokine receptor interaction	1.85	4.34e-10
IL-17 signaling pathway	1.82	0.0011
Toll-like receptor signaling pathway	1.81	0.0011
TNF signaling pathway	1.78	0.0012

vesh *et al.* [19] reported cytokine changes in chronic PACG patients in the Indian population, such as a reduced TNF-alpha and increased IP-10, while TNF-alpha was increased in acute angle-closure glaucoma patients [20]. These studies indicated that the TNF-alpha level might be associated with the disease course. Another study reported that IL-13 was found to be more abundant in AH of primary open-angle glaucoma (POAG) patients [21]. Although the pathology of PACG and POAG is not the same, IL-13 may play a role in POAG and PACG, such as the progressive loss of retinal ganglion cells and their axons.

In DM patients, 11 cytokines were significantly increased in their AH despite their lack of ocular symptoms. Similar to our results, the aqueous levels of IL-8, MCP-1 and TNF-alpha were higher in diabetic macular edema patients [22,23], and lower levels of IL-5 and IL-8 were associated with improved diabetic macular edema [23].

In response to cataract surgery, IL-2 was increased in all groups, and was correlated with intraocular inflammation. Inflamed uveal and retinal tissues were detected in patients with certain types of uveitis [24] despite an increase in the level of VEGF and PDGF-BB in the PACG, HM and DM groups compared with the control group. VEGF has been associated with pathological angiogenesis and increased vascular permeability in eye diseases such as diabetic retinopathy and age-related macular degeneration [25]. Retinal vascular function damage is well known in both glaucoma and diabetic retinopathy, while the role of

PDGF-BB in the maintenance of retinal vasculature was just recognized [26,27]. In mice and rabbits, it was shown that the retina-specific expression of PDGF-B was associated with severe neovascularization and retinal detachment [28,29].

In response to cataract surgery, pre-existing ocular disease conditions were associated with more significant and distinguishing inflammatory reactions. Four cytokines were significantly altered in the control group, 3 in the DM group, 9 in the PACG group, and 8 in the HM group. Patients with PACG and HM had a stronger reaction than the control and DM groups. Surprisingly, DM patients did not show a stronger inflammation in reaction to surgical stimuli.

After the first-eye cataract surgery, only bFGF was significantly upregulated in the HM group compared with the controls, while 3 cytokines in the PACG group and 6 cytokines in the DM group were significantly different from the control group. The level of bFGF was significantly higher in the PACG and HM groups. The level of bFGF was also increased in the irises of patients with neovascular glaucoma and in the aqueous humor of patients with exfoliation syndrome or exfoliative glaucoma [30,31]. In an animal model of photic-induced retinopathy, bFGF up-regulation was associated with the protection of retinal functions [32]. Nonetheless, the exact source of bFGF and its role in the eye remains to be studied.

In the PACG group, the level of IP-10 and MCP-1 was significantly lower after the first-eye surgery. In acute angle-closure glaucoma patients, the level of IP-10 was found to be significantly higher [20]. It was also reported that IP-10 in AH was positively correlated with IOP reduction after trabeculectomy [33]. Taken together, these findings indicate that IP-10 might be correlated with the IOP of glaucoma patients. Furthermore, MCP-1 was also reported to play a role in IOP regulation. The enhanced expression of MCP-1 was correlated with trabecular meshwork cell contractile activity, potentially implicated in the pathobiology of abrupt IOP elevation [34].

To our knowledge, this is the first study comparing the cytokine profiles of patients with different ocular and systemic disease conditions to identify the comorbidity-specific cytokines in each group. However, due to the nature of human-related studies, the underlying molecular mechanisms responsible for the findings reported here are largely unknown. Therefore, the precise functions of those cytokines and their downstream targets should be further elucidated in the future. Furthermore, from the data reported it is not evident a dramatic surge of inflammatory cytokines after the first surgery, and their clustering was not so pronounced to allow a characterization of the different conditions. Last but not least, it is unlikely that all the proteins responsible for intraocular inflammation related to the comorbidities are identified using cytokine assay. Mass-spectrometry-assisted proteomics approaches on a larger sample size could have returned results describing better the different conditions at the different time points. As a propaedeutic study, the findings are envisioned to provide a useful reference point for future studies. Further studies with larger samples are warranted to establish more solid patterns of variations in cytokine expression associated with different comorbidities.

5. Conclusions

In summary, our data show that pre-existing ocular or systemic conditions could lead to intraocular inflammation even before surgery, particularly in patients with type 2 DM. Strikingly, pre-existing ocular or systemic disease conditions led to distinguishing inflammatory reactions in the second eye after the first eye surgery. PACG and HM patients showed a change in some cytokine expression to topical stimuli compared with controls, while fewer cytokines appear to be altered in DM patients. These findings suggest that ophthalmologists should pay close attention to the inflammatory response in the second eye, especially in patients with pre-existing ocular conditions.

Author Contributions

JT designed the project, performed the analysis and wrote the manuscript. HL processed the experimental data, performed the analysis, wrote the manuscript and designed the figures. MS checked the experimental data, performed the analysis and revised the manuscript. JT, QL, HC and HpC helped recruiting the patients and collected samples. XZ collected the patients data. VP supervised the project and revised the manuscript. HpC performed the surgery, supervised the project and acquired funding. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was performed according to local ethical approval protocol no. EC. D (BG) .016.03.1. Informed consent was obtained from all subjects enrolled in the study. The study was in accordance with the guidelines of the ethical commission of Shanghai East Hospital of Tongji University.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Gurnani B, Kaur K. Phacoemulsification. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC. 2022.

[2] Furino C, Boscia F, Niro A, D'Addario M, Grassi MO, Saglimbene V, *et al*. Diabetic macular edema and cataract surgery: phacoemulsification combined with dexamethasone intravitreal implant compared with standard phacoemulsification. Retina. 2021; 41: 1102–1109.

[3] Scheers D, Van Os L, Ní Dhubhghaill S, Wouters K, Tassignon M. Clinically significant pseudophakic cystoid macular edema after bag-in-the-lens implantation. Journal of Cataract and Refractive Surgery. 2020; 46: 606–611.

[4] Hayashi K, Yoshida M, Hayashi S, Hirata A. Posterior vitreous detachment after cataract surgery in eyes with high myopia: an optical coherence tomography study. Japanese Journal of Ophthalmology. 2022; 66: 167–172.

[5] Wakefield D, Lloyd A. The role of cytokines in the pathogenesis of inflammatory eye disease. Cytokine. 1992; 4: 1–5.

[6] Hayashi K, Ohno-Matsui K, Futagami S, Ohno S, Tokoro T, Mochizuki M. Choroidal Neovascularization in Highly Myopic Eyes after Cataract Surgery. Japanese Journal of Ophthalmology. 2006; 50: 345–348.

[7] Stamer WD, Williams AM, Pflugfelder S, Coupland SE. Accessibility to and Quality of Human Eye Tissue for Research: a Cross-Sectional Survey of ARVO Members. Investigative Opthalmology & Visual Science. 2018; 59: 4783–4792.

[8] Goel M, Picciani RG, Lee RK, Bhattacharya SK. Aqueous humor dynamics: a review. The Open Ophthalmology Journal. 2010; 4: 52–59.

[9] McLaren JW, Ziai N, Brubaker RF. A Simple Three-compartment Model of Anterior Segment Kinetics. Experimental Eye Research. 1993; 56: 355–366.

[10] To CH, Kong CW, Chan CY, Shahidullah M, Do CW. The mechanism of aqueous humour formation. Clinical and Experimental Optometry. 2002; 85: 335–349.

[11] Tripathi RC, Millard CB, Tripathi BJ. Protein composition of human aqueous humor: SDS-PAGE analysis of surgical and postmortem samples. Experimental Eye Research. 1989; 48: 117–130.

[12] Fleenor DL, Shepard AR, Hellberg PE, Jacobson N, Pang IH, Clark AF. TGFbeta2-induced changes in human trabecular meshwork: implications for intraocular pressure. Investigative Ophthalmology and Visual Science. 2006; 47: 226–234.

[13] Klenkler B, Sheardown H. Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. Experimental Eye Research. 2004; 79: 677–688.

[14] Ozcan AA, Ozdemir N, Canataroglu A. The aqueous levels of TGF-beta2 in patients with glaucoma. International Ophthalmology. 2004; 25: 19–22.

[15] Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, *et al*. Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle among Subjects with Impaired Glucose Tolerance. New England Journal of Medicine. 2001; 344: 1343–1350.

[16] Taechameekietichai T, Chansangpetch S, Peerawaranun P, Lin SC. Association between Daily Niacin Intake and Glaucoma: National Health and Nutrition Examination Survey. Nutrients. 2021; 13: 4263.

[17] Shchuko AG, Zaitseva NV, Yurieva TN, Chernykh ER, Mikhalevich IM, Shevela EY, *et al*. Intraocular Cytokines and their Correlations with Clinical Parameters in Patients with Myopic Choroidal Neovascularization. Ophthalmologica. 2017; 237: 96–104.

[18] Shchuko AG, Zaytseva NV, Yur'eva TN, Chernykh ER, Mikhalevich IM, Grigor'eva AV. Role of immunological factors in the development of myopic choroidal neovascularization. Vestnik Oftalmologii. 2016; 132: 5–14. (In Russian)

[19] Duvesh R, Puthuran G, Srinivasan K, Rengaraj V, Krishnadas S, Rajendrababu S, *et al*. Multiplex Cytokine Analysis of Aqueous Humor from the Patients with Chronic Primary Angle Closure Glaucoma. Current Eye Research. 2017; 42: 1608–1613.

[20] Tong Y, Zhou YL, Zheng Y, Biswal M, Zhao PQ, Wang ZY. Analyzing cytokines as biomarkers to evaluate severity of glaucoma. International Journal of Ophthalmology. 2017; 10: 925–930.

[21] Vidal-Villegas B, Burgos-Blasco B, Santiago Alvarez JL, Espino-Paisán L, Fernández-Vigo J, Andrés-Guerrero V, *et al*. Proinflammatory Cytokine Profile Differences between Primary Open-Angle and Pseudoexfoliative Glaucoma. Ophthalmic Research. 2022; 65: 111–120.

[22] Lee WJ, Kang MH, Seong M, Cho HY. Comparison of aqueous concentrations of angiogenic and inflammatory cytokines in diabetic macular oedema and macular oedema due to branch retinal vein occlusion. British Journal of Ophthalmology. 2012; 96: 1426–1430.

[23] Shiraya T, Kato S, Araki F, Ueta T, Miyaji T, Yamaguchi T. Aqueous cytokine levels are associated with reduced macular thickness after intravitreal ranibizumab for diabetic macular edema. PLoS ONE. 2017; 12: e0174340.

[24] Hooks JJ, Chan CC, Detrick B. Identification of the lymphokines, interferon-gamma and interleukin-2, in inflammatory eye diseases. Investigative Ophthalmology and Visual Science. 1988; 29: 1444–1451.

[25] Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO. Vascular endothelial growth factors and angiogenesis in eye disease. Progress in Retinal and Eye Research. 2003; 22: 1–29.

[26] Lindahl P, Johansson BR, Levéen P, Betsholtz C. Pericyte Loss and Microaneurysm Formation in PDGF-B-Deficient Mice. Science. 1997; 277: 242–245.

[27] Mudhar HS, Pollock RA, Wang C, Stiles CD, Richardson WD. PDGF and its receptors in the developing rodent retina and optic nerve. Development. 1993; 118: 539–552.

[28] Koyama N, Watanabe S, Tezuka M, Morisaki N, Saito Y, Yoshida S. Migratory and proliferative effect of platelet-derived growth factor in rabbit retinal endothelial cells: Evidence of an autocrine pathway of platelet-derived growth factor. Journal of Cellular Physiology. 1994; 158: 1–6.

[29] Seo MS, Okamoto N, Vinores MA, Vinores SA, Hackett SF, Yamada H, *et al*. Photoreceptor-Specific Expression of Platelet-Derived Growth Factor-B Results in Traction Retinal Detachment. The American Journal of Pathology. 2000; 157: 995–1005.

[30] Gartaganis SP, Georgakopoulos CD, Exarchou AM, Mela EK, Lamari F, Karamanos NK. Increased aqueous humor basic fibroblast growth factor and hyaluronan levels in relation to the exfoliation syndrome and exfoliative glaucoma. Acta Ophthalmologica Scandinavica. 2001; 79: 572–575.

[31] Miao H, Hou X, Hwang D, Tao Y. Vascular Endothelial Growth Factor, Basic Fibroblast Growth Factor, and Pigment Epithelium-Derived Factor Expression in the Neovascular Iris in Retinal Diseases. Journal of Ophthalmology. 2018; 2018: 8025951.

[32] Agarwal N, Martin E, Krishnamoorthy RR, Landers R, Wen R, Krueger S, *et al*. Levobetaxolol-induced up-regulation of Retinal bFGF and CNTF mRNAs and Preservation of Retinal Function against a Photic-induced Retinopathy. Experimental Eye Research. 2002; 74: 445–453.

[33] Burgos-Blasco B, Vidal-Villegas B, Saenz-Frances F, Fernandez-Vigo JI, Andres-Guerrero V, Espino L, *et al*. Cytokine profile in tear and aqueous humor of primary open-angle patients as a prognostic factor for trabeculectomy outcome. European Journal of Ophthalmology. 2021. (in press)

[34] Choi JA, Ju HH, Kim JE, Lee J, Jee D, Park CK, *et al*. Cytokine profile and cytoskeletal changes after herpes simplex virus type 1 infection in human trabecular meshwork cells. Journal of Cellular and Molecular Medicine. 2021; 25: 9295–9305.