Original Research

Cytokine Profiling in Aqueous Humor of Glaucoma Patients and in Retinas from an Ex Vivo Glaucoma Animal Model

Jiancen Tang1,†, Mengsha Sun1,†, Yuan Feng2, Verena Prokosch2,*, Hongping Cui1,†,*

1Department of Ophthalmology, Shanghai East Hospital, Tongji University School of Medicine, 200120 Shanghai, China
2Department of Ophthalmology, Faculty of Medicine and University Hospital of Cologne, University of Cologne, 50937 Cologne, Germany
*Correspondence: verena.prokosch@uk-koeln.de (Verena Prokosch); hpceu@vip.163.com (Hongping Cui); hanhan.liu@uk-koeln.de (Hanhan Liu)
†These authors contributed equally.

Abstract

Background: Although the current role of cytokines and neuroinflammation in glaucoma remains obscure, it represents an expanding field in research. The purpose of this study was to analyze cytokines in the aqueous humor (AH) of glaucoma patients and in retinas from an ex vivo glaucoma animal model, to aid in determining the role of neuroinflammation in glaucoma. Methods: AH samples were collected from 20 patients during cataract surgeries (controls: n = 10, age: 70.3 ± 9.742; glaucoma: n = 10, age: 66.5 ± 8.073) in Shanghai East Hospital, an affiliate of Tongji University, between September 2018 and March 2019 and analyzed in duplicate by Luminex cytokine polystyrene color bead-based multiplex assay. Retinas from female Sprague-Dawley rats (n = 6) were harvested ex vivo and cultured with or without 60 mmHg of hydrostatic pressure for 24 hours. Retinal ganglion cells (RGCs) were quantified using Brn3a staining. Cytokines in the retina and culture medium were analyzed by rat cytokine array (Abcam). Results: At baseline, patients with primary angle closure glaucoma (PACG) have significantly lower levels of IL-6 and IP-10 and a higher level of PDGF-BB in their AH, compared to the controls. Postoperatively, patients with PACG have significantly higher levels of IL-1ra, IL-13, and MIP-1α and a lower level of IL-6. Elevated hydrostatic pressure led to significant RGC loss in the retina, ex vivo, as well as the upregulation of ciliary neurotrophic factor (CNTF), IL-6, IL-10, IL-4, and TIMP-1 alongside the downregulation of PDGF-AA, MMP-8, TNF-α, and IFN-γ. Furthermore, eight cytokines were detected as being downregulated in the culture medium, including PDGF-AA, MMP-8, and IL-4. Conclusions: Proinflammatory cytokines showed changes in both AH and ex vivo. Further studies are needed on the role of these cytokines and their corresponding signaling pathways in both neurodegeneration and glaucoma.

Keywords: glaucoma; neurodegeneration; cytokine; inflammation; retina culture; retinal ganglion cell

1. Introduction

Glaucoma is a group of optic neuropathies characterized by progressive loss of retinal ganglion cells and their axons and represents a leading cause of blindness. Glaucoma can be classified into two broad categories: open-angle glaucoma and angle-closure glaucoma [1]. Elevated intraocular pressure (IOP) is the main risk factor in both types of glaucoma, however, adequate IOP reduction cannot halt the progress of the disease in most patients. Additionally, the pathophysiology remains obscure [2].

Increasing evidence suspects neuroinflammation, an innate immunological response of the nervous system that comprises microglia, astrocytes, cytokines, and chemokines, as a key process in neurodegenerative disease [3–7]. High IOP is a potential stimulus associated with the production of proinflammatory cytokines, such as chemokine (C-C motif) ligand 2 (CCL2), CCL1, CCL1, Interleukin-10 (IL-10), Interleukin-6 (IL-6), and activated transforming growth factor-beta 2 (TGF-β2) [8]. Increased levels of TGF-β2 and tumor necrosis factor-α (TNF-α), and IL-6, interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (IL-12), interferon-γ (IFN-γ), interferon-α (IFN-α), vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein-1 (MCP-1), alongside the chemokine (C-X-C motif) ligand 9 (CXCL9) have even been found in the aqueous humor (AH) of glaucoma patients [9–14]. It is indicated that these cytokines are produced locally by ocular tissues [15].

Although the exact source of the inflammatory cytokines and their roles in neuroinflammation is unclear, the cytokine levels in AH have been reported to be associated with the clinical prognosis of retinal disorders [16–19]. However, it is difficult to verify these results and to study the molecular mechanisms of the findings in relation to glaucoma in human ocular tissues since human ocular material can only be obtained postmortem and human patients usually have other onset systematic diseases. Thus, the experimental glaucoma models provide the possibility to investigate the precise molecular and cellular changes in a standardized manner. Most of the progress in the research of glaucoma has been based on experimentally inducible
glaucoma animal models [20–24]. Ex vivo retina models are common research tools that provide the additional positive effect of improved experimental standardization and a reduction in the number of animals that are used in the research [25,26].

The purpose of this study was to first analyze the cytokines in the AH of patients with primary angle closure glaucoma (PACG). Secondly, to analyze the cytokines in the retinas from an ex vivo glaucoma model. The largely unspecified roles of neuroinflammation in glaucoma prompted us to further compare the cytokine profiles between glaucoma patients and in the ex vivo animal model, as well as their corresponding signaling pathways. Better insight into the intraocular condition in glaucoma and the glaucoma model is fundamental for optimizing future research in both clinical and basic science.

2. Materials and Methods

2.1 Patient Recruitment

A total of 20 patients, including seven males (14 eyes) and thirteen females (26 eyes), underwent cataract surgery from September 2018 to March 2019 in the ophthalmology department at Shanghai East Hospital and were included in this study. Participants were adults with PACG and their age-matched controls had age-related cataracts (10 patients/group). PACG was defined using the criteria from the International Society for Geographical and Epidemiological Ophthalmology (ISGEO) [27]. 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, 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., Kniz, Switzerland). Patients with histories of acute glaucoma attack, ocular surgery, an advanced visual field defect, and uncontrolled IOP were excluded. All the PACG patients enrolled in the present study had controlled IOP with only pilocarpine eyedrops (assessed by 24 h IOP profile). Exclusion criteria included previous trauma, fundus pathology, retinal diseases, other autoimmune disease, and a history of previous ocular surgery. This prospective study was approved by the research ethics boards at Shanghai East Hospital of Tongji University (EC. D (BG) .016.03.1-2018-007), and adhered to the tenets of the Declaration of Helsinki. All participants provided written informed consent prior to participation in the study.

2.2 Aqueous Humor Collection

A total of 40 aqueous humor samples were collected at the outset of cataract surgeries from both eyes of 20 patients. The cataract surgery and the aspiration of the AH were carried out in sterile conditions by the same surgeon and under a surgical microscope for ophthalmology (Leica M844 F40 / F20, Wetzlar, Germany). The cytokines from the AH of the first eye, before the first eye surgery, were considered the baseline, while those from the second eye after the first eye surgery were defined as postoperation.

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 “2 o’clock” using a 15-degree angle blade (Model: 8065921501, Alcon, Geneva, Switzerland). Through the corneal incision, approximately 150 μL of the AH was aspirated slowly using a 1 mL syringe attached to a 30-gauge needle, on a plane approximately parallel to the long axis of the elliptical lens. 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, chemokines, and growth factors were measured in the AH samples using Luminex cytokine polystyrene color bead-based multiplex assay, in duplicate.

The measured cytokines, chemokines, and growth factors were as follows: Interleukin-10 (IL-10), interleukin-13 (IL-13), interleukin-12p70 (IL-12p70), interleukin-15 (IL-15), interleukin-17 (IL-17), interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), eotaxin (CCL11), interferon gamma-induced protein 10 (IP-10), monocyte chemotactractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), regulated upon activation, normal T cell expressed and presumably secreted (RANTES), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF-BB), and vascular endothelial growth factor (VEGF).

2.4 Ex Vivo Glaucoma Model

Age-matched female Sprague-Dawley rats (n = 12, 250–300 g) were used in this study. All animals were housed in standard accommodation provided by the Animal Institute of the Faculty of Medicine of the University of Cologne. Food and water were provided ad libitum on a 12-hour diurnal cycle.

All experimental procedures complied with the German animal welfare law, which is in line with the European Community law, and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animal protocols used in this study were reviewed and approved by the governmental body responsible for ani-
PACG patients had their IOP medically controlled throughout the study using only pilocarpine in the normal range. There is no significant difference in IOP between groups before or after the cataract operation. n = 10. IOP, intraocular pressure; ns, not significant. PACG, primary angle closure glaucoma.

mal welfare in the state of North Rhine-Westphalia, Germany (Paragraph 4.21.003, Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen). Sprague-Dawley rats were sacrificed under a CO₂ atmosphere. The eyes were removed immediately and placed in phosphate-buffered saline (PBS, pH 7.4). The anterior segment of the eye was removed under the microscope and each whole retina was carefully explanted. The retina was laid flat on Millipore filters (Millipore, Millicell, Cork, Ireland) with the retina ganglion cell side facing up. The vitreous body was subsequently removed using forceps.

The retinal explants were transferred to a Lumox dish 50 (Sarstedt, Nümbrecht, Germany). Every two retinal explants were assigned to a dish and were cultured in a 5 mL culture medium. The culture medium was Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 (DMEM/F12; Gibco BRL, Eggenstein, Germany) with an additional 10% FCS and 1% penicillin/streptomycin. The control groups and the IOP groups were placed in the same pressure incubation chamber. The chambers were put in an incubator (Heracell 240i, Thermo Scientific, Niederelbert, Germany) at 37 °C in a 5% CO₂ humidified atmosphere for 24 hours. The control group was cultured without additional pressure, although the IOP group was cultured under fluctuating pressure for the first 6 hours (pressure in the chamber was increased to 60 mmHg, then reduced gradually every 15 minutes until 0 mmHg), and the other 18 hours were without pressure.

2.5 Quantification of Retinal Ganglion Cells

Retinal ganglion cells (RGCs) were determined by immunohistochemical staining against the brain-specific homeobox/POU domain protein 3A (Brn3a, sc-8429, Santa Cruz Biotechnology, Dallas, TX, USA) using retinal flatmounts. One-quarter of each retinal sample was carefully separated under a microscope. After washing in PBS, the retinal tissue was fixed in 4% formalin solution for 30 minutes (Carl Roth, Karlsruhe, Germany) at room temperature and in 30% sucrose solution overnight. The retinas were permeabilized with PBS-T (0.3% Triton x-100 in PBS) for 30 minutes. Subsequently, the tissues were blocked with Blotto solution (1% milk powder and 0.3% Triton x-100 in PBS) for 30 minutes at room temperature and incubated with the primary antibody (1:200; mouse Anti-Brn3a monoclonal antibody, EMD Millipore, Darmstadt, Germany) diluted in Blotto solution at 4 °C overnight. After thorough washing with PBS-T, the retinal samples were incubated with the secondary antibody (1:1000; goat anti-mouse IgG Alexa Fluor™ 488, Abcam, Cambridge, UK) for 1 hour at room temperature. The retinal samples were flat mounted on the objective, the RGCs were visualized using a fluorescent microscope (Zeiss Axio Imager.M2, Carl Zeiss Microscopy Deutschland GmbH, Oberkochen, Germany) at 20× magnification. Photos were taken at the same six positions for each retinal quarter. The number of Brn3a-positive cells was counted using ImageJ (imageJ Fiji v_1, https://imagej.nih.gov/ij/).

2.6 Rat Cytokine Antibody Array in Retinas and Culture Medium

The rest of the rat retinas from 12 eyeballs were lysed by T-PER tissue protein extraction reagent. After protein quantification by BCA assay, retinal protein samples from both groups were pooled equally into three biological replicates, represented by R1, R2, and R3 (N = 3 replicates per group), the culture medium was pooled equally into three biological replicates per group. Total protein samples of 250 µg per biological replicate were added to 1 mL of 1X Blocking Buffer (final volume) and subsequently subjected to the array membrane of the rat cytokine antibody array (ab133992, Abcam, Cambridge, UK), the procedure was carried out following the manufacturer’s manual.
Fig. 2. Aqueous humor cytokine profiles for PACG and control patients at baseline and postoperation. (A) Heatmap depicts the baseline hierarchical clustering of the differentially expressed cytokines in the PACG group compared to the control group. The relative abundance of the cytokines is represented as heat maps for each cluster. (B) Differentially expressed cytokines in PACG and control patients at baseline. (C) Heatmap depicts the postoperative hierarchical clustering of the differentially expressed cytokines in the PACG group compared to the control group. (D) Differentially expressed cytokines in the control and PACG groups postoperatively. n = 10, *p < 0.05. CTRL: control; PACG, primary angle closure glaucoma.

2.7 Bioinformatics, Functional Annotation, Pathways Analyses, and Statistics

Unsupervised hierarchical clustering analysis was performed using the z-scores of the cytokine concentrations following Euclidean distance (linkage = average; preprocess with k-means) and illustrated in a heat map. The significant differentially expressed cytokines, chemokines, and growth factors (fold change cutoff of ≥1.5 or <2/3) in both PACG patients and the glaucoma animal model were further subjected to the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) for protein–protein association networks [28] and pathways analysis. Statistical analysis and presentation were performed by GraphPad Prism (version 9.2, Mac OS X, GraphPad Software, San Diego, CA, USA, www.graphpad.com). Data values are presented as mean ± SD, Mann–Whitney tests were used to compare the age and operation interval of the patients, while an unpaired t-test was used to compare the RGC numbers between groups. A value of p < 0.05 was considered statistically significant.

3. Results

3.1 Patient Demographics

In this study, seven male (14 eyes) and thirteen female (26 eyes) patients were recruited and distributed into two groups: patients diagnosed with PACG (PACG, n = 10) and a control group of cataract participants (CTRL, n = 10, Table 1). There was no significant difference in age, gender, and the interval and operative times between the groups. All PACG patients had their IOP medically controlled with only pilocarpine eyedrops without prior glaucoma surgery (Fig. 1). All patients recovered well from the phacoemulsification without intraoperative and postoperative complications.

3.2 Cytokine Profiling of AH from the Controls and Patients with PACG at Baseline and Postoperation

Luminex cytokine polystyrene color bead-based multiplex assay was used to quantify and distinguish AH cytokine profiles acquired from patients during their first and second cataract surgeries. The AH acquired from the first operation was assumed as the baseline and those acquired from the second operation were set as postoperative.

There is a subtle separation in cytokine profile between the PACG vs. controls (Fig. 2A). At baseline, 3 of the 27 cytokines were found to be significantly differentially expressed in the AH samples of glaucoma patients compared to the control group (Fig. 2B). Patients with PACG has significantly lower levels of IL-6 (FC = 0.37) and IP-10 (FC = 0.447) and a significantly higher level of PDGF-BB (FC = 1.508) in their AH, compared to the controls.
In the second eye, there was a clearer separation in cytokine profile between the PACG and control groups (Fig. 2C). Four cytokines were found to be significantly differentially expressed in the AH samples between the PACG and the control groups (Fig. 2D). Patients with PACG had significantly higher levels of IL-1ra (FC = 2.0), IL-13 (FC = 2.152), and MIP-1α (FC = 2.0) and a significantly lower level of IL-6 (FC = 0.425) in their AH, compared to the controls.

### 3.3 Significant RGC Loss Ex Vivo in the Glaucoma Animal Model

Culturing under fluctuating hydrostatic pressure for 24 h resulted in the number of RGCs in the retinal samples being significantly reduced (167.52 ± 39.52 RGC/field) compared to the control (205.59 ± 28.38 RGC/field, Fig. 3, **p < 0.0001, n = 6, mean ± SD)."
Fig. 3. Significant RGC loss ex vivo in the glaucoma animal model. (A,B) Representative fluorescence microscopy of Brn3a positive RGCs in retinal explants after culturing 24 h with or without fluctuating pressure. (C) Compared to the control (205.59 ± 28.38 RGC/field), culturing under fluctuating pressure resulted in significant RGC loss in retinal explants (167.52 ± 39.52 RGC/field). n = 6, ****p < 0.0001. RGCs, retinal ganglion cells. Scale bar = 50 μm.

3.6 Functional Enrichment Analysis of Significantly Altered Cytokines in PACG Patients and Glaucoma Animal Model

The differentially expressed cytokines from both the patients and the glaucoma animal model were respectively entered into Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) for protein–protein association networks (Fig. 6 and Table 2) [28]. Using STRING analysis (Version 11, https://string-db.org/), pathways such as the IL-17 signaling pathway, viral protein interaction with cytokine, and cytokine receptor and cytokine–cytokine receptor interaction were shown to be deregulated in all groups. Whereas pathways such as the TNF signaling pathway and toll-like receptor signaling pathway were altered in the PACG baseline group and glaucoma model, while the inflammatory bowel disease signaling pathway and JAK/STAT signaling pathway were altered in the PACG postoperative group and glaucoma model. Nonetheless, there are pathways that were exclusively deregulated in the glaucoma animal model, such as the MAPK, PI3K–Akt, HIF-1, neurotrophin, and apoptosis signaling pathways.

4. Discussion

The aim of this study was to first profile the cytokines in the AH of patients diagnosed with PACG in comparison to non-PACG patients at both the baseline and after cataract surgery. Luminex cytokine polystyrene color bead-based multiplex assay was used to quantify and distinguish 27 cytokines simultaneously. Our results showed a differential expression of AH cytokines in PACG patients both at baseline and postoperatively. At baseline, an increased abundance of PDGF-BB was observed, while IL-6 and IP-10 were decreased. After cataract surgery, IL-6 remained lower, whereas the abundance of IL-1ra, IL-13, and MIP-1alpha increased. Changes in the signaling pathways involved in the Toll-like receptor signaling pathway, TNF signaling pathway, cytokine–cytokine receptor interaction, IL-17 signaling pathway, cytosolic DNA-sensing pathway, viral protein interaction with cytokine and cytokine receptor, inflammatory bowel disease, and JAK/STAT signaling pathway were found in patients with PACG compared to the control group.

The molecular changes in humans cannot be examined in a standardized manner, whereas using experimental glaucoma models allows the precise molecular and cellular changes to be investigated in a standardized manner. The second objective was to profile the cytokines in the retina of an ex vivo glaucoma animal model and then compare the cytokine profiles of the PACG patients and the animal model.

Although ex vivo models do not represent the intricacy of glaucomatous disease [29,30], subjecting retinal explants from rodents to elevated hydrostatic pressure is able to simulate some of the milieu of the retinal ganglion cells under glaucoma as well as the complexity of the internal cellular processes. This model has been used repeatedly in our
Fig. 4. Cytokine profiling of retinal samples from the glaucoma animal model and controls. (A) Representative cytokine antibody array membranes of the controls and the glaucoma model. (B) Heat map depicts the hierarchical clustering of the differentially expressed cytokines in the glaucoma model compared to the control group. (C) Eight cytokines were significantly upregulated in the glaucoma model (fold change cutoff of >1.5, n = 3). (D) Seven cytokines were significantly downregulated in the glaucoma model (fold change cutoff of <2/3, n = 3). *p < 0.05.
Eight cytokines were downregulated in the culture medium of the glaucoma model compared to the control (fold change cutoff of <2/3, n = 3).

The list of the differentially expressed cytokines subjected to STRING analysis to reveal functional interaction.

Previous studies to study the pathogenesis of glaucoma and to test potential neuroprotective treatments [31–35]. To assess how well the results obtained from this model can be transferred to glaucoma in humans, a better understanding of the similarities and differences between the *ex vivo* model and glaucoma patients is required. Culturing under fluctuating hydrostatic pressure led to significant loss of RGCs and changes in the cytokine profile. The abundance of CNTF, CINC-2α, IL-4, IL-6, IL-10, Fas ligand, b-NGF, and TIMP-1 was increased, while IFN-γ, TNF-α, MMP-8, L-selectin, RAGE, PDGF-AA, and prolactin R were decreased in the retinal samples from the glaucoma model.

In our study, we found that patients with PACG have some degree of inflammatory factor change in their eyes.
before surgery. The level of IL-6 was decreased in PACG patients both at baseline and postoperatively. A meta-analysis concluded an overall reduction in AH IL-6 levels in patients with POAG and pseudoxfoliative glaucoma (PXG) compared to controls [36,37]. However, IL-6 was increased in the ex vivo model, as well as in early optic nerve head injury and inflammatory stimulation [38,39]. Perhaps the difference in the course of the diseases and properties of IL-6 could explain the discrepancy since it is a pleiotropic cytokine that possesses proinflammatory and antiapoptotic properties. IL-6 plays a central role in host defense mechanisms by regulating acute phase reactions. It has been reported to protect retinal ganglion cells from pressure-induced apoptosis by counteracting proapoptotic stimuli from retinal glial cells [38,40,41]. In addition to IL-6 itself, proteins related to IL-6, such as the CNTF, influence the survival of retinal cells [42], which is also significantly increased in the glaucoma model in this study. CNTF prolongs the survival of retinal ganglion cells against different injuries [43–46]. Both IL-6 and CNTF promote axonal growth in retinal ganglion cells in optic nerve crush or transaction models [39,47,48].

Interferon-induced protein 10 (IP-10) is another cytokine that was significantly decreased in the AH of PACG patients at baseline. IP-10 belongs to the CXC chemokine family, which can induce chemotaxis, cell growth, apoptosis, angiogenesis, and inflammation. In other studies, the IP-10 level was found to be increased in the AH of patients with chronic PACG, as well as in patients with acute angle closure glaucoma (AACG) [49,50]. However, the exact role of IP-10 in glaucoma pathology is largely unclear.

Platelet-derived growth factor-BB (PDGF-BB) was significantly upregulated in the PACG group, whereas in the ex vivo model, PDGF-AA was significantly downregulated in both the retinal samples and the culture medium. The expression level of the PDGF family increased under hypoxic conditions and is believed to be associated with angiogenesis under hypoxia [51]. PDGF-BB is a potent inducer of proinflammatory cytokines [52]. In AACG patients who underwent trabeculectomy, those with consistently elevated IOP had significantly increased PDGF-CC and -DD levels in their AH compared to those with reduced IOP after trabeculectomy [53]. However, despite controlling IOP, glaucoma patients still had higher PDGF-BB in their tear samples as well as in their AH [54,55]. The PDGF-AA level was downregulated in the serum of PXG patients [37], yet upregulated in the AH of patients with neovascular glaucoma, phakic POAG, and phakic exfoliation glaucoma [56,57]. PDGF-AA has shown neuroprotective effects on RGCs both in vitro and in vivo in a hypertension model, while it also inhibits hydrogen peroxide-induced retinal cell death [58].

The AH cytokine profile in the second eye after the first eye cataract surgery reflects the inflammatory condition in PACG patients. IL-6 remained significantly decreased, while the levels of PDGF-BB and IP-10 were not significantly different compared to the control. The levels of IL-1α, IL-13, and MIP-1α were significantly increased in the PACG group compared to the control. IL-1α was also found to be increased in the AH of POAG patients [59]. The interleukin-1 (IL-1) family is a central mediator of innate immunity and inflammation [60], and has been linked to neurodegenerative and neuroinflammatory diseases, such as Alzheimer’s disease, Parkinson’s disease, acute neurodegeneration in the central nervous system, and retinal degenerative diseases [61–64]. IL-1α is an anti-inflammatory competitive receptor antagonist [65–67]. An age-related increase in IL-1α was also observed in donor eyes [68]. The levels of IL-10 and IL-1β were found to be increased in the trabecular meshwork of POAG patients and seem to play a role in glaucoma pathology. IL-1α also protects trabecular meshwork (TM) cells against oxidative stress [69,70]. IL-13 is another anti-inflammatory cytokine that was also significantly increased in the AH in this study, as well as in another study on PACG patients [71]. IL-13 is associated with AMD and proliferative diabetic retinopathy [72,73]. However, its role in glaucoma and RGCs has been less studied. MIP-1α was also significantly higher in the AH in this study and in the AH from POAG eyes [55]. Macrophage inflammatory protein-1alpha (MIP-1α) acts produced by macrophages and monocytes after they are stimulated with proinflammatory cytokines, such as IL-1β, are crucial for immune responses towards infection and inflammation [74]. These results indicate that PACG patients show stronger intraocular inflammatory reactions toward topical stimuli compared with the controls.

It is clear that cytokine levels vary among studies in patients with the same diagnosis, in not only the different stages and severity of disease but also the variability in ages, treatments of the recruited patients, and the method used to analyze the cytokines have a distinct impact on the results. Commonalities among studies are that glaucoma patients have shown distinguished inflammatory response profiles in their AH compared to the control. Indeed, similar changes are observed in the signaling pathways in the retinal samples from the glaucoma model to patients with PACG, such as in the Toll-like receptor signaling pathway, TNF signaling pathway, cytokine–cytokine receptor interaction, IL-17 signaling pathway, and JAK/STAT signaling pathway; furthermore, there are also changes in pathways seen exclusively in the glaucoma model, such as in the MAPK signaling, apoptosis, neurotrophin signaling, PI3k-Ark signaling, and HIF-1 signaling pathways. However, the mechanisms involved need further investigation.

Human retinal samples are difficult to obtain. The AH is the most frequently sampled ocular material in examinations and clinical studies of ocular disease, including diseases in the posterior segment of the eye. It is widely assumed by ophthalmology researchers that the AH cytokine levels are influenced by the retina. The AH cytokine levels
have been reported to be correlated with retinal conditions, such as age-related macular degeneration, diabetic macular edema, chronic central serous chorioretinopathy, and glaucoma [19,75–81].

In this study, the cytokine changes in the culture medium partially reflect the cytokine changes in the retinal samples. A relative decrease in the abundance of IL-10, IL-4, IL-1r6, MMP-8, L-selectin, RAGE, and PDGF-AA was observed in the culture medium. In our previous study, changes in the retinal proteome were also detected in the vitreous body in an in vivo glaucoma model [33]. Due to the size of the rodent eyeball, it is difficult to collect the AH from small animals, the exact correlation between cytokines in the retina and the AH needs to be explored in bigger animals.

To the best of our knowledge, this is the first study to compare the cytokine profiles of glaucoma patients with an ex vivo model. The alterations in cytokine profiles seem to be quite different in the retinal samples from the glaucoma model and the AH samples from PACG patients. This is probably because the ex vivo model represents an acute phase of retinal degeneration, while the AH samples were not collected immediately after the operation. Nonetheless, similar cytokine changes in the ex vivo glaucoma model can be seen in the AH from glaucoma patients in other studies. For instance, an increase in IFN-γ and MIP-1α was found in the AH, while IL-4 was increased in the tears of POAG patients [55]. The levels of PXG, fractalkine, IL-10, and IL1Ra were significantly upregulated in serum, while the level of PDGF-AA was downregulated [37].

5. Conclusions

The different cytokines between patients with PACG and the controls confirm that eyes with PACG retained a mild inflammation and in response to external stimulation, such as the cataract surgery, a stronger inflammatory reaction was also observed in eyes with PACG. The cytokine profile for the culture medium partially represents the cytokine changes in the retina. The glaucoma model simulates similar cytokine and pathway changes not only in PACG patients but also in AAGC, POAG, and PXG. It is a practical tool to study some of the specific changes involved in glaucoma pathology. However, it is also important to address the differences between this model and glaucoma patients, thus, caution should be taken when translating the results from this model to the glaucoma pathology in human patients.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

JT and YF performed the measurements, HC, VP and HL were involved in planning and supervised the work, JT, MS, YF and HL processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. JT, MS, VP and HL wrote the paper with input from all authors. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was performed according to local ethical approval protocol no. EC. D (BG).016.03.1-2018-007. 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.

Acknowledgment

Not applicable.

Funding

This research was funded by National Natural Science Foundation of China (81870634) and Deutsche Forschungsgemeinschaft (DFG), grant number PR1569-1-1.

Conflict of Interest

The authors declare no conflict of interest.

References


[43] Meyer-Franke A, Kaplan MR, Pfeifer FW, Barres BA. Charac-


[60] Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunological Reviews. 2018; 281: 8–27.


