CLINICAL RELATIONSHIP BETWEEN CYTOKINE CONCENTRATION IN ATRIAL FLUID AND PRIMARY GLAUCOMA

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ABSTRACT

Objective*: Glaucoma is a group of diseases characterized by optic nerve atrophy and visual field defects in common. Atrial fluid is inextricably linked to the development and progression of glaucoma, and the detection of cytokines in atrial fluid is one of the current hot topics in glaucoma research. Researchers hope to find one or several biomarkers in atrial fluid to answer clinical questions such as screening and early diagnosis of glaucoma in high-risk groups. Previously, the main obstacle limiting the detection of cytokines in atrial fluid was the small volume of atrial fluid specimens, while the liquid-phase suspension microarray technology has solved this problem by enabling multi-factor detection in small volume samples. In addition, there are few studies on the correlation between atrial fluid cytokine concentrations and the prognosis of trabeculectomy surgery in glaucoma patients, and it would be helpful to identify factors associated with the prognosis of trabeculectomy surgery for the follow-up management of glaucoma patients after surgery.*

Methods: *1.90 participants were selected to collect atrial fluid specimens(30 patients with POAG, 30 patients with PACG (including 15 patients with APACG and 15 patients with CPACG) and 30 patients with senile cataract).2.IL-6, IL-8, IFN-γ, MCP-1, TNF-α, Cathepsin D, sNCAM and other molecules were detected in the atrial fluid specimens using Luminex liquid-phase suspension microarrays. 3. 60 patients with primary glaucoma underwent trabeculectomy by the same surgeon and were followed up for 24 months after surgery, with IOP as the main index, and the correlation between cytokine concentrations in atrial fluid and the prognosis of trabeculectomy was analyzed using the Kaplan-Meier method and Cox proportional risk regression model.*

Results: *1. Cathepsin D and sNCAM (P<0.001) concentrations in the atrial fluid of patients in the POAG group were significantly higher than those in the senile cataract group; IFN-γ (P=0.02) concentrations were significantly lower than those in the senile cataract group.2.Cathepsin D, sNCAM, IL-6, IL-8 and MCP-1 (P<0.001) and TNF-α (P=0.001) concentrations in atrial fluid were significantly higher in the PACG group than in the senile cataract group.3.The concentrations of sNCAM, IL-6, IL-8, MCP-1 (P<0.001) and TNF-α (P=0.007) in the atrial fluid were significantly higher in the PACG group than in the POAG group.4.The concentration of IL-6 (P=0.02) in the atrial fluid of patients in the APACG group was significantly higher than that in the CPACG group.5. Cathepsin D (ϱ=-0.67, P<0.001), IL-6 (ϱ=-0.78, P<0.001), IL-8 (ϱ=-0.62, P=0.001), MCP-1 (ϱ=-0.69, P<0.001) and TNF-α (ϱ=-0.69, P<0.001) in the atrial fluid of patients with POAG were significantly correlated with MD values.6. The follow-up study suggested that the number of previous anti-glaucoma drug types was a risk factor for trabeculectomy (RR=14.01, P=0.02), while atrial cytokine concentrations did not correlate with trabeculectomy.*

Conclusion: *1.Liquid-phase suspension microarray technology is a good way to quantify cytokine concentrations in atrial water. 2.Altered cytokine concentrations in atrial water in patients with POAG may reflect damage to the trabecular meshwork and optic nerve. 3.Altered atrial cytokine concentrations in PACG patients may be associated with blood-atrial water barrier disruption, intraocular ischemia and hypoxia, and ischemia-reperfusion injury. 4.The number of types of antiglaucoma drugs used previously is a risk factor for trabeculectomy surgery.*

Keywords: *Glaucoma, cytokines, atrial aqueous, trabeculectomy.*

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Introduction

Glaucoma is a group of diseases commonly characterized by optic nerve atrophy and visual field defects and is the first irreversible blinding eye disease in the world(1). Primary glaucoma is a type of glaucoma whose etiology has not yet been fully elucidated, and is divided into primary open-angle glaucoma (POAG) and primary closed-angle glaucoma (PACG) depending on whether the anterior chamber is closed or open at the time of IOP elevation; primary closed-angle glaucoma is divided into acute closed-angle glaucoma (APACG) and chronic closed-angle glaucoma (CPACG) depending on the urgency of the onset and clinical course[2]. (CPACG). In recent years, more and more scholars consider glaucoma as a neurodegenerative pathology. It has been shown that the prevalence of glaucoma is higher in the AD and PD populations; and Sadum found that retinal ganglion cell death is also present in the eyes of AD patients.

Elevated intraocular pressure is the most important risk factor in the development of glaucoma, and elevated intraocular pressure is often the result of altered atrial aqueous dynamics, so atrial aqueous is closely associated with the development of glaucoma. In recent years, there has been an increasing number of studies on atrial fluid, and cytokine concentrations in atrial fluid are altered in a variety of ocular diseases, such as open-angle glaucoma, neovascular glaucoma, age-related macular degeneration, and high myopia, and some researchers have suggested that alterations in these cytokines in atrial fluid may be involved in the onset and progression of ocular diseases, and or may affect the prognosis of surgery, therefore, studying the changes of these cytokines in atrial fluid can help us to understand the mechanisms of disease onset and development[3].

The detection of cytokines in the atrial fluid of glaucoma patients is one of the popular ophthalmic studies, such as the study of inflammatory factors and oxidative stress factors in the atrial fluid of glaucoma patients. Previously, the main factor limiting the detection of cytokines in atrial fluid was the atrial volume, especially for patients with primary angle-closure glaucoma, which has a short eye axis, shallow anterior chamber, and small anterior chamber volume, and only a small amount of cytokines can be detected if enzyme-linked immunosorbent assay (ELISA) is used.

In recent years, the advent of assay technologies, such as liquid-phase suspension microarrays, has made it possible to detect multiple factors in small volume samples. The application of liquid-phase suspension microarrays, on the other hand, allows for multi-factor detection in small volume samples with higher sensitivity[4].

Seven cytokines, including interleukin-6 (IL-6), interleukin-8 (IL-8), interferon-gamma (IFN-γ), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), histone proteinase D (Cathepsin D), and soluble neural cell adhesion molecule (sNCAM), were selected for this study. Their concentrations in the atrial fluid of primary glaucoma patients and aged cataract patients were measured. We also investigated the mechanism and clinical significance of the changes of cytokine concentrations in atrial fluid in primary glaucoma, and investigated the relationship between cytokine concentrations in atrial fluid and the prognosis of trabeculectomy surgery through follow-up studies.

Materials and methods

General materials

Ethical review and informed consent

This study is a clinical trial study and has been approved by the Ethics Committee of our Hospital. Participants were required to sign the informed consent form voluntarily. Participants were informed of the purpose and process of the study before participating in the study.

Study population

Inclusion criteria:

POAG:

- Affected eye with glaucomatous optic papillary damage and/or retinal nerve fiber layer defect;
- Affected eye with glaucomatous visual field defect;
- Intraocular pressure >21 mmHg;
- Atrial angle examination with an open anterior chamber angle.

APACG:

- The patient has the ocular anatomical features that occur in closed-angle glaucoma;
- Acute IOP elevation with atrial angle closure;
- Examination of the contralateral eye in a patient with monocular onset reveals the same ocular anatomical features that occur in primary closed-angle glaucoma;
- Ocular examination may include decreased visual acuity, pain, congestion, corneal edema, dilated pupils, iris atrophy, and other ocular signs caused by acute IOP elevation signs.

CPACG:

- Ocular anatomical features for the occurrence of closed-angle glaucoma;
- Symptoms of recurrent mild to moderate IOP elevation or asymptomatic;
- Narrowing of the atrial angle and closure of the atrial angle in the state of high IOP;

• Visible damage to the optic papilla and visual field; 5. no signs of ischemic damage caused by acute high IOP in the anterior segment.

Exclusion criteria:

Patients with glaucoma:

• Secondary glaucoma;

• Previous history of ocular surgery in the operated eye;

• Combined ocular diseases other than senile cataract, such as uveitis, iris neovascularization, trauma, tumors, etc.;

• Combined diabetes, systemic infections, autoimmune diseases, etc.;

• Combined neurodegenerative diseases such as Alzheimer's disease, Parkinson's, multiple sclerosis, etc.;

• Collection of atrial volume Less than 50 µL or incomplete clinical information.

Cataract patients:

• Secondary cataract;

• History of previous eye surgery in the operated eye;

• Combination of other eye diseases, such as glaucoma, uveitis, iris neovascularization, trauma, tumor, etc.;

• Combination of diabetes, systemic infection, autoimmune diseases, etc.;

• Combination of neurodegenerative diseases such as Alzheimer's disease, Parkinson's, multiple sclerosis, etc.;

• Collection of atrial volume less than 50µL or incomplete clinical information.

Ocular examination and atrial fluid collection

Intraocular pressure measurement

IOP was measured using a non-contact IOP meter TX-F (Canon, Japan).

Before the measurement, it was explained to the participants that there would be airflow to the eyes during the IOP measurement, and the participants were instructed not to move their eyes and heads, and were assisted to place their jaws on the bracket with their foreheads pressed against the headband and relaxed sitting position, then the focusing handle was moved and the alignment point was placed in the alignment mark, and air was injected to display the IOP value, which was measured 3 times, calculate the average value that is the IOP value of the IOP measurement.

Eye axis measurement

The IOL Master was used to measure the axial length of the eye. The participant was asked to sit in a seated position, the height of the instrument was adjusted to help the participant sit comfortably, the participant was assisted to place the lower jaw in the mandibular rest, the forehead was pressed against the forehead rest, the position of the mandibular rest was adjusted so that the eye was in the operable range, the participant was asked to look at the fixation lamp and hold it still, the axial length of the participant's eye was measured, the measurement was taken 5 times, and the average value was calculated as the axial length of the eye for that measurement. The average value was calculated as the eye-axis length of the measurement.

Visual field examination

Before the examination, the participants were explained the procedure of visual field testing in detail, brought into the dark room, took a sitting position, covered the non-testing eye with an eye shield, assisted the participants to place their chin on the cheek rest, asked the participants to look at the fixation lamp without moving their eyes, and pressed the sensor when they saw the blinking point.

If the false-positive error rate or false-negative error rate exceeded 20%, the participant was allowed to rest for 15 minutes and then retested, and the MD value was recorded after the test was completed.

Atrial fluid collection

All atrial fluid samples were collected between 8 a.m. and 12 p.m. All procedures were performed by the same surgeon, and 50-100 µL of undiluted atrial fluid samples were collected by anterior chamber puncture 1 mm inside the corneal limbus during trabeculectomy and cataract ultrasound emulsification before breaking the blood-atrial fluid barrier, avoiding the mixing of blood and other fluids during collection, in sterile The atrial fluid samples were collected in sterile Eppendorf tubes, wrapped with sealing film and numbered, and stored in a -80 degree refrigerator within 10 minutes after removal for testing.

Luminex liquid-phase suspension microarray technology

Luminex liquid-phase suspension chip technology, also known as suspension array and flow-through fluorescence technology, is based on the principle of coloring each microsphere with 2-3 fluorescent dyes to produce 100-500 different colors of microbeads through different dye ratios, and each different color of microbeads is coupled with a specific protein antibody; when the microbeads coupled with the specific protein antibody react with the protein in the sample When the microbeads coupled with the specific protein antibody react with the protein in the sample, then the biotin-labeled detection antibody is added, and the signal is amplified by the connection of streptavidin and biotin labeled with phaeohemoglobin (PE), and the complex formed after the whole reaction includes the fluorescence of the microbeads and the fluorescence signal of PE; during the detection, the first beam is red laser, which can identify the microbeads, and the second beam is green laser, which can detect the strength of the PE fluorescence signal. detects the strength of the PE fluorescence signal; finally the intensity of the fluorescence signal will be processed into a digital signal, and the concentration of each protein can be calculated after analysis by professional software.

Postoperative follow-up of trabeculectomy patients

Sixty patients with primary glaucoma (30 POAG patients and 30 PACG patients) after trabeculectomy surgery were followed up postoperatively, and the main index of the follow-up was IOP.

During the follow-up, the criteria for successful surgery were defined in this study as:

- Criterion A: IOP ≤21 mmHg and no postoperative IOP-lowering medication;
- Criterion B: IOP ≤18 mmHg and no postoperative IOP-lowering medication.

Once the patient's IOP exceeded the criteria or started using IOP-lowering drugs to control IOP, it was recorded as a failure and the follow-up was terminated. If the patient's operated eye required other eye surgery during the follow-up process, the last follow-up time was recorded as the censored value. The reason for criterion B is that a more stringent criterion was defined considering that the number of cases of patients with surgical failure may be relatively small in the short follow-up period, which is not conducive to data analysis.

Statistical methods

Data were analyzed using SPSS17.0 (IBM-SPSS, Chicago, IL) statistical software, and the chi-square test was used for gender and eye category; one-way ANOVA was used for differences in age between the three groups, and the Mann-Whitney U test was used for two-by-two comparisons of IOP, eye axis, and cytokine concentrations in the atrial fluid of the three groups because they did not conform to a normal distribution or had unequal variance between groups.

The MD values of the POAG and PACG groups were normally distributed with equal variance, so the independent sample t-test was applied. Bonferroni correction was used for multiple analyses. The correlation between cytokine concentration in atrial fluid and the prognosis of trabeculectomy was analyzed using the Kaplan-Meier method and the Cox proportional risk regression model, and P<0.05 was considered a statistically significant difference.

Results

Comparison of general conditions

A total of 90 participants were included and divided into three groups: 30 patients in the POAG group, 30 patients in the PACG group (15 patients in APACG and 15 patients in CPACG) and 30 patients in the senile cataract group (Table 1).

General Information	Group Cataract	Group POAG	Group PACG
n	30	30	30
Eye Separation (L/R)	16/14	15/15	13/17
Gender (M/F)	14/16	15/15	16/14
Age, y	65.12±2.35	63.12±3.21	64.31±4.11
Preoperative IOP, mmHg	13.23±1.43	23.12±2.12	25.31±2.54
Eye axis length, mm	24.32±0.25	25.36±0.47	22.65±0.76
MD value, dB	Not measured	-17.41±0.25	-18.32±0.65
Types of anti-glaucoma drugs, n (%)			
Β-blockers	0	25 (83.3)	26 (86.7)
Cholinergic drugs	0	0 (0)	5 (16.7)

Table 1: Basic patient information.

There was no statistical difference between the three groups in terms of eye type (χ^2 =0.35, P=0.84), gender (χ^2=0.13, P=0.94), and age (F=0.70, P=0.50); preoperative IOP: preoperative IOP was significantly higher in the POAG group (P<0.001) and PACG group (P=0.02) than in the senile cataract group, while IOP in the PACG and POAG groups was not statistically different (P=0.02). There was no statistical difference between the PACG and POAG groups (P=0.69); axial length: the axial length in the PACG group was significantly smaller than that in the POAG (P<0.001) and cataract groups (P<0.001), and there was no statistical difference between the axial length in the POAG and age-related cataract groups (P=0.35); MD values: there was no statistical difference between the MD values in the POAG and PACG groups (t=0.33, P=0.74); there were no statistical differences in gender, age, preoperative IOP, axial length and MD values between the APACG and CPACG groups (Table 2).

General Information	APACG	CPACG	P
n	15	15	
Gender (M/F)	5/10	6/9	1.000
Age, y	65.82±2.34	64.36±2.45	0.73
Preoperative IOP, mmHg	25.72±1.35	24.26±1.47	0.65
Eye axis length, mm	22.42±0.35	23.16±0.57	0.05
MDvalue, dB	-13.21±0.15	-20.51±0.35	0.08

Table 2: Comparison of APACG and CPACG baseline information.

Comparison of cytokine concentrations in atrial fluid in each group of patients

Comparison of cytokine concentrations in atrial fluid in patients with POAG and senile cataract

From the above experiments, it can be seen that the change pattern of anterior chamber depth in the three gThe concentrations of cathepsin D (P<0.001) and sNCAM (P< 0.001) in the atrial water of patients in the POAG group were significantly higher than those in the senile cataract group; the concentration of IFN-γ (P=0.015) was significantly lower than that in the senile cataract group; the concentrations of the remaining cytokines were not statistically different between the two groups, as shown in Table 3.

Comparison of atrial fluid cytokine concentrations between PACG and senile cataract patients

The concentrations of cathepsin D (P<0.001), sNCAM (P<0.001), IL-6 (P<0.001), IL-8 (P<0.001), MCP-1 (P<0.001) and TNF-α (P=0.001) in the atrial fluid of patients in the PACG group were significantly higher than those in the senile cataract group; the concentrations of IFN-γ in the two groups were not statistical differences between the two groups, see Table 3.

Comparison of cytokine concentrations in atrial fluid between POAG and PACG patients

The concentrations of sNCAM (P<0.001), IL-6 (P<0.001), IL-8 (P<0.001), MCP-1 (P<0.001) and TNF-α (P=0.007) in atrial fluid were significantly higher in the PACG group than in the POAG group; the concentrations of cathepsin D and IFN-γ were not statistically different between the two groups, as shown in Table 3.

Cytokine	Group Cataract	Group POAG	Group PACG
Cathepsin D (mg/ml)	0.17 (0.16,0.19)	0.22 (0.18,0.24)	0.21 (0.20,0.23)
sNCAM (μg/ml)	7.68 (7.39,9.28)	10.62 (7.48,12.77)	17.11 (14.31,19.27)
IL-6 (pg/ml)	4.56 (2.82,11.21)	1.63 (1.15,3.47)	63.12 (61.52,65.93)
IL-8 (pg/ml)	4.65 (3.45,8.02)	5.68 (4.20,9.21)	78.31 (26.23,120.76)
IFN-γ (pg/ml)	0.96 (0.78,1.20)	0.78 (0.58,0.94)	0.85 (0.75,0.97)
TNF-α (pg/ml)	0.58 (0.52,0.63)	0.57 (0.53,0.67)	0.90 (0.65,1.30)
MCP-1 (pg/ml)	873.52 (674.12,1132.63)	1223.49 (900.35,1574.13)	4435.00 (2986.73,5530.12)

Table 3: Cytokine concentration in atrial fluid in three groups of patients.
Note: Values are expressed as medians (first quartile, fourth quartile).

Comparison of atrial fluid cytokine concentrations between APACG and CPACG patients

The concentration of IL-6 (P=0.03) in the atrial fluid of patients in the APACG group was significantly higher than that in the CPACG group; the concentrations of the remaining cytokines were not statistically different between the two groups, as shown in Table 4.

Cytokine	Group APACG	Group CPACG	P value
Cathepsin D (mg/ml)	0.22 (0.20,0.24)	0.23 (0.21,0.25)	0.48
sNCAM (μg/ml)	17.68 (14.63,20.25)	16.21 (12.89,18.88)	0.18
IL-6 (pg/ml)	278.45 (635.42,7498.01)	129.12 (3.65,332.15)	0.03
IL-8 (pg/ml)	122.35 (70.52,212.68)	42.92 (10.63,387.34)	0.23
IFN-γ (pg/ml)	0.88 (0.74,0.94)	0.85 (0.73,0.95)	0.54
TNF-α (pg/ml)	1.22 (0.82,1.74)	0.78 (0.50,0.94)	0.06
MCP-1 (pg/ml)	4412.20 (3492.04,5594.72	4166.48 (1272.13,5527.43)	0.32

Table 4: Comparison of cytokine concentrations in APACG and CPACG room water.
Note: Values are expressed as medians (first quartile, fourth quartile).

Correlation between cytokine concentrations in atrial water and biological parameters in POAG and PACG patients

Cathepsin D (ϱ=-0.67, P<0.001), IL-6 (ϱ=-0.78, P<0.001), IL-8 (ϱ=-0.62, P=0.001), MCP-1 (ϱ=-0.69, P<0.001) and TNF-α (ϱ=-0.69, P<0.001) concentrations in the atrial fluid of patients with POAG were significantly correlated with MD values. were significantly correlated with MD values, as shown in Table 5. The remaining cytokine concentrations were not correlated with MD values; all cytokine concentrations were not correlated with age, preoperative IOP, or axial length, as shown in Table 5.

	Age (y)		IOP (mmHg)		AL (mm)		MD (mm)	
	ϱ	P	ϱ	P	ϱ	P	ϱ	P
Cathepsin D	0.42	0.06	-0.13	0.50	0.26	0.22	-0.67	0.01
sNCAM	-0.19	0.39	-0.04	0.85	0.08	0.70	-0.43	0.06
IL-6	0.17	0.42	-0.03	0.88	0.14	0.51	-0.78	0.01
IL-8	0.10	0.64	-0.03	0.91	-0.04	0.85	-0.62	0.01
IFN-γ	-0.07	0.76	-0.18	0.40	0.11	0.60	0.09	0.67
TNF-α	0.34	0.10	-0.07	0.74	0.10	0.65	-0.69	0.01
MCP-1	-0.06	0.78	0.09	0.68	0.16	0.46	-0.69	0.01

Table 5: Correlation between cytokine concentration in atrial water and biological parameters in patients with POAG.

Note: The correlation parameters ϱ and P values were obtained from.

Spearman rank correlation test

There was no correlation between atrial cytokine concentration and age, preoperative IOP, axial length, and MD values in patients with POAG, as shown in Table 6.

	Age(y)		IOP(mmHg)		AL(mm)		MD(mm)	
	ϱ	P	ϱ	P	ϱ	P	ϱ	P
Cathepsin D	0.09	0.62	0.48	0.11	-0.05	0.78	0.05	0.80
sNCAM	0.09	0.63	-0.04	0.81	0.04	0.82	0.27	0.13
IL-6	-0.12	0.52	-0.10	0.57	-0.18	0.32	0.37	0.04
IL-8	0.02	0.91	-0.13	0.46	-0.15	0.41	0.08	0.67
IFN-γ	-0.08	0.68	-0.14	0.44	-0.11	0.55	0.27	0.14
TNF-α	-0.06	0.11	-0.17	0.36	0.03	0.88	0.16	0.38
MCP-1	-0.11	0.55	-0.21	0.25	-0.06	0.73	0.30	0.09

Table 6: Correlation between cytokine concentration in atrial fluid and biological parameters in PACG patients.

Note: The correlation parameters ϱ and P values were obtained from Spearman rank correlation test.

Comparison of time distribution of successful surgery between trabeculectomy patient groups

The postoperative follow-up time was 24 months. 4 patients were lost in the POAG group, with a rate of 13.33%; 5 patients were lost in the PACG group, with a rate of 16.67%. The reasons for the lost visits were that they lost contact with each other and did not come back to the hospital for review. At standard A: the mean time to successful surgery in the POAG group was 21.46 months with 95% confidence interval (20.32, 22.45); the mean time to successful surgery in the PACG group was 19.26 months with 95% confidence interval (17.26, 21.27), and the difference in the distribution of time to successful surgery between the two groups was not statistically significant (χ^2=2.56, P=0.11) (Figure 1).

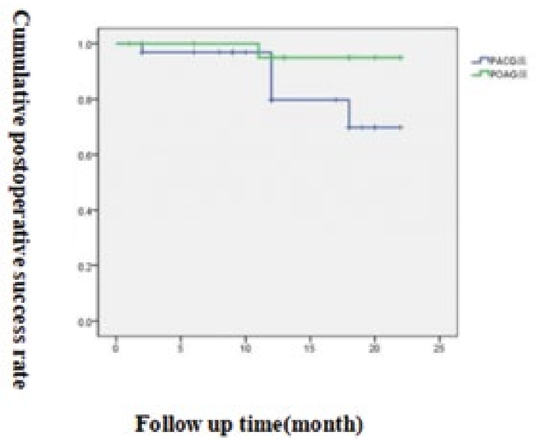

Figure 1: Follow-up survival curves for patients with POAG and PACG (Criterion A).

At standard B: the mean time to surgical success in the POAG group was 19.68 months with 95% confidence interval (17.65, 21.53); the mean survival time in the PACG group was 16.18 months with 95% confidence interval (13.62, 18.76), and the difference in the distribution of time to surgical success between the two groups was not statistically significant (χ^2=3.22, P=0.07) (Figure 2).

Cox proportional risk regression model analysis showed that when surgical success was defined as criterion B, i.e., IOP ≤18 mmHg, and no postoperative IOP-lowering drugs were used, the number of types of antiglaucoma drugs previously used was a risk factor for trabeculectomy failure in the POAG group, with a relative risk ratio (RR) value of 14.01, P=0.02, i.e., the more types of antiglaucoma drugs used before surgical treatment. There was no correlation between the concentration of cytokines in the atrial fluid and the prognosis of trabeculectomy in both the

PACG and POAG groups, and no correlation between age, gender, preoperative intraocular pressure and axial length and the prognosis of trabeculectomy.

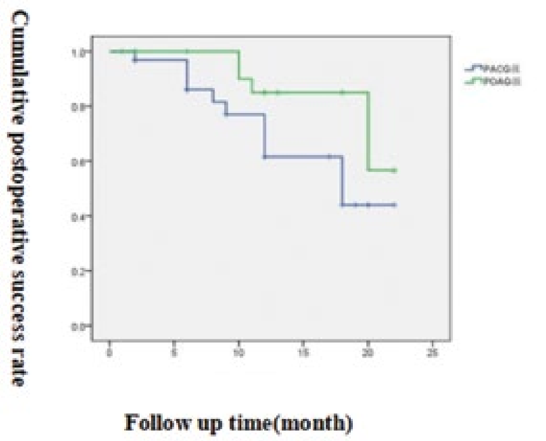

Figure 2: Follow-up survival curves for patients with POAG and PACG (Criterion B).

Discussion

POAG is a disease with insidious onset and not easily diagnosed at an early stage, and optic nerve damage is already severe at the time of consultation, causing a great burden to the patient's family and society[5]. In recent years, more and more scholars consider glaucoma as a neurodegenerative pathology, grouped with neurodegenerative diseases such as AD, PD and MS. Toshihiro[6, 7] examined AD markers in the atrial fluid of open-angle glaucoma, such as α2-macroglobulin, apolipoprotein AI, and apolipoprotein E, and found that these cytokine concentrations were significantly elevated in the atrial fluid of open-angle glaucoma. This trial suggests that this is a good direction to investigate, and that early retinal ganglion cell loss may affect the concentration of cytokines in the atrial fluid, and these cytokines may become sensitive early diagnostic indicators.

In this study, we found that the concentrations of Cathepsin D and sNCAM in the atrial fluid were significantly higher in the POAG group than in the senile cataract group; the concentration of IFN-γ was significantly lower than in the senile cataract group; the concentrations of MCP-1, IL-6, IL-8, and TNF-α were not statistically different between the two groups. First, these results cannot be explained simply by the disruption of the blood-atrial water barrier, because not all cytokine concentrations were elevated in the atrial water of POAG patients, and although Bonferroni correction of the P values was performed in this study to make the results more conservative, there were still many cytokine concentrations that were not statistically different between the POAG and senile cataract groups, and IFN-γ concentrations concentrations were even lower in the POAG group. Secondly, the cytokines with significantly higher concentrations in the atrial fluid in the POAG group, including cathepsin D and sNCAM, were found to be associated with nerve damage and neurodegenerative lesions in this study, and patients with systemic or local infections, immune system disorders, and neurodegenerative lesions (such as AD, PD, and MS) were excluded from the inclusion of participants, allowing the exclusion of these confounding factors. Therefore, the present study suggests that changes in these cytokines may be associated with the development of glaucoma. Cytokine concentration changes in the atrial fluid are complex and sensitive, and any tissue damage or changes in the eye may cause cytokine concentration changes in the atrial fluid. For POAG, the IOP is not as high as PACG and the tissue damage is less severe, so this study suggests that the cause of cytokine concentration changes in the atrial fluid in POAG patients is more simple compared to PACG. The altered cytokine concentration in the atrial fluid of POAG patients may reflect the condition of the optic nerve and trabecular meshwork.

Cathepsin D is an aspartic protease, mainly found in lysosomes, which plays an important role in intracellular homeostasis[8]. Lin's study[9] found that in the immediate anterior segment, the production of reactive oxygen clusters in lysosomes leads to increased lysosomal membrane permeability, which releases Cathepsin D from lysosomes into the cytosol, leading to trabecular meshwork (TM) cell death, which may be the mechanism by which oxidative stress leads to a decrease in TM cells.

These findings suggest that altered Cathepsin D concentrations in atrial fluid of POAG patients are associated with trabecular meshwork and optic nerve damage. Interferon-gamma (IFN-γ), a pro-inflammatory factor produced by activated T cells and NK cells, has immunomodulatory effects that inhibit cell growth and induce the expression of class I major histocompatibility complex antigen molecules. IFN-γ was also found to be associated with nerve damage[10] and involved in the development of neurodegenerative pathologies such as AD. Zhou et al[11] found that low concentrations of IFN-γ in atrial water facilitated the survival of RGC. In this study, sNCAM cell adhesion molecules, which belong to the immunoglobulin superfamily and are involved in cell-cell and cell-extracellular matrix associations, were also selected as study proteins(12). Cell adhesion molecules play an important role in neuroinflammation and may be associated with neurodegenerative diseases(13). However, there is a lack of information on the concentration of cell adhesion molecules in the atrial fluid of glaucoma patients. In this study, we concluded that POAG belongs to the same neurodegenerative changes and may have a similar pathological process to other neurodegenerative diseases. The sNCAM concentration in the atrial fluid of POAG patients was significantly higher than that of the aged cataract group, which may be related to the degenerative changes of the optic nerve caused by neuroinflammation.

In conclusion, the present study suggests that the mechanisms responsible for the altered concentrations of these cytokines in the atrial water of patients with POAG may be more biased toward optic nerve and trabecular meshwork injury.

PACG is the main type of primary glaucoma in China, and epidemiological surveys have found that PACG has the highest prevalence in Asian populations at approximately 1.20%(14). PACG is often associated with increased intraocular pressure due to atrial angle obstruction and closure.

In this study, we found that the concentrations of Cathepsin D, sNCAM, IL-6, IL-8, MCP-1, and TNF-α in the atrial fluid were significantly higher in the PACG group than in the age-related cataract group; the concentrations of sNCAM, IL-6, IL-8, MCP-1, and TNF-α were significantly higher than in the POAG group. In particular, the increase in inflammation-related factors was most prominent. In this paper, we discussed that the altered cytokine concentrations in the atrial water of POAG patients were mainly related to optic nerve and trabecular meshwork cell injury, while the altered cytokine concentrations in the atrial water of PACG patients could no longer be explained by optic nerve and trabecular meshwork cell injury alone. This study summarized several mechanisms of elevated cytokine concentrations in the atrial water of PACG, including: blood-atrial water barrier disruption, ischemia-hypoxia and retinal ischemia-reperfusion injury. Tissue ischemia can cause ischemic-hypoxic injury such as disturbance of the intracellular environment, electrolyte overload, acidosis and cytoskeletal destruction(15), which may cause elevated concentrations of pro-inflammatory factors in the process(16). Hypoxia can induce IL-6 production(17); under hypoxia, iris pigment epithelial cells produce more IL-6 and MCP-1; high intraocular pressure in glaucoma patients causes retinal blood flow obstruction, which likewise causes retinal ischemia, and elevated pro-inflammatory factors in retinal ischemia(18), which is the same as the results of the present study. These studies suggest that ischemia and hypoxia occurred in the eyes of PACG patients, and that ischemia and hypoxia caused a significant increase in the concentration of pro-inflammatory factors in the atrial fluid.

RIR injury is the resumption of blood perfusion to the retina when the intraocular pressure is lowered after elevated IOP causes retinal ischemia and hypoxia, but the reperfused blood flow to the retina continues to cause a series of tissue damage, such as increased free radicals causing oxidative damage, mitochondrial dysfunction, and inflammatory response(19-21). In this process, cell adhesion molecules (CAM) play an important role, and CAM is an important factor that regulates the movement of inflammatory cells from the blood to the site of inflammation. In addition, the damage to the optic nerve and trabecular meshwork discussed in this paper is also a cause, but personally, I believe that for PACG, the disruption of the blood-atrial water barrier, intraocular ischemia and hypoxia, and ischemia-reperfusion injury are the main causes of elevated cytokine concentrations in atrial water.

In this study, IL-6 concentrations in atrial fluid were significantly higher in the APACG group than in the CPACG group, while the remaining cytokine concentrations were not statistically different between the two groups. However, it is noteworthy that cytokine concentrations in atrial fluid were also increased in CPACG patients and were essentially the same as those in PACG patients analyzed in the article, especially inflammatory factors, which were increased hundreds or thousands of times. Some studies have shown that atrial water flash glow and atrial cells are significantly lower in CPACG patients than in APACG patients(22). However, the present study found that the concentration of inflammatory factors in atrial water was also significantly higher in CPACG patients, which suggests to us that more severe inflammation occurs in the anterior segment of the eye in CPACG patients as well. The inflammatory response in the eye may have a driving role in the development of glaucoma, with leukocyte aggregation in the retina found in a dog model of glaucoma. In addition, elevated concentrations of pro-inflammatory factors in atrial fluid may cause adhesions to form between the iris and the lens, and post-iris adhesions will lead to pupillary block and peripheral anterior adhesions. These studies suggest that pro-inflammatory factors in atrial fluid play an important role in the development, progression, and surgical prognosis of glaucoma. Therefore, inflammation of the anterior segment of the eye in patients with CPACG also needs to be taken into account during clinical treatment.

In this study, we found that Cathepsin D, IL-6, IL-8, MCP-1 and TNF-α concentrations in atrial fluid correlated with MD values in patients with POAG. The results of the present study are similar to those of Inoue[23], who also showed that biomarkers of AD: transthyretin (TTR), apolipoprotein AI (Apo AI), and apolipoprotein E (Apo E) correlated with MD values, which may be related to the previously discussed alteration of cytokine concentrations in atrial water of POAG patients due to optic nerve damage related. However, this is not sufficient to demonstrate the exact relationship between these cytokines and the degree of visual field defects, and studies with larger samples and longer follow-up are needed. In addition, since most of the patients included in this study were patients with intermediate to advanced glaucoma, all with high MD values, further experiments could include patients according to glaucoma stage and compare the differences in atrial fluid concentrations of these cytokines in patients with different stages of glaucoma. If these cytokine concentrations can really reflect the degree of visual field defects, it will help clinicians to accurately determine the degree of glaucoma progression.

Previous studies have shown that changes in the concentration of cytokines in atrial fluid in glaucoma patients can affect the prognosis of trabeculectomy surgery, for example, Toshihiro[24] et al found that MCP-1 was associated with the prognostic outcome of trabeculectomy surgery through the detection of cytokines in preoperative atrial fluid and a 3-year postoperative follow-up study, and that patients with higher concentrations of MCP-1 in preoperative atrial fluid were more likely to have elevated postoperative intraocular pressure. Barbara and his colleagues also found that high concentrations of IL-6 and TNF-α in the atrial fluid were associated with poor postoperative prognosis[25]. However, this study did not find any cytokine concentrations associated with trabeculectomy prognosis, and the analysis may be due to the following reasons: 1. small sample size and insufficient follow-up time, which will be increased in the next phase of the study and prolonged; 2. anti-inflammatory drugs were applied preoperatively and postoperatively, which may have influenced the results. However, this again proves that the preoperative and postoperative application of anti-inflammatory drugs is very clinically relevant.

Although no correlation was found between atrial cytokine concentrations in glaucoma patients and the prognosis of trabeculectomy, the number of anti-glaucoma drug classes was found to be a risk factor for trabeculectomy failure (criterion B) in a postoperative follow-up study of POAG patients with a relative risk ratio (RR) value of 14.01. Broadway found that by anti-glaucoma filtration surgery of POAG patients with at least 6 months of follow-up, found no difference between a 90% success rate for surgery with brief use of antiglaucoma medications (i.e., no longer than 2 months) and a 93% success rate for surgery in patients using only β-blockers, and a significantly lower 72% success rate for surgery when both β-blockers and pupil constrictors were used in combination, and when β-blockers, pupil constrictor, and adrenergic agonist were used in combination, the success rate of surgery was further reduced to 45%. This is similar to the results of the present study. The reason for this analysis may be due to the subclinical inflammation of the bulbar conjunctiva when long-term or multiple anti-glaucoma drugs are used in combination.

In conclusion, this study found that the number of types of antiglaucoma drugs previously used was a risk factor for trabeculectomy failure; while cytokine concentrations in the atrial fluid did not correlate with the prognosis of trabeculectomy.

Conclusion

Liquid-phase suspension microarray technology enables the detection of multiple cytokines in small volume samples and is a good way to quantify cytokines in atrial water. The altered cytokine concentration in atrial water of POAG patients may reflect damage to trabecular meshwork cells and optic nerve. Altered cytokine concentration in atrial fluid in PACG patients may be related to disruption of the blood-atrial fluid barrier, intraocular ischemia-hypoxia and ischemia-reperfusion injury.

The number of types of antiglaucoma drugs used previously is a risk factor for trabeculectomy surgery, while the cytokine concentration in atrial water does not correlate with the prognosis of trabeculectomy surgery.

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