

# Aqueous Humor Growth Factor Levels and Trabeculectomy Outcomes in Primary Open-Angle Glaucoma Patients: A 2-Year Prospective Study

Tianwei Qian<sup>1-5</sup>, Mingshui Fu<sup>1-5</sup>, Luyao Ye<sup>1-5</sup>, Jingxiao Du<sup>1-5</sup>, Xun Xu<sup>1-5</sup>, and Zhihua Zhang<sup>1-5</sup>

<sup>1</sup> Department of Ophthalmology, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China

<sup>2</sup> National Clinical Research Center for Eye Diseases, Shanghai, China

<sup>3</sup> Shanghai Key Laboratory of Ocular Fundus Diseases, Shanghai, China

<sup>4</sup> Shanghai Engineering Center for Visual Science and Photomedicine, Shanghai, China

<sup>5</sup> Shanghai Engineering Center for Precise Diagnosis and Treatment of Eye Disease, Shanghai, China

**Correspondence:** Zhihua Zhang, Department of Ophthalmology, Shanghai General Hospital, Shanghai Jiao Tong University, No. 100 Haining Road, Shanghai 200080, China. e-mail: [drzhihuazhang@126.com](mailto:drzhihuazhang@126.com)

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**Purpose:** Maintenance of a filtering bleb is essential for long-term intraocular pressure control after trabeculectomy. Surgical site fibrosis and excessive extracellular matrix production are common causes of trabeculectomy failure, mediated by several growth factors. We aimed to evaluate the levels of five growth factors and their correlation with trabeculectomy outcomes in patients with primary open-angle glaucoma (POAG).

**Methods:** We collected aqueous humor samples intraoperatively from patients with POAG who underwent trabeculectomy and measured the concentrations of transforming growth factor- $\beta$  (TGF- $\beta$ ), acidic fibroblast growth factor (aFGF), insulin-like growth factor-1, vascular endothelial growth factor, and platelet-derived growth factor using multiplexed immunoassay kits. Intraocular pressure was measured with Goldmann applanation tonometry at 1 week and at 1, 3, 6, 12, 18, and 24 months after trabeculectomy. We allocated the eyes based on surgical outcome into a success or failure group.

**Results:** Significantly high levels of aFGF and TGF- $\beta$  were observed in the failure group (both  $P < 0.0001$ ) and were significant risk factors for trabeculectomy outcomes. Higher success rates were observed over the 24-month follow-up period in eyes with low aFGF and TGF- $\beta$  levels compared to eyes with high levels ( $P = 0.0031$  and  $P = 0.0007$ , respectively). The levels of TGF- $\beta$  were significantly positively correlated with aFGF.

**Conclusions:** In POAG patients, high aFGF and TGF- $\beta$  levels were significant risk factors for trabeculectomy failure.

**Translational Relevance:** Modulation of aFGF and TGF- $\beta$  expression may have potential clinical applications after filtration surgery.

## Introduction

Primary open-angle glaucoma (POAG) is one of the leading causes of irreversible blindness globally and is projected to affect about 80 million people worldwide by 2040.<sup>1</sup> POAG is a neurodegenerative disease characterized by progressive loss of retinal ganglion cells, resulting in optic nerve head (ONH) deformation and visual field defects.<sup>2,3</sup> Elevated intraocular pressure (IOP) is closely related to optic nerve damage.

The mainstay of treatment to delay the progression of POAG is IOP control within a target range, achieved by medications, laser therapy, or glaucoma filtration surgery.<sup>4</sup> Trabeculectomy is a commonly performed glaucoma filtration surgery that can control IOP by providing an artificial aqueous humor (AH) outflow path from the anterior chamber to the subconjunctival space, forming a filtering bleb.<sup>5</sup> Maintenance of the filtering bleb is essential for long-term IOP control and surgical success.<sup>6,7</sup> Excessive extracellular matrix (ECM) production and fibrosis at the surgical

site are the most common causes of trabeculectomy failure.<sup>8</sup>

Important growth factors such as, transforming growth factor- $\beta$  (TGF- $\beta$ ),<sup>9</sup> insulin-like growth factor-1 (IGF-1),<sup>10</sup> acidic fibroblast growth factor (aFGF),<sup>11</sup> vascular endothelial growth factor (VEGF),<sup>12</sup> and platelet-derived growth factor (PDGF)<sup>13</sup> can play an essential role in wound healing and fibrotic processes in various tissues. In several studies, increased levels of these growth factors have been observed in the AH of patients with glaucoma.<sup>12,14-17</sup> Furthermore, some studies have reported that growth factors could play a role in glaucoma by affecting ECM remodeling; for example, TGF- $\beta$  could alter ECM homeostasis in the trabecular meshwork (TM), increasing resistance to outflow.<sup>18</sup> Other studies have shown increased IGF-1 levels associated with high fibronectin levels in the AH of patients with glaucoma,<sup>17</sup> and a role for VEGF in wound healing as an initial activator of fibrosis.<sup>19</sup> Furthermore, TGF- $\beta$ , aFGF, and PDGF, all derived from mononuclear cells, could increase fibroblast activity and enhance the secretion of ECM components.<sup>6,20</sup>

The proportion of Chinese patients with glaucoma who have open-angle glaucoma is lower than in European and American countries; however, it is worrisome that the incidence of open-angle glaucoma increases as the population ages. To date, though, the correlation between AH levels of TGF- $\beta$ , IGF-1, aFGF, VEGF, and PDGF and trabeculectomy outcomes in Asian patients with POAG has not been thoroughly evaluated. In this study, we measured the levels of TGF- $\beta$ , IGF-1, aFGF, VEGF, and PDGF in the AH of Chinese patients with POAG at the time of trabeculectomy and investigated the correlation between growth factor levels and surgical outcomes after trabeculectomy.

## Methods

### Patients

This study was approved by the Shanghai General Hospital Institutional Review Board and conducted in accordance with the tenets of the Declaration of Helsinki. We obtained written informed consent from each patient. For this prospective cohort study, we consecutively selected patients diagnosed with POAG who required trabeculectomy for IOP control and were seen at Shanghai General Hospital ophthalmology clinics from January 2018 to December 2019. All patients suspicious for POAG underwent ophthalmic evaluation, including visual acuity measurement, pupil examination, slit-lamp biomi-

croscopy, IOP measurement using Goldmann applanation tonometry, visual fields (Humphrey Visual Field Analyzer; Carl Zeiss Meditec, Dublin, CA), gonioscopy, optic nerve photographs (VISUCAM 200; Carl Zeiss Meditec), and ONH and retinal nerve fiber layer examination (CIRRUS HD-OCT; Carl Zeiss Meditec).<sup>21</sup> An experienced glaucoma expert diagnosed POAG based on the criteria of open anterior chamber angle, optic nerve head glaucomatous changes, elevated IOP (>21 mmHg), and visual field damage.<sup>4,22</sup> The indications for trabeculectomy were an IOP exceeding the target pressure despite maximum tolerated medical therapy or progression of glaucomatous damage.<sup>14,22</sup> All patients included in this study agreed to undergo trabeculectomy for IOP reduction.

Study participants were selected based on the following inclusion criteria: (1) definite diagnosis of POAG and indications for trabeculectomy; (2) signed informed consent accepting trabeculectomy; (3) no apparent lens opacity or need for combined cataract surgery; (4) no intraocular injections of anti-VEGF agents and no diabetes; and (5) agreement to complete the 24-month follow-up period. The exclusion criteria were (1) presence of ocular diseases other than POAG; (2) optic nerve degeneration not due to glaucoma; (3) significant lens opacity, age-related macular degeneration, retinal vascular diseases, or inherited retinopathy; (4) history of previous ocular surgery; (5) history of ocular inflammation or infection within 2 months; or (6) serious systemic diseases, such as cancer or renal failure. Medication history was determined from the patients' medical records. The standard medication treatment for eyes with POAG included some or all of the following according to the patients' IOP and ophthalmic status: (1) topical prostaglandin analogs, (2) topical  $\beta$ -adrenergic antagonists, (3) topical  $\alpha$ -adrenergic agonists, (4) topical or oral carbonic anhydrase inhibitors, and (5) topical parasympathomimetic agents.

### Surgical Technique and Postoperative Care

One experienced surgeon (MF) performed a trabeculectomy in all patients included in this study, and the main steps of the operation were similar to those described in our previous study.<sup>23</sup> Under topical gel anesthesia, the surgeon created a fornix-based conjunctival flap from 11 to 1 o'clock, followed by dissection of a limbus-based 4  $\times$  4-mm scleral flap. A sponge soaked with 5-fluorouracil (25 mg/mL) was placed underneath the conjunctival flap for 3 minutes, followed by irrigation of the surgical area with balanced salt solution. Subsequently, a sclerostomy

and basal iridectomy were performed. The scleral flap was sutured with a single, interrupted 10-0 nylon suture at the two corners and adjustable sutures at the center of the two sides to allow minimal leakage during anterior chamber reconstruction. Finally, the conjunctiva was closed using a 10-0 nylon suture.

Topical antibiotic and nonsteroidal anti-inflammatory medications were given four times daily for 1 week. Topical corticosteroids were used four times daily for the first week and then tapered over the next month. No additional antimetabolites were used during follow-up. We performed postoperative interventions according to bleb appearance based on two previous studies.<sup>24,25</sup> First, we performed ocular massage; in cases where the postoperative IOP did not reach target despite this treatment, we loosened the adjustable sutures with forceps. If the bleb became encapsulated and associated with a rise in IOP, we performed bleb needling and added IOP-lowering medications. In cases of inadequate IOP control despite these measures, we conducted additional surgical procedures as required.

### Aqueous Humor Collection

Generally, AH samples (at least 50  $\mu$ L) were collected at the beginning of the trabeculectomy through a limbal paracentesis. All collections were carefully performed without touching any intraocular tissues. AH samples were immediately frozen in liquid nitrogen and transferred to a  $-80^{\circ}\text{C}$  environment until analyzed.

### Measurement of Growth Factor Levels in Aqueous Humor

Five types of growth factors—IGF-1, aFGF, VEGF, PDGF, and active TGF- $\beta$ —were detected using a multiplex assay (Luminex Screening Human Magnetic Assay; R&D Systems, Inc., Minneapolis, MN) performed according to the manufacturer's instructions. For each assay, AH samples were diluted at 1:2, and 50  $\mu$ L of the diluted sample was added to each well. Fluorescence intensity was acquired and analyzed using Luminex xPONENT 3.1 software (Luminex Corporation, Austin, TX).

### Outcome Measures

Preoperative IOP and all topical antiglaucoma medications used in this study were recorded before surgery. Patients were followed up at 1 week and at 1, 3, 6, 12, 18, and 24 months postoperatively. At each postoperative visit, we measured IOP levels using

Goldmann applanation tonometry and recorded the number of antiglaucoma medications used and any additional surgeries performed. We divided patients into two groups, success and failure, based on surgical results. The success group was subdivided into complete success and qualified success. We defined failure as IOP  $> 21$  mmHg or a  $<20\%$  reduction from baseline IOP at two consecutive follow-up visits  $\geq 3$  months after surgery; IOP  $\leq 5$  mmHg at two consecutive follow-up visits  $\geq 3$  months after surgery; reoperation for glaucoma required; or loss of light perception vision. We defined success if the above failure criteria were not met with or without supplemental medications.<sup>26–28</sup> We defined reoperation for glaucoma as additional glaucoma surgery requiring a return to the operating room, such as tube shunt placement. We also regarded cyclodestruction as a reoperation for glaucoma, whether performed in the clinic or operating room. Ocular massage, loosening of adjustable sutures, and bleb needling were performed using a slit lamp and were not considered additional glaucoma surgery.<sup>29,30</sup> For the definition of surgical failure, IOP values obtained  $\geq 3$  months after trabeculectomy were used to avoid the effect of short-term postoperative IOP fluctuations.

### Statistical Analysis

Commercially available SPSS Statistics 22.0 (IBM Corporation, Chicago, IL) and Prism 8.01 (Graph-Pad Software, San Diego, CA) were used for the statistical analyses. Categorical variables (e.g., gender) were expressed as numbers and frequencies, and the differences were assessed using Fisher's exact test. The continuous variables (e.g., age, IOP, growth factor levels) were expressed as the mean  $\pm$  standard deviation (SD). The Kolmogorov–Smirnov test was used to determine whether continuous variables fit with a normal distribution. The unpaired *t*-test was used for normally distributed data, and the Wilcoxon signed-rank test was used for abnormal distributed data. Univariate and multivariate logistic regression analyses were used to identify risk factors for surgical failure. If any growth factor was determined to be a risk factor for trabeculectomy failure, we divided the patients into two groups according to the ratio, and Kaplan–Meier survival curves were used to display the success rate of endpoints over time between the groups. Linear regression analysis was used to assess the relationship between growth factor levels. The coefficient of determination  $R^2$  was used to express the proportion of the variation in the dependent variable explained by the regression model. All statistical tests were two sided, with  $P < 0.05$  considered statistically significant.

## Results

### Patients

We included 67 eyes (67 patients) in this study, and all participants completed the 24-month follow-up period. We classified 49 eyes (73.1%) into the success group, and 18 eyes (26.9%) into the failure group during the follow-up period. The clinical characteristics of both groups are shown in Table 1. There were no significant differences between the groups in gender, age, preoperative IOP, HbA1c, axial length, central corneal thickness, or the number of antiglau-

coma eyedrops used before surgery. Similarly, there were no significant differences in the levels of VEGF, PDGF, and IGF-1 between the success and failure groups; however, the levels of aFGF ( $P < 0.0001$ ) and TGF- $\beta$  ( $P < 0.0001$ ) were both significantly higher in the failure group compared to the success group (Table 1).

Table 2 shows the postsurgical IOP-lowering interventions in the two groups. There was no significant difference in ocular massage or needling ( $P = 0.1803$  and  $P = 0.2010$ , respectively) between the groups. Ocular massage was required in three eyes (6.1%) in the success group and three eyes (16.7%) in the failure

**Table 1.** Characteristics of the 67 Previous POAG Patients

Characteristics	All	Success Group	Failure Group	<i>P</i>
Patients (eyes), <i>n</i>	67 (67)	49 (49)	18 (18)	—
Male/female, <i>n</i>	35/32	27/22	8/10	0.4388
Age (y), mean $\pm$ SD	45.91 $\pm$ 5.12	46.31 $\pm$ 5.28	44.83 $\pm$ 4.62	0.3001
Preoperative IOP (mmHg), mean $\pm$ SD	29.06 $\pm$ 2.67	29.20 $\pm$ 2.74	28.67 $\pm$ 2.50	0.4691
HbA1c (%), mean $\pm$ SD	4.96 $\pm$ 0.53	4.99 $\pm$ 0.56	4.88 $\pm$ 0.45	0.4316
Axial length (mm), mean $\pm$ SD	24.97 $\pm$ 1.20	25.02 $\pm$ 1.21	24.83 $\pm$ 1.18	0.5715
Central corneal thickness ( $\mu$ m), mean $\pm$ SD	566.81 $\pm$ 28.37	565.57 $\pm$ 29.43	570.17 $\pm$ 25.73	0.5607
Number of antiglaucoma eyedrops, mean $\pm$ SD	2.67 $\pm$ 0.66	2.69 $\pm$ 0.62	2.61 $\pm$ 0.78	0.6527
VEGF (pg/mL), mean $\pm$ SD	638.92 $\pm$ 272.89	628.23 $\pm$ 291.23	668.03 $\pm$ 220.05	0.6005
PDGF (pg/mL), mean $\pm$ SD	23.58 $\pm$ 6.54	23.94 $\pm$ 6.83	22.61 $\pm$ 5.73	0.4629
IGF-1 (ng/mL), mean $\pm$ SD	6.02 $\pm$ 1.30	5.95 $\pm$ 1.24	6.24 $\pm$ 1.48	0.4223
aFGF (pg/mL), mean $\pm$ SD	27.48 $\pm$ 12.52	23.18 $\pm$ 8.23	39.20 $\pm$ 14.78	<b>&lt;0.0001</b>
TGF- $\beta$ (pg/mL), mean $\pm$ SD	2397.59 $\pm$ 899.40	2112.92 $\pm$ 823.96	3172.54 $\pm$ 595.09	<b>&lt;0.0001</b>

Bold values indicate significant results.

**Table 2.** Postoperative Interventions and Complications in the Success and Failure Groups

	Success Group, <i>n</i> (%)	Failure Group, <i>n</i> (%)	<i>P</i>
Postoperative interventions			
Ocular massage	3 (6.1)	3 (16.7)	0.1803
Suture lysis	4 (8.2)	6 (33.3)	<b>0.0104</b>
Needling revision	5 (10.2)	4 (22.2)	0.2010
Complications			
Corneal epitheliopathy	1 (2.0)	1 (5.6)	0.4536
Hyphema	1 (2.0)	1 (5.6)	0.4536
Iridocyclitis	1 (2.0)	2 (11.1)	0.1115
Subconjunctival hemorrhage	3 (6.1)	2 (11.1)	0.4910
Shallow anterior chamber	1 (2.0)	0 (0)	0.5414
Transconjunctival oozing	1 (2.0)	1 (5.6)	0.4536
Malignant glaucoma	0 (0)	0 (0)	—
Choroidal detachment	0 (0)	0 (0)	—
Endophthalmitis	0 (0)	0 (0)	—

Bold value indicates a significant result.

**Table 3.** Results of Univariate Analysis of Trabeculectomy Outcomes

Variable	RR	95% CI	<i>P</i>
Age	0.945	0.849–1.051	0.297
Gender	1.019	0.344–3.020	0.974
Preoperative IOP	1.069	0.8701–1.314	0.527
HbA1c	1.572	0.636–3.891	0.327
Axial length	0.878	0.561–1.375	0.570
Central corneal thickness	1.006	0.987–1.025	0.555
Number of antiglaucoma eyedrops	0.824	0.359–1.891	0.791
VEGF	0.904	0.305–2.679	0.856
PDGF	1.041	0.354–3.069	0.941
IGF-1	1.413	0.477–4.185	0.533
aFGF	15.059	3.092–73.351	<b>0.001</b>
TGF- $\beta$	35.062	4.279–287.277	<b>0.001</b>

Bold values indicate significant results.

group, and needling was required in five eyes (10.2%) in the success group and four eyes (22.2%) in the failure group. Four eyes (8.2%) in the success group and six eyes (33.3%) in the failure group required suture lysis, and this difference was significant ( $P = 0.0104$ ).

Surgical complications in both groups are also shown in Table 2. There was no significant difference between the two groups regarding all complications, with eight adverse events in 49 eyes in the success group and seven adverse events in 18 eyes in the failure group. All adverse events were either managed medically or recovered without treatment. No severe complications, such as endophthalmitis, were found during follow-up.

### Correlation Between Growth Factors and Surgical Results

We performed univariate and subsequent multivariate analyses to validate the significant association between growth factor levels, other potential risk factors, and trabeculectomy results. Univariate analysis revealed that the AH levels of aFGF and TGF- $\beta$  were significant prognostic factors for trabeculectomy outcomes ( $P = 0.001$ , relative risk [RR] = 15.059 and  $P = 0.001$ , RR = 35.062, respectively); however, age, gender, preoperative IOP, HbA1c, axial length, central corneal thickness, the number of antiglaucoma eyedrops, VEGF, PDGF, and IGF-1 were not (Table 3). The five growth factors were included in the subsequent multivariate analysis, and results showed that aFGF and TGF- $\beta$  were significant risk factors for trabeculectomy outcomes ( $P = 0.023$ , RR = 8.346 and  $P = 0.018$ , RR = 16.781, respectively), whereas the other three factors were not (Table 4). The results from univariate and multivariate analyses indicated that high aFGF and TGF- $\beta$  levels could be considered significant risk factors for trabeculectomy failure.

**Table 4.** Results of Multivariate Analysis of Trabeculectomy Outcomes

Variable	RR	95% CI	<i>P</i>
VEGF	0.893	0.225–3.542	0.872
PDGF	1.047	0.262–4.185	0.949
IGF-1	1.264	0.335–4.777	0.730
aFGF	8.346	1.189–59.172	<b>0.023</b>
TGF- $\beta$	16.781	1.349–208.705	<b>0.018</b>

Bold values indicate significant results.

We subsequently assigned study participants into one of two groups based on the levels of aFGF and TGF- $\beta$  in the AH. A total of 34 eyes with aFGF levels less than or equal to the median value ( $\leq 23.04$  pg/mL) were included in group L of aFGF, and 33 eyes with aFGF levels higher than the median value ( $> 23.04$  pg/mL) were included in group H of aFGF. Postoperative outcomes, including postoperative IOP, use of antiglaucoma eyedrops, and success rates, are shown in Table 5. Similarly, 34 eyes with TGF- $\beta$  levels less than or equal to the median value ( $\leq 2506.68$  pg/mL) were included in group L of TGF- $\beta$ , and 33 patients with TGF- $\beta$  levels higher than the median value ( $> 2506.68$  pg/mL) were included in group H of TGF- $\beta$ ; the postoperative outcomes for these groups are shown in Table 6. Kaplan–Meier survival plots were drawn, and the Mantel–Cox test was used to investigate the overall success rate between groups L and H each for aFGF and TGF- $\beta$  (Fig. 1). Eyes with low aFGF and TGF- $\beta$  levels had significantly higher success rates over the 24-month follow-up than eyes with high levels ( $P = 0.0031$  and  $P = 0.0007$ , respectively).

**Table 5.** Comparison of Surgical Results Between Groups With Low and High Levels of aFGF

Characteristic	POAG With Low Levels of aFGF, Group L	POAG With High Levels of aFGF, Group H	<i>P</i>
Patients, <i>n</i>	34	33	—
aFGF level (pg/mL), mean ± SD	18.63 ± 3.13	36.60 ± 12.02	<b>&lt;0.0001</b>
Probability of success, <i>n</i> (%)			
6 mo	34 (100)	30 (90.9)	0.0720
12 mo	33 (97.1)	26 (78.8)	<b>0.0211</b>
18 mo	32 (94.1)	22 (66.7)	<b>0.0045</b>
24 mo	30 (88.2)	19 (57.6)	<b>0.0046</b>
IOP 24 mo postoperatively, mean ± SD	15.21 ± 3.76	18.76 ± 4.98	<b>0.0016</b>
Number of glaucoma eyedrops 24 mo postoperatively, mean ± SD	0.32 ± 0.53	1.06 ± 0.83	<b>&lt;0.0001</b>

Bold values indicate significant results.

**Table 6.** Surgical Results Between Groups With Low and High Levels of TGF- $\beta$ 

Characteristic	POAG With Low Levels of TGF- $\beta$ , Group L	POAG With High Levels of TGF- $\beta$ , Group H	<i>P</i>
Patients, <i>n</i>	34	33	—
TGF- $\beta$ level (pg/mL), mean ± SD	1706.66 ± 552.36	3109.46 ± 569.16	<b>&lt;0.0001</b>
Probability of success, <i>n</i> (%)			
6 mo	33 (100)	29 (90.9)	0.1528
12 mo	33 (97.1)	24 (78.8)	<b>0.0052</b>
18 mo	32 (94.1)	21 (66.7)	<b>0.0022</b>
24 mo	31 (88.2)	18 (57.6)	<b>0.0007</b>
IOP at 24 mo postoperatively, mean ± SD	15.03 ± 3.37	18.94 ± 5.12	<b>0.0004</b>
Number of glaucoma eyedrops at 24 months postoperatively, mean ± SD	0.35 ± 0.54	1.03 ± 0.85	<b>0.0002</b>

Bold values indicate significant results.

### Correlation Between aFGF and TGF- $\beta$ Levels

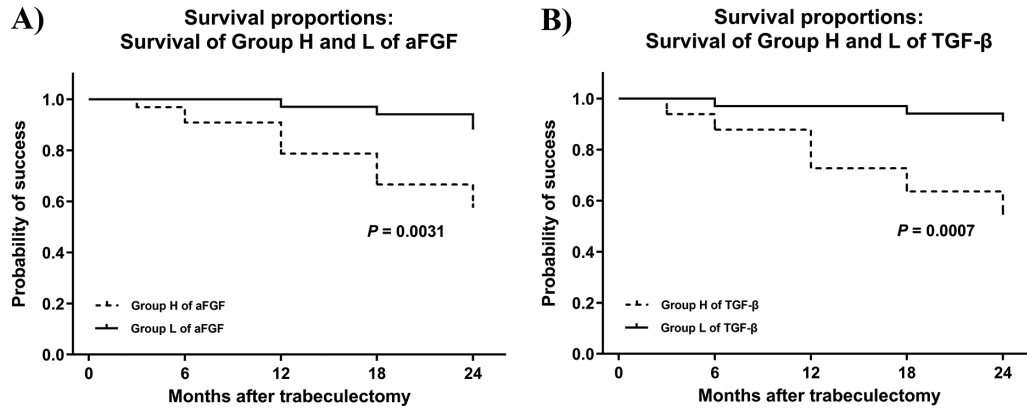
We evaluated the relationship between the levels of aFGF and TGF- $\beta$  in the AH (Fig. 2). The levels of TGF- $\beta$  were significantly positively correlated with aFGF levels ( $P < 0.0001$ ,  $R^2 = 0.5841$ ). No correlation was found between other growth factors in the same way.

## Discussion

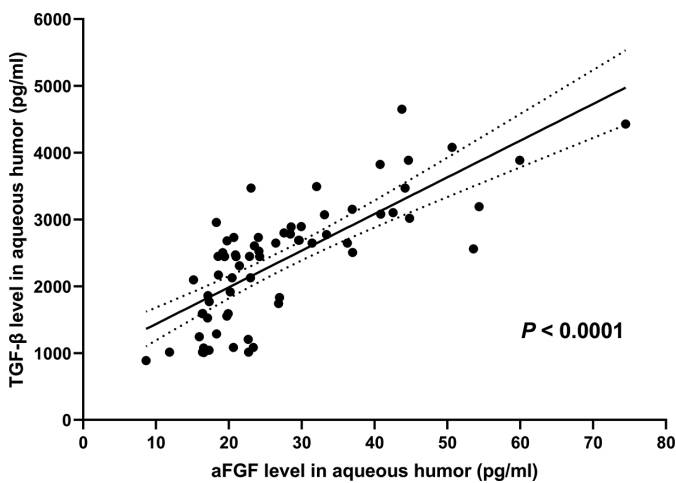
POAG is an optic neuropathy leading to a loss of axons at the ONH<sup>2,31</sup> and is one of the major causes of blindness worldwide. Elevated IOP is caused by

high resistance to AH outflow generated in the juxtacanalicular region of the TM.<sup>18,32</sup> Trabeculectomy is currently the prevailing operative technique for lowering IOP in patients with POAG. The key to trabeculectomy success is an unobstructed artificial drainage route from the anterior chamber to the subconjunctival space, which allows the AH to drain smoothly. Therefore, the inhibition of scarring after trabeculectomy has been attracting increasing attention in recent years.

The growth factors evaluated in this study regulate cell proliferation and ECM production; thus, alterations in these factors profoundly impact scar formation.<sup>33,34</sup> The ECM serves multiple functions, such as storage and delivery of growth factors and cytokines, tissue repair, and various physiological functions.<sup>34</sup>



**Figure 1.** Kaplan-Meier survival plot of two groups according to aqueous humor aFGF and TGF- $\beta$  levels. (A) Eyes with aFGF  $\leq 23.04$  pg/mL (group L of aFGF, 34 eyes; *solid line*) and eyes with aFGF  $> 23.04$  pg/mL (group H of aFGF, 33 eyes; *dashed line*). The probability of trabeculectomy success for eyes with low aFGF levels was significantly higher than for eyes with high aFGF levels ( $P = 0.0031$ ). (B) Eyes with TGF- $\beta \leq 2506.68$  pg/mL (group L of TGF- $\beta$ , 34 eyes; *solid line*) and eyes with TGF- $\beta > 2506.68$  pg/mL (group H of TGF- $\beta$ , 33 eyes; *dashed line*). The probability of trabeculectomy success for eyes with low TGF- $\beta$  levels was significantly higher than for eyes with high TGF- $\beta$  levels ( $P = 0.0007$ ).



**Figure 2.** The relationship between AH levels of aFGF and TGF- $\beta$  in all 67 eyes. The *diagonal line* shows the significant correlation between the two factors ( $P < 0.0001$ ,  $R^2 = 0.5841$ ). The *dotted line* represents the 95% confidence interval.

Abnormal ECM reconstruction during wound healing contributes to scar formation; therefore, excessive ECM production after trabeculectomy could increase the fibrotic process at the site of surgery leading to surgical failure. Thus, the levels of TGF- $\beta$ , IGF-1, aFGF, VEGF, and PDGF may be closely related to the surgical success rate. Our results show that aFGF and TGF- $\beta$  levels were significantly higher in the failure group; thus, they confirm this hypothesis and are similar to those of previous studies.<sup>35–37</sup>

FGF is one of the important factors of tissue fibroblasts. FGF signaling plays a crucial role in multiple cellular processes and the movement of FGF ligands to target cells in the ECM.<sup>38</sup> FGF-stimulated endothe-

lial cells may play a role in growth, differentiation, and involution modes during angiogenesis by altering the adhesivity or mechanical integrity of the ECM.<sup>39</sup> In an animal experiment, halofuginone and pirfenidone could decrease FGF levels, resulting in delayed wound healing in glaucoma filtration surgery.<sup>37</sup> A comparative study conducted by Turgut et al.<sup>40</sup> also showed that low FGF levels effectively suppressed subconjunctival scarring.

TGF- $\beta$  is a group of multifunctional cytokines involved in wound repair, migration, proliferation, apoptosis, and fibrosis.<sup>41</sup> They could promote the expression of multiple ocular hypertensive mediators in TM cells, including genes associated with fibrosis and cellular contractility. These effects are mediated by the induction of canonical (Smad) and non-canonical (mitogen-activated protein kinase, Rho GTPase) signaling cascades.<sup>42</sup> Tellios et al.<sup>43</sup> found that TGF- $\beta$ -induced increased collagen expression was associated with phosphorylation of phosphatase and tensin homolog (PTEN) at residues Ser380/Thr382/383 in TM cells, and TGF- $\beta$ -induced collagen expression by TM cells was prevented by exogenous overexpression of a mutated form of PTEN with enhanced phosphatase activity. This finding proved that TGF- $\beta$  in the AH is the main cause of TM fibrosis in patients with POAG. In addition to fibrosis, TGF- $\beta$  may also be involved in damage to the ONH.<sup>18</sup>

Our linear regression analysis results showed that TGF- $\beta$  levels were significantly positively correlated with aFGF. Xiao et al.<sup>44</sup> found that TGF- $\beta$ 1-induced fibroblast proliferation was mediated by the FGF-2/extracellular signal-regulated kinase pathway. In pulmonary fibrosis, FGF and TGF- $\beta$  may also have

a synergistic relationship. Shimbori et al.<sup>45</sup> demonstrated that FGF-1 might have preventive and therapeutic effects on TGF- $\beta$ 1-driven pulmonary fibrosis by inhibiting myofibroblast differentiation. Our results indirectly suggest that aFGF may be upregulated by the activation of TGF- $\beta$  in AH. Based on this, we propose that there may be an interactive and cooperative relationship between AH aFGF and TGF- $\beta$  in the progression of scarring after trabeculectomy. Modulation of AH composition or TGF- $\beta$  suppression may in turn reduce aFGF and improve surgical outcomes. Methods applicable in clinical settings must be elucidated in glaucoma eyes with high aFGF undergoing surgery.

Several limitations apply to this study. First, although the study was prospectively designed, the sample size was relatively small because the prevalence of POAG in China is relatively low compared with European and American populations. However, to the best of our knowledge, this is the first study to simultaneously analyze these five growth factors in Chinese eyes with POAG. Second, AH was collected at the beginning of trabeculectomy only, and we do not know whether there were changes in growth factor levels immediately after the operation or during follow-up. Future animal studies are needed to verify our results by dynamic observation. Third, there are three TGF- $\beta$  isoforms present in mammals, TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. Tamm et al.<sup>46</sup> found that TGF- $\beta$ 1 may play a role in differentiating TM cells toward a myofibroblast-like cell type by modulating the expression of alpha-smooth muscle actin. TGF- $\beta$ 2 is a strong inducer of ECM and a modifier of the actin cytoskeleton in TM cells.<sup>47</sup> Clinical studies are needed to clarify the specific isoform that interacts with TGF- $\beta$  in postoperative scarring. Nevertheless, preliminary results from this study showed that aFGF and TGF- $\beta$  levels correlated with surgical results, providing room for further research aimed at targeting valuable therapeutic factors.

## Conclusions

In the AH, aFGF and TGF- $\beta$  levels were correlated with trabeculectomy outcomes. High aFGF and TGF- $\beta$  levels were significant risk factors for trabeculectomy failure, and targeting their expression may have potential clinical applications in reducing fibrotic reactions after glaucoma surgery for patients with POAG. In the future, possible therapeutic strategies targeting aFGF and TGF- $\beta$  can be considered to improve the success rate of trabeculectomy for the treatment of POAG.

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