Potential Effects of Angiogenesis-related Factors on the Severity of APAC and Surgical Outcomes of Trabeculectomy

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Abstract

Background. Acute primary angle closure (APAC) caused by an abrupt closure of trabecular meshwork can develop into angle closure glaucoma due to uncontrolled IOP. Under reduced oxygen tension, hypoxia inducible transcription factors will be accumulated. EPO (erythropoietin) and PDGF (platelet derived growth factor) families are thought to be associated with angiogenesis under hypoxic condition.

Methods. Concentrations of EPO, PDGF-AA, -BB, -CC and -DD collected in aqueous humor samples were measured. Before operations, correlations between target proteins and IOP were detected between APAC (acute primary angle closure) and cataract patients. Based on the post-operative follow-up, the effects of EPO and PDGF family members on the successful rate of trabeculectomy were tested.

Results. The levels of EPO, PDGF-CC and -DD were significantly elevated in the APAC group compared to the cataract group. During the post-operative follow-up, EPO, PDGF-CC and -DD showed significant differences between the success and failure groups. In multivariable linear regression analyses, failed filtration surgery was more likely in APAC eyes with higher EPO level. The Kaplan-Meier survival plot suggested that the success rate in eyes with low EPO level was significantly higher than that in eyes with high EPO level.

Conclusion. The levels of EPO, PDGF-CC and -DD were significantly elevated in failure group. EPO level correlated with preoperative IOP and numbers of eyedrops, and higher EPO level in aqueous humor is a risk factor for trabeculectomy failure. It can be a biomarker to estimate the severity of APAC and the success rate of surgery. The investigation of mechanism of EPO in APAC a may have potential clinical applications for the surgical treatment of APAC.

Introduction

Acute primary angle closure (APAC), characterized by typical symptoms and clinical signs, is usually caused by an abrupt closure of trabecular meshwork in the anterior chamber angle. Patients usually refer to emergency management complaining about eye redness, ocular pain or discomfort, blurring of vision, frontal headache, nausea and vomiting. It was estimated that about 50% of eyes developed angle closure glaucoma due to uncontrolled IOP after an APAC attack. The sharp rise of IOP in APAC results in an inefficient supply of oxygen and nutrients, which can lead to atrophy of anterior structures, the inflammation of anterior chamber, ischemia and hypoxia of the whole eye [1]. Under reduced oxygen tension, hypoxia inducible transcription factors will be accumulated and lead to over expression of a large variety of target genes such as vascular endothelial growth factor (VEGF), erythropoietin (EPO) and platelet-derived growth factor (PDGF) [2–4].

VEGF is a well-known and potent vasculogenesis and angiogenesis cytokine, which has been found to increase in the aqueous humor of APAC eyes due to hypoxia [5, 6]. Except for VEGF, EPO and PDGF families are also thought to be associated with angiogenesis under hypoxic condition. EPO is a pleiotropic cytokine that was first identified as being essential for red blood cell (RBC) production. It plays
a key role in the modulation of the response to injury, inflammation, and tissue hypoxia via the inhibition of apoptosis [7]. The PDGF family includes four homodimers, PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD, which are important players in ocular diseases involving the degeneration of retinal neuronal and vascular cells [8, 9]. EPO and PDGFs elevations have been detected in primary open angle glaucoma and neovascular glaucoma [10, 11]. However, there has been no research describing the changes of EPO and PDGF levels in APAC eyes. Recent studies have shown that both EPO and PDGF have neuroprotective functions such as anti-apoptotic [12, 13], anti-oxidant [14] and anti-inflammatory properties [15, 16]. Thus, the fluctuations of their levels in aqueous humor may correlate with prognosis of trabeculectomy in APAC eyes. In this study, we aimed to measure the expression of EPO and PDGF family (PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD) in APAC eyes and determine their effects on APAC's surgical success rate.

Methods

Subjects

This study was conducted in accordance with the tenets of declaration of Helsinki, and it was approved by the Institutional Review Board of the Shanghai General Hospital. Patients were recruited from Dec 1, 2017 to Mar 31, 2018, prospectively and consecutively. Written and oral informed consent was obtained from each patient and their families, respectively.

All participants underwent a thorough ophthalmic examination including slit-lamp biomicroscopy, IOP measurement by Goldmann applanation tonometry, ultrasound biomicroscopy (UBM) as well as gonioscopy and fundus examinations. Enrolled APAC patients had experienced an APAC attack within one month before hospital admission. Even once the pupillary block was released, they still had uncontrolled IOP under standardized anti-glaucomatous medications. Patients with secondary angle closures, other ocular diseases or histories of previous ocular surgeries were excluded. Cataract patients, who were enrolled as the control group, had no history of IOP higher than 21 mmHg or glaucomatous optic neuropathy.

Surgical Technique and Postoperative Care

For all of the previous APAC patients, trabeculectomy combined with cataract surgery was performed by one experienced surgeon [17]. First, a fornix-based conjunctival flap was made, a limbus-based 4×4-mm scleral flap was dissected, and then a sponge soaked with 5-fluorouracil (25 mg/mL) was placed underneath the conjunctival flap for 3 minutes. Next, 250 mL balanced salt solution was used to wash the surgical area. Then, cataract extraction was performed by phacoemulsification and intraocular lens implantation according to the surgeon’s preferred technique. Finally, a trabeculectomy and a basal iridectomy were performed. After the procedure, the scleral flap was sutured using two 10-0 nylon sutures at the corners, and the centers of the two sides were closed using two adjustable sutures to allow minimal leakage during anterior chamber reconstruction. Finally, the conjunctiva was closed using a 10-0 nylon suture. A topical antibiotic and topical nonsteroidal anti-inflammatory medication were given four times daily for 1 week. Topical corticosteroids were used every 2 to 3 hours for the first week and then
tapered over the next 1 to 2 months. Postoperative interventions were performed for bleb formation. At first, ocular massages were performed. Then adjustable sutures were loosened with forceps. If the bleb became encapsulated and associated with a rise in IOP, needling was carried out without antimetabolites. In the case of postoperative IOP measurements > 21 mmHg, despite receiving an ocular massage and having any adjustable sutures loosened (if needed), IOP-lowering medication was added. In cases of inadequate IOP control, additional surgical procedures were performed as required.

**Aqueous Humor Collection and Analysis**

Generally, 80-120μl aqueous humor was needed for an examination and analysis. The 30-gauge needle and tuberculin syringe were used to withdraw the aqueous humor via limbal paracentesis. Aqueous humor samples from APAC and cataract patients were collected at the start of the trabeculectomy and cataract surgery, respectively. The aqueous humor was collected with caution to avoid touching any intraocular tissue. The collection was frozen in liquid nitrogen immediately and, then, transferred within 24 hours into a -80℃ environment until the analysis was completed.

EPO and PDGF family members (PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD) were detected by the Luminex Screening Human Magnetic Assay (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer’s instructions of the Bio-Plex 200 System (Bio-Rad Laboratories, Inc., Hercules, CA). The standard curve was automatically produced using the Bio-Plex Manager 5.1.0.0 software. The fluorescence intensities of both the sample and background were detected after the aqueous humor samples had been diluted in a 1:2 ratio. The final levels of the EPO and PDGF family members were analyzed after subtracting the background levels.

**Outcome Measures**

The IOPs of all the participants were analyzed a half hour before each surgical procedure, and all procedures were performed during a similar time frame in the afternoon. After operation, all APAC patients were followed up at 1 week and 1, 3, 6, 12 and 18 months. Goldmann tonometry and slit lamp biomicroscopy were performed at each post-operative follow-up. The surgical failure was defined as IOP >21 mmHg or administration of anti-glaucoma medications after 3 months or an additional glaucoma surgery [18,19]. The ocular massage, loosening of adjustable sutures and bleb needling were performed at the slit lamp and were not considered as an additional glaucoma surgery. If the IOP of one visit was lower than 21 mmHg without the use of topical anti-glaucoma medication, the visit was defined as a “success”.

**Statistical Analysis**

Commercially available SPSS software (version 26.0.0.0, SPSS Inc., Chicago, IL, USA) was used to analyze the results. The Kolmogorov-Smirnov test was used to test the normality of the continuous variable. The median (M) value and interquartile range (IQR) were used to describe the distribution of EPO, PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD. A Mann-Whitney U test was used to perform the
comparisons of target proteins and the clinical characteristics between the two groups. A Spearman correlation test was performed to determine the correlation between target proteins and associated factors.

After operation, a Mann-Whitney U test was used to perform the comparisons of EPO and PDGF families between success and failure groups. If any target protein was detected to have a significant difference between the two groups, it was included in the multiple linear regressions to characterize the effects after adjustment. If any protein showed significance after multiple linear regressions, the patients would be divided into two groups according to the protein level. The Kaplan Meyer survival curves were used to display the success rate of the end points over time between the low and high protein groups. Also, in the failure group, a Spearman test was used to examine the correlation between significant-protein(s) and the IOP and the time of failure before the use of any drug or surgical treatment. A p-value less than 0.05 was regarded as being statistically significant.

Results

Patients

In total, 55 patients participated in the present study, of which 35 were previous APAC patients and 20 were non-glaucomatous cataract patients. The clinical characteristics of patients are shown in Table 1. Since each data group was non-normally distributed, we used M and IQR values to present the data distribution. There were no significant differences between the APAC group and the control group regarding age (p = 0.630) or sex (p = 0.138). The IOP of these two groups had a significant difference (p < 0.001).

| Characteristics          | Previous APAC | Cataract | P       |
|--------------------------|---------------|----------|---------|
| No. Patients (No. Eyes)  | 35 (35)       | 20 (20)  | /       |
| Age, Medium (IQR), years| 70 (14)       | 67 (20)  | 0.630   |
| Sex [n (%)]              |               |          | 0.138   |
| Male                     | 12 (34.3%)    | 11 (55.0%)|         |
| Female                   | 23 (65.7%)    | 9 (45.0%) |         |
| IOP ,Medium (IQR), mmHg | 31.0 (13.0)   | 14.0 (2.0) | < 0.001*|

Note:Mann-Whitney U test test was used to investigate the difference of age, gender and IOP between two groups. *P < 0.05

APAC = acute primary angle closure, No.=number, IOP = intraocular pressure, IQR = interquartile-range
EPO and PDGF

The EPO and four PDGF family members were investigated in the aqueous humor samples of all 57 patients. It was found that the EPO and two PDGF family members, PDGF-CC and PDGF-DD, had significant differences between the APAC and the control group (Table 2). The Spearman correlation analysis between the levels of five target proteins and age, sex, IOP, number of preoperative antiglaucoma eyedrops as well as interval between the onset of APAC and trabeculectomy is shown in Table 3. In the previous APAC group, EPO had a significant correlation with pre-operation IOP and number of preoperative eyedrops, PDGF-AA and PDGF-BB only had a significant correlation with pre-operation IOP, while PDGF-DD had a significant correlation with sex and interval between the onset of APAC and trabeculectomy.

Table 2
Aqueous Humor Analysis of EPO and PDGF Family Members Concentration in Two Groups

| Analyte       | APAC      | Cataract  | Median difference (95% CI) | P       |
|---------------|-----------|-----------|---------------------------|---------|
| EPO, Medium (IQR), pg/ml | 36.46 (16.74) | 25.96 (10.50) | 9.00 (0.00-10.50) | 0.009*  |
| PDGF-AA, Medium (IQR), pg/ml | 1.92 (0.74) | 1.62 (0.78) | 0.18 (-0.16-0.54) | 0.302   |
| PDGF-BB, Medium (IQR), pg/ml | 0.18 (0.04) | 0.16 (0.02) | 0.00 (0.00-0.20) | 0.135   |
| PDGF-CC, Medium (IQR), pg/ml | 1.90 (2.02) | 1.44 (0.51) | 0.40 (0.14–0.72) | 0.002*  |
| PDGF-DD, Medium (IQR), pg/ml | 0.10 (0.12) | 0.04 (0.00) | 0.04 (0.02–0.12) | < 0.001* |

Note: Mann-Whitney U test was used to investigate the difference of EPO, PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD between APAC and cataract patients’ aqueous humor. *P < 0.05

APAC = acute primary angle closure, IQR = interquartile-range
Table 3
Correlations Between Target Proteins and Associated Factors in Two Groups

| Spearman Correlation | Previous APAC |
|----------------------|--------------|
|                      | Target Protein (pg/ml) | Age | Gender | Preoperative IOP | Number of preoperative eyedrops | Interval between the onset of APAC and trabeculectomy |
| EPO                  | r | -0.177 | -0.137 | 0.343* | 0.404* | -0.048 |
|                      | P | 0.309 | 0.433 | 0.044 | 0.016 | 0.786 |
| PDGF-AA              | r | 0.132 | -0.101 | 0.444* | 0.225 | -0.230 |
|                      | P | 0.449 | 0.562 | 0.008 | 0.193 | 0.183 |
| PDGF-BB              | r | -0.125 | -0.142 | 0.515* | 0.293 | -0.324 |
|                      | P | 0.473 | 0.417 | 0.002 | 0.087 | 0.058 |
| PDGF-CC              | r | 0.152 | -0.153 | 0.317 | 0.115 | -0.217 |
|                      | P | 0.384 | 0.382 | 0.064 | 0.512 | 0.211 |
| PDGF-DD              | r | 0.029 | -0.348* | 0.137 | 0.086 | -0.454* |
|                      | P | 0.876 | 0.040 | 0.432 | 0.624 | 0.006 |

Note: Spearman correlation test was used to evaluate the correlations between EPO, PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD and demographic characteristic in previous APAC group. *P < 0.05

Surgical Results

After operation, all of the APAC patients finished their follow-ups after 18 months. During the follow-up period, 24 eyes of the 35 previous APAC eyes (68.6%) were assigned to the success group, and 11 eyes (31.4%) were assigned to the failure group. The age, gender, numbers of preoperative eyedrops, the interval between the onset of APAC and trabeculectomy and pre-operation IOP showed no significant differences between the two groups (Table 4).
| Characteristics                                             | Success group | Failure group | P    |
|-------------------------------------------------------------|---------------|---------------|------|
| No. Patients (%)                                            | 24 (68.6%)    | 11 (31.4%)    | /    |
| Age, Medium (IQR), years                                    | 72 (13.5)     | 65 (18.0)     | 0.061|
| Sex [n (%)]                                                 |               |               | 0.283|
| Male                                                        | 10 (41.7%)     | 2 (18.2%)     |      |
| Female                                                      | 14 (58.3%)     | 9 (81.8%)     |      |
| IOP, Medium (IQR), mmHg                                     | 34.5 (16.3)    | 40.0 (21.0)   | 0.123|
| No. of Preoperative Eyedrops, Medium (IQR)                  | 2 (1.8)        | 2 (2.0)       | 0.985|
| Interval between the Onset of APAC and Trabeculectomy, Medium (IQR), days | 7.75 (14.0) | 9 (7.0) | 0.113 |

Note: Mann-Whitney U test test was used to investigate the difference of age, gender, IOP, numbers of preoperative eyedrops, and interval between the onset of APAC and trabeculectomy between two groups. *P < 0.05

APAC = acute primary angle closure, No.=number, IOP = intraocular pressure, IQR = interquartile-range
Table 5
Aqueous Humor Analysis of EPO and PDGF Family Members Concentration in Success and Failure Groups

| Analyte        | Success | Failure | Median difference (95% CI) | P       |
|----------------|---------|---------|---------------------------|---------|
| EPO, Medium (IQR), pg/ml | 28.44 (10.50) | 48.60 (40.68) | -16.74 (-37.22~6.24) | 0.008*  |
| PDGF-AA, Medium (IQR), pg/ml | 1.62 (0.71) | 2.14 (1.18) | -0.50 (-1.06 ~ 0.04) | 0.074   |
| PDGF-BB, Medium (IQR), pg/ml | 0.16 (0.04) | 0.18 (0.12) | -0.02 (-0.06 ~ 0.00) | 0.164   |
| PDGF-CC, Medium (IQR), pg/ml | 1.70 (0.42) | 2.46 (7.14) | -0.72 (-2.32~0.30) | 0.003*  |
| PDGF-DD, Medium (IQR), pg/ml | 0.08 (0.12) | 0.18 (0.28) | -0.060 (-0.20 ~ 0.00) | 0.045*  |

Note: Mann-Whitney U test was used to investigate the difference of EPO, PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD between success and failure patients’ aqueous humor. *P < 0.05

APAC = acute primary angle closure, IQR = interquartile-range

Three eyes (12.5%) in the success group and two eyes (18.1%) in the failure group received ocular massages. Five eyes (20.8%) in the success group and five eyes (45.5%) in the failure group received suture lysis. The differences were not significant. There was a slightly greater number of eyes receiving needling in the failure group (4/11 eyes) than in the success group (3/24 eyes), but the difference was not significant (p = 0.1013). In the failure group, two patients underwent glaucoma valve implantation, and two underwent ciliary body photocoagulation; two needed two kinds of intraocular pressure-lowering medication to control their IOP, and five needed one kind. All post-operation patients who used IOP-lowering medications had their IOP controlled within the normal range.

Correlation Between EPO/PDGFs and Surgical Outcomes

We first determined the potential changes in EPO and PDGF levels between the success and failure groups. Our results showed that the EPO, PDGF-CC and PDGF-DD levels were significantly elevated in the failure group. Then the age and content of EPO, PDGF-CC, and PDGF-DD were included in the multiple linear regression analyses (Table 6). Failed filtration surgery was more likely in APAC eyes with higher EPO level (p = 0.021). Other factors, including age and the levels of PDGF-CC and PDGF-DD, were not associated with the outcome of trabeculectomy. Results indicated that high EPO level was a significant
risk factor for trabeculectomy failure. We, subsequently, divided the patients into two groups according to the levels of these two proteins, respectively. To EPO, 17 eyes with EPO levels lower than the median value (<36.46 ng/mL) were included in group EPO-L, and the other 18 eyes were divided in group EPO-H. The Kaplan-Meier survival plots were drawn to investigate the success rate between the EPO-L and EPO-H groups (Fig. 1). It showed that patients with low EPO levels had significantly higher success rates over the 18-month follow-up period compared to those with high EPO levels: with p = 0.003 through Breslow (Generalized Wilcoxon) test.

| Factors       | Standardized coefficient | Unstandardized coefficient | p    |
|---------------|--------------------------|----------------------------|------|
| Age           | 0.269                    | 0.013                      | 0.086|
| EPO, pg/ml    | -0.371                   | -0.003                     | 0.021*|
| PDGF-CC, pg/ml| -0.112                   | -0.005                     | 0.577|
| PDGF-DD, pg/ml| -0.280                   | -0.280                     | 0.086|

Note: Multivariable linear regression analyses were performed with outcome of surgery as the dependent variables and age, EPO, PDGF-CC and PDGF-DD as independent variables. *P < 0.05.

**Discussion**

APAC is an ophthalmic emergency characterized by the sharp rise of IOP and ischemia of the eye. Aqueous humor contains various protein factors, and the composition of those proteins changes dramatically with IOP elevation and ischemia [20]. EPO is a hematopoietic glycoprotein playing an important role in the modulation of the response to tissue hypoxia via the inhibition of apoptosis [7, 21]. In a biological study, EPO proved to be effective in preventing the loss of retinal ganglion cells (RGCs) due to pressure elevation [22]. Researches also showed that PDGFs play critical roles in many ocular neovascular diseases and RGCs as well as degeneration diseases, such as glaucoma, retinopathy of prematurity and retinitis pigmentosa [23, 24]. Recently, the PDGF-AA protein has been shown to rescue RGCs from death in a glaucoma mice model [25]. Moreover, PDGF-CC has proved to be critically required for neuronal survival in an ischemia-induced stroke model [26]. Thus, the levels of EPO and PDGFs could change to cope with the IOP fluctuation induced hypoxia.

In the present study, we found that EPO and two members of the PDGF families (PDGF-CC and -DD) showed significant elevation in the APAC group than in the control group. In other clinical researches, EPO and PDGF were found to increase in the aqueous humor of patients with primary angle closure glaucoma, primary open angle glaucoma and neovascular glaucoma [10, 27]. Mokbel et al pointed out that EPO level increased in aqueous of these glaucoma patients as a result of glaucoma damage and not a cause [28]. In our study, when the APAC attacked, the sudden elevation of IOP caused transient retinal hypoxia. The
anoxic environment induced an increase in EPO and PDGF-CC/-DD levels in the eye mainly to serve their neuroprotective functions [15, 29, 30]. Moreover, the EPO level was found to significantly correlate with pre-operation IOP and numbers of preoperative eyedrops. Taken together, it is reasonable to postulate that the EPO level might reflect the severity of the disease in APAC eyes. In severe cases with higher IOP and more usage of medications, EPO was consistently produced to protect retinal cells in a more severe oxygen-deficient environment.

In addition to the neuro- and tissue-protection abilities, EPO and PDGF were proven to associate with the fibrosis and inflammation process in different organs [15, 16, 31–33]. Thus, the fluctuation of these proteins could correlate with the outcome of trabeculectomy. Bearing this in mind, we first determined the EPO and PDGFs levels in the surgical failure group and in the success group, and the content of EPO, PDGF-CC and PDGF-DD showed a significant elevation in the failure group. To further evaluate which proteins were the most relevant risk factors for surgical failure, we performed multiple linear regressions analyses. The result revealed that the level of EPO was significant risk factor for trabeculectomy failure. The Kaplan-Meier survival plots also confirmed that a lower probability of success was achieved in the APAC group with a higher EPO level. Since EPO significantly correlated with preoperative IOP and numbers of antiglaucoma medications usage, it is possible that eyes with higher preoperative IOP and more usage of eyedrops had worse conditions. In those conditions, retinal ganglion cells called for much higher level of EPO to fight against the oxygen-deficient environment. Some studies have shown the anti-fibrosis [33] and anti-inflammation [16] abilities of EPO. However, various factors in the aqueous humor were found rising during acute angle closure, such as matricellular proteins and inflammation factors. The former can cause the extracellular deposition of fibrillary and obstruct the outflow route [34, 35], and the latter can cause the function loss of the blebs [5]. Therefore, we speculated that even though EPO was more elevated to protect the RGCs in severe cases, the level of EPO was not high enough to completely reverse the fibrosis, and it ultimately resulted in surgical failure. Researches should further analysis the correlations between EPO and other factors affecting the outcome of trabeculectomy.

The study is translational to clinical practice in that it reminds us that EPO therapy is a double-edged sword in the treatment of eye disease. Different modes of EPO administration, such as systemic delivery or intravitreal injection, have given positive results for ocular disorders [2, 36, 37]. There are emerging evidences for the neuroprotection effect of EPO both in vivo and in vitro [21, 38, 39]. The positive roles that EPO may play in APAC under higher dose, such as anti-apoptotic [12, 13], anti-oxidant [14], and anti-inflammatory [15, 16] make it an ideal treatment in APAC. However, its role in angiogenesis, causing neovascularization is a dreaded occurrence in the eye [40]. Future animal studies should be performed to verify our theory and, hopefully, get the optimal dosage and regime of EPO treatment after APAC.

There were several limitations in our current study. First, although we performed postoperative follow-ups for patients, due to ethical limitations, we were unable to detect the EPO and PDGF levels in their aqueous humor after surgery. Therefore, we cannot conclude a causal relationship between the EPO and the surgical outcomes. Second, the sample size of this study was too small to perform a subgroup analysis, and the number of subjects in each group was slightly uneven (although the differences in age and sex...
between the groups were not significant). Future research should be performed to check if there is any difference in the EPO and PDGF levels between the different types of failures, and to check if there is any correlation between the levels of EPO and PDGF and the time of IOP rise. However, to our knowledge, this is the first study evaluating the levels of EPO and PDGF family members in APAC eyes. A higher successful rate of trabeculectomy was found in the low-EPO level group.

In conclusion, the levels of EPO, PDGF-CC and PDGF-DD were significantly elevated in eyes suffering from APAC. Eyes with higher EPO levels had lower successful rates of trabeculectomy, so the EPO content in aqueous humor may be used as a predictor of APAC prognoses. Further studies are necessary to investigate the molecular mechanism(s) and histological evidence of EPO pathogenesis in APAC and targeting its expression may have potential clinical applications for the surgical treatment of APAC.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the Shanghai General Hospital in accordance with the tenets of declaration of Helsinki. Written and oral informed consent was obtained from each patient and their families, respectively.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests

Authors’ contributions

ZZ conceptualized the idea, contributed to study design, result interpretation, analysis, manuscript writing, and proofreading. BK recorded and arranged the data of patients before operation and in follow-up test. MF diagnosed and enrolled the subjects. MZ performed the trabeculectomy. JW performed most of the analysis of the results and writing of the manuscript. XX helped in designing the study and revising the manuscript.

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