• Clinical Research •

Increased aquaporin-1 levels in lens epithelial cells with primary angle-closure glaucoma

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Abstract

• AIM: To determine the levels of aquaporin-1 (AQP-1) in the lens epithelial cells (LECs) of primary glaucoma and to clarify its correlation with lens thickness.

• METHODS: This study comprised 64 eyes of 64 patients with primary glaucoma, who were divided into 3 groups: 25 eyes of 25 patients with acute primary angle-closure glaucoma (APACG), 19 eyes of 19 patients with chronic primary angle-closure glaucoma (CPACG) and 20 eyes of 20 patients with primary open angle glaucoma (POAG). This study also included 12 eyes of 12 patients with senile cataract as controls. The levels of AQP-1 in LECs were examined by real-time quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry. The lens thickness was measured by A-scan ultrasonography.

• RESULTS: The AQP-1 mRNA levels of LECs were 0.84±0.27, 0.69±0.34, 0.44±0.19 and 0.51±0.21 in APACG, CPACG, POAG and senile cataract group, respectively. The levels of AQP-1m RNA were significantly higher in PACG groups compared with those in senile cataract and POAG group (all P<0.05). The immunohistochemistry showed the AQP-1 expression were strong-positive in PACG groups, but weak-positive in senile cataract and POAG group. A positive correlation was found between AQP-1 mRNA levels and the lens thickness (r=0.645, P<0.001).

• CONCLUSION: These findings show that the higher expression of AQP-1 in LECs may contribute to increased lens thickness, which might be associated with the occurrence and development of PACG.

• KEYWORDS: aquaporin-1; lens epithelial cells; lens thickness; primary angle-closure glaucoma
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INTRODUCTION

Glaucoma affects more than 70 million people worldwide with about 10% being bilaterally blind, making it the leading cause of irreversible blindness[1]. Although primary open angle glaucoma (POAG) is more prevalent, primary angle-closure glaucoma (PACG) causes more blindness and accounts for 70% to 90% of primary glaucoma in Chinese primary hospitals[2].

The main ocular risk factor for angle closure includes a crowded anterior segment, with a shallow central anterior chamber, a thicker and more anteriorly positioned len, and short axial length of the eyeball[3-5]. The most satisfactory explanations for the shallower anterior chamber is the increase in lens thickness and more anterior position of the lens with age. Many studies have shown that the lens thickness in PACG is greater than in normal subjects[6-7]. The lens play a pivotal role in the pathogenesis of PACG, although the mechanisms responsible for the thicker lens are poorly understood.

The aquaporins (AQPs) are a family of water channels that facilitate bidirectional osmotic water transport across cell plasma membranes and the transport of glycerol and other small solutes[8-9]. The important roles of AQPs in ocular tissues have been consistently reported. For example, corneal thickness was remarkably reduced in AQP-1 null mice and increased in AQP-5 null mice[10]. Similar mechanisms of AQP-dependent fluid transport could apply in lens. The lens capsule is a kind of basement membrane that completely wraps the ocular lens. An epithelial cell monolayer extends from the anterior pole of the lens to its equatorial region. Fluid is assumed to enter the lens at the poles and leave at the equatorial region, facilitated by its extensive internal communication *via* gap junctions and AQPs[11]. Two main AQPs are expressed in the lens: AQP-0

in the posterior pole and in nuclear fibers, and AQP-1 at the anterior pole in epithelial cells[12]. The purpose of this study was to investigate changes in AQP-1 expression in human lens epithelial cells (LECs) of primary glaucoma compared with senile cataract and its correlation with lens thickness.

SUBJECTS AND METHODS

Patients Selection This study was approved by the Ethical Review Committee of Zhongshan Ophthalmic Center and adhered to the tenets of the Declaration of Helsinki. Participants were recruited as a consecutive sample of patients at the Glaucoma Department and Cataract Department. The study included a total of 64 eyes from 64 patients with primary glaucoma combined with cataract and 12 eyes from 12 age-matched patients with simple senile cataract. The 64 eyes with primary glaucoma include 25 eyes with acute primary angle-closure glaucoma (APACG), 19 eyes with chronic primary angle-closure glaucoma (CPACG) and 20 eyes with POAG. Only patients in the age group of 60-80y were included in the study. Exclusion criteria were patients with diabetes mellitus, hypertension and eyes with uveitis, high myopia, pseudoexfoliation, traumatic cataract and subluxated cataract. Cataract surgery alone or combined trabeculectomy were performed under local anesthesia using a standardized technique[13]. The anterior lens capsule, approximately 6 mm in diameter, was removed from the anterior region of the lens capsule during phacoemulsification and stored immediately at -80℃ for future use.

RNA Extraction and Real-time Quantitative Polymerase Chain Reaction Total RNA was extracted from LECs by using Trizol isolation reagent (Takara, Japan) and reverse transcribed into cDNA with a cDNA Synthesis Kit (Thermo Fisher, USA). Quality assessment and concentration of RNA extracts was done by NanoDrop Products (Thermo Fisher, USA) before cDNA preparation. The housekeeping gene GAPDH served as a control. The primers for GAPDH were as follows: F: 5'-GCAGGGGGGAGCCAAAAGGGT-3', R: 5'-TGGGTGGCAGTGATGGCATGG-3', primers for AQP-1 were as follows: F: 5'-GTCCAGGACAACGTGAAGGT-3', R: 5'-GAGGAGGTGATGCCTGAGAG-3'. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed with a LightCycler 480 SYBR Green I Master and Roche LightCycler 480 real-time system. The RT-qPCR reaction mixtures contained a total volume of 20 μL, which included 10 μL of SYBR Green I Master Mix, 0.4 μL of PCR forward primer, 0.4 μL of PCR reverse primer, 2 μL of cDNA template and 7.2 μL of dH_2O. The PCR conditions consisted of 45 cycles of denaturation at 95℃ for 10s and annealing at 60℃ for 20s and extension at 72℃ for 20s. All groups were performed in triplicate. Analysis of relative gene expression data using RT-qPCR and the 2-ΔCt method.

Immunohistochemistry All excised anterior lens capsule were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Immunohistochemical studies were performed on 5 μm thick paraffin section. After blocking with goat serum in PBS, the slides were incubated with rabbit anti-AQP1 (1:500, Abcam, USA) in blocking solution overnight at 4℃. After extensive washing in PBS, slides were incubated with secondary biotinylated antibody for 30min at 37℃, then with avidin-peroxidase for 10min at 37℃, DAB was used as chromogen. Slides were counterstained with haematoxylin. Positive cells were identified by a brown stain. Negative controls used substitution of the primary antibody with PBS. Images were captured by a confocal laser scanning microscope system (Zeiss Axioplan2 imaging, Germany).

A-scan Ultrasonography After topical anesthesia, A-scan (Nidek US-1800, Japan) was performed with the patient in a supine position and low ambient light. The patient was asked to fixate on a fixation in front of them. The 10 MHz probe was then placed on the center of the cornea perpendicularly, taking care not to indent the cornea. Using manual freezing the scan, ten consecutive measurements of lens thickness, anterior chamber depth, and axial length were obtained. An average of ten measures was used for analysis.

Statistical Analysis The data were processed and statistically analyzed by SPSS (V. 16.0, USA). Categorical covariates were assessed individually using the χ^2 test. All distributed data were presented as mean±standard deviation (SD). One way ANOVA was performed for the comparison of four groups. Pearson correlation was used to assess the associations between AQP-1 mRNA levels and lens thickness. $P<0.05$ were considered as statistically significant.

RESULTS

This study included 25 eyes with APACG, 19 eyes with CPACG, 20 eyes with POAG and a control group of 12 eyes with senile cataract. The basic information about the patients was listed in Table 1.

A-scan ultrasonography showed mean lens thickness in POAG and senile cataract groups were 4.63±0.34 and 4.61±0.48 mm, respectively. In contrast, mean lens thickness in APACG and CPACG groups were significantly thicker than those in other two groups (5.43±0.31 and 5.31±0.24 mm, respectively) (all $P<0.001$). However, no significant differences were found between APACG and CPACG groups, POAG and senile cataract groups (both $P>0.05$) (Figure 1).

Increased Aquaporin-1 mRNA Expression in Lens Epithelial Cells of Patients in Primary Angle-closure Glaucoma RT-qPCR analysis showed the relative AQP-1 mRNA levels of LECs in POAG and senile cataract groups were 0.44±0.19 and 0.51±0.21, respectively. In contrast, AQP-1 mRNA levels in APACG (0.84±0.27) and CPACG (0.69±0.34) groups were significantly higher than those in other two groups (all

Table 1 Characteristics of the subjects

Subgroups	APACG	CPACG	POAG	Cataract	*P*
No. of eyes	25	19	20	12	N/A
Age, a (SD)	68.9 (4.6)	67.4 (5.6)	70.4 (5.2)	69.2 (5.6)	>0.05
Sex (M/F)	6/19	8/11	8/12	4/8	>0.05
Lens thickness, mm (SD)	5.43 (0.31)	5.31 (0.24)	4.63 (0.34)	4.61 (0.48)	<0.001

Data are expressed as the mean (SD).

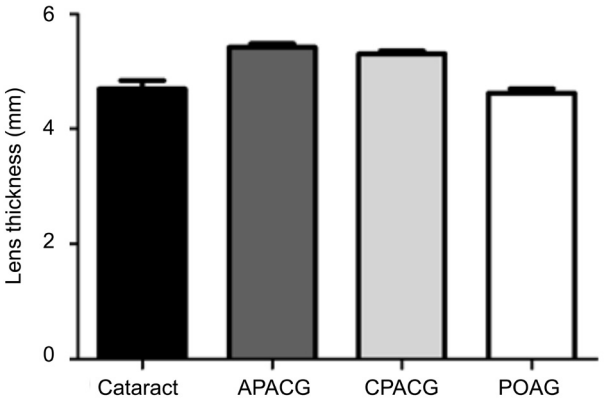

Figure 1 Levels of lens thickness of the four groups Mean lens thickness in APACG group and CPACG group were significantly thicker than those in senile cataract and POAG groups (P<0.001).

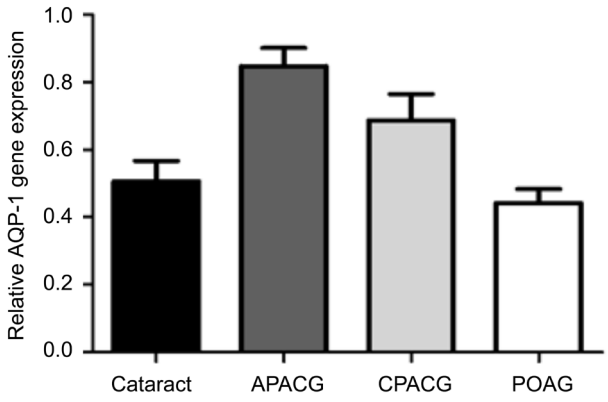

Figure 2 Levels of AQP-1 mRNA in the LECs of the four groups AQP-1 mRNA levels in APACG and CPACG group were significantly higher than those in senile cataract and POAG groups. The AQP-1 mRNA levels in APACG group were also significantly higher than those in CPACG group (P<0.05).

P<0.05). The AQP-1 mRNA levels in APACG group were also significantly higher than those in CPACG group (P<0.05). However, the difference between the POAG and senile cataract group was not statistically significant (P>0.05) (Figure 2).

Increased Aquaporin-1 Protein Expression in Lens Epithelial Cells of Patients with Primary Angle-closure Glaucoma We next evaluated the protein expression of AQP-1 in these four groups with immunohistochemistry staining. The results showed the staining of AQP-1 was strong-positive in APACG and CPACG groups, while weak-positive in POAG and senile cataract groups, consistent with the expression at the mRNA level. The darkest staining level was in APACG group (Figure 3).

High Levels of Aquaporin-1 mRNA Positively Correlated with Lens Thickness The Pearson linear correlation analysis showed the levels of AQP-1 mRNA were significant correlated with lens thickness (r=0.645, P<0.001) (Figure 4). Increased lens thickness were correlated with high levels of AQP-1 mRNA in PACG groups.

DISCUSSION

Our study showed that the AQP-1 mRNA levels of LECs were significantly higher in PACG groups than those in senile cataract and POAG groups. However, there was no significant difference between POAG and senile cataract groups. The study also found that high levels of AQP-1 mRNA in LECs were closely correlated with increased lens thickness in PACG groups.

As we know that PACG results from abnormality of the iris, the lens, and posterior segment structures. Pupillary block is the most common mechanism of angle closure and is caused by resistance to aqueous humor flow from the posterior to anterior chambers at the pupil[14]. The most satisfactory explanations for the pupillary block is the thicker and more anteriorly positioned lens compared with normal subjects[6-7]. Among the different subtypes of PACG, Mimiwati and Fathilah[15] showed that the thickest lenses were seen in the APACG eyes. The lens was thinner in CPACG eyes compared to the APACG ones. Our study also found the lens thickness in APACG eyes were the thickest among the four groups. Chen *et al*[16] found that the mean lens thickness of PACG was greater than 5.0 mm and that of the normal eyes 4.5 mm, and the lens thickness and lens/axial length factor tended to increase from normal to primary angle closure (PAC) to PACG. Also in another prospective ASOCT study, Guzman *et al*[17] reported that lens vault was greatest in the acute PAC group, followed by PAC, PACG, and PAC suspect, respectively. In our study, the mean lens thickness of PACG patients were significantly thicker than the senile cataract and POAG eyes, a finding consistent with other studies.

As discussed below, AQPs play important roles in all ocular tissues. In cornea, AQPs play a fundamental role in transmembrane water movements across the cornea and conjunctiva into the tear film and therefore are important in maintaining tear film osmolarity and stromal layer thickness. In AQP-1 null mice, reduced osmotic water influx from aqueous humor to the stroma combined with normal movement to

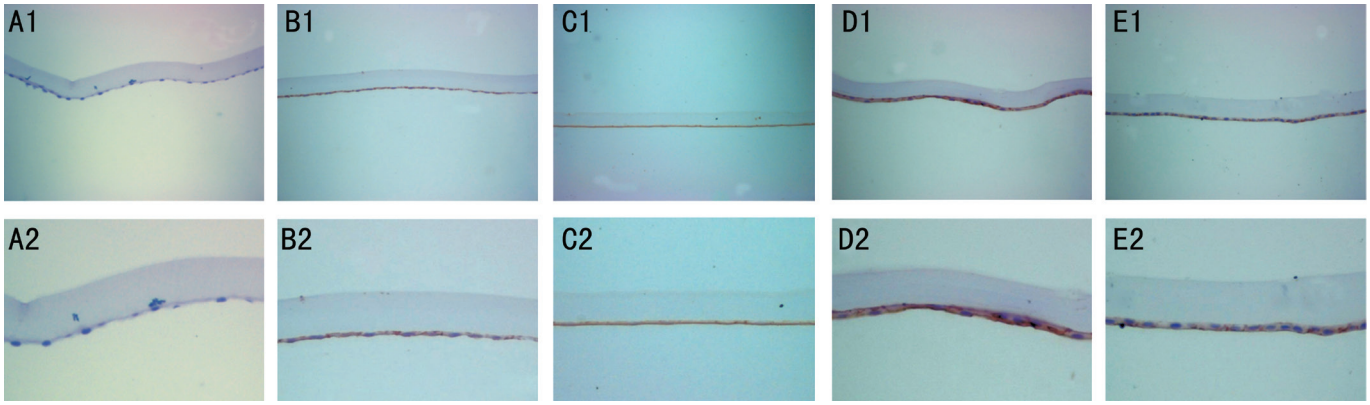

Figure 3 AQP-1 expression in LECs of senile cataract and primary glaucoma eyes A1: Immunohistochemistry showing no AQP-1 staining was observed when the primary antibody was omitted (negative control) (×200); A2: High magnification of A1 (B1) (×400). AQP-1 protein weak-positive expression in LECs of senile cataract (×200); B2: High magnification of B1 (×400); C1: AQP-1 protein weak-positive expression in LECs of POAG (×200); C2: High magnification of C1 (×400); D1: AQP-1 protein strong-positive expression in LECs of APACG (×200); D2: High magnification of D1 (×400). E1: AQP-1 protein strong-positive expression in LECs of CPACG (×200); E2: High magnification of E1 (×400).

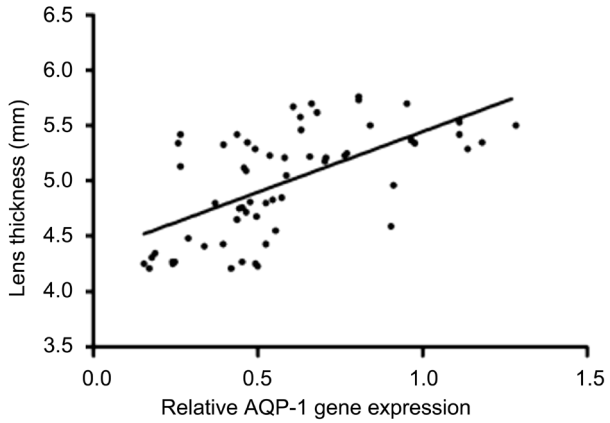

Figure 4 Relationship between levels of AQP-1 mRNA in LECs and lens thickness Levels of AQP-1 mRNA in LECs were significant correlated with lens thickness (r =0.645, P<0.001).

the tear film *via* AQP-5 is predicted to produce a chronically dehydrated and thinned cornea. In AQP-5 null mice, the reduced rate of osmotically driven water efflux from the stroma to the tear layer is predicted to produce an increase in corneal thickness[10]. Likewise, the capsule as a collagen matrix is highly permeable to water, and LECs contain abundant AQP-1[18-19]. Lens volume changes during accommodation are accompanied by rapid water movements presumably through AQP channels[11]. Localization of both ion channels and AQP is important in establishing the water flux necessary for the circulation system[20]. In our study, we found that the AQP-1 mRNA expression levels of LECs in PACG groups were significantly higher than those in senile cataract and POAG groups. However, there was no significant difference between POAG and senile cataract groups. Furthermore, we also found that high levels of AQP-1 mRNA in LECs were related to increased lens thickness in the four groups. The higher expression of AQP-1 in LECs may be predicted to produce increased osmotic water influx from aqueous humor into the lens, which make the lens thicker in PACG groups.

However, several limitations in this study should be recognized. First, we only detected the expression of AQP-1 in LECs at the level of transcription, so the protein level of the AQP-1 needs to be confirmed. Second, the exact regulatory mechanism *via* AQP-1 in LECs and the process of water circulation in lens needs more research. Finally, the sample size of our study is limited, we need to expand the sample size for further study.

In conclusion, we found that the AQP-1 mRNA levels of LECs were significantly higher in PACG groups and they were correlated with increased lens thickness. The up-regulated gene might be associated with the occurrence and development of PACG. Larger studies with larger sample sizes are needed to confirm and further support these findings and their clinical importance.

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