Occurrence of Oxidative Stress and Premature Senescence in the Anterior Segment of Acute Primary Angle-Closure Eyes

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Received: August 12, 2021
Accepted: December 27, 2021
Published: January 25, 2022

Citation: Ye D, Xu Y, Shi Y, et al. Occurrence of oxidative stress and premature senescence in the anterior segment of acute primary angle-closure eyes. *Invest Ophthalmol Vis Sci.* 2022;63(1):34. https://doi.org/10.1167/iovs.63.1.34

Purpose. To explore whether oxidative stress and premature senescence occur in the anterior segment of acute primary angle-closure (APAC) eyes after increased intraocular pressure.

Methods. The eye samples of 21 APAC patients, 22 age-related cataract patients, and 10 healthy donors were included. Aqueous humor (AqH), iris, and anterior lens capsule samples were collected. The levels of oxidative stress markers and senescence-associated secretory phenotype (SASP)–related cytokines in AqH were estimated using relevant reagent kits and multiplex bead immunoassay technique. The intensity of relevant markers in anterior segment tissues was examined by immunofluorescence- and senescence-associated β-galactosidase (SA-β-gal) staining.

Results. Oxidative stress marker levels elevated significantly in the AqH of APAC eyes. Reactive oxygen species (ROS) and 8-hydroxydeoxyguanosine levels were positively correlated with preoperative peak intraocular pressure and age, whereas reduced glutathione/oxidized glutathione (GSH/GSSH) ratio was negatively correlated with both parameters. The levels of several SASP-related cytokines were markedly increased. ROS and malondialdehyde levels were positively correlated with the levels of some SASP-related cytokines, whereas superoxide dismutase level and GSH/GSSH ratio showed an opposite trend. The number of cells positive for oxidative mitochondrial DNA damage and apoptosis-related markers increased in the iris and anterior lens capsule of the APAC group. Senescence-associated markers (p16, p21, and p53) and SA-β-gal activity were increased in the iris of the APAC group.

Conclusions. Oxidative stress and premature senescence occurred in the anterior segment of APAC patients, suggesting that they may be involved in the development of pathological changes in the anterior segment of APAC eyes.

Keywords: oxidative stress, premature senescence, acute primary angle-closure, anterior segment

Acute primary angle-closure (APAC) is an ophthalmic emergency that endangers eyesight. It is characterized by rapid elevation in intraocular pressure (IOP) due to the obstruction of outflow in the aqueous humor (AqH).[1] High levels of IOP in APAC patients can lead to extreme ocular or periocular pain, conjunctival congestion, corneal edema, and a series of pathological changes in the anterior segment, including iris atrophy, mid-dilated pupil, and

"glaukomflecken," and these pathological changes cannot be reversed even after control of the IOP.[2–4] Despite decades of in-depth investigation, the molecular mechanism underpinning increased IOP in the pathology of APAC remains unclear, preventing improvements in treatments.

Oxidative stress is defined as an imbalance between oxidants (reactive oxygen species [ROS]) and antioxidants or the production of oxidants at levels exceeding physiological values.[5] A certain amount of ROS is elementary for maintaining normal physiological functions, whereas excess ROS damages the cells.[6] A previous study demonstrated that oxidative stress reactions occurred in the retina of an acute IOP elevation glaucoma model.[7] However, it is not clear whether acute elevated IOP could induce oxidative stress in the anterior segment of APAC patients. ROS are effective activators of p53 function, which is believed to act on growth arrest and replicative senescence or apoptosis.[6] At high doses of ROS, p53 is activated and targets apoptosis-related genes.[8] Acute elevation of the perfusion pressure reportedly induces an increase in nitric oxide production in the trabecular meshwork of the anterior segment perfusion human donor eye model.[9] Nitric oxide can react with ROS to form peroxynitrite, which can induce apoptosis.[10] Thus oxidative stress-induced apoptosis may be involved in anterior segment injury caused by acute ocular hypertension. However, there is no report on oxidative stress–induced apoptosis and the expression of related factors in anterior segment tissues of APAC patients, especially in iris and anterior lens capsule.

Oxidants can also trigger stress-induced premature senescence (SIPS),[11,12] which could be activated by p53 at relatively lower doses of ROS.[8] Previous studies showed that the cyclin-dependent kinase inhibitor p16/INK4A mRNA expression was significantly higher in the retina of an acute IOP mouse model, and p53 played an indispensable role in driving retinal ganglion cells toward senescence.[13] Thus we can infer that acute high IOP has a distinct relationship with cellular senescence, and there is a high probability that SIPS occurs in the anterior segment of APAC patients with acute elevated IOP in the anterior chamber. However, the occurrence of cellular senescence in human APAC eyes and the factors associated with it have not been clarified.

In the present study, we examined the levels and relationship between oxidative stress markers and senescence-associated secretory phenotype (SASP)–related cytokines in the AqH of APAC patients. Furthermore, we examined apoptosis and senescence markers induced by oxidative stress in the iris and anterior lens capsule to investigate whether oxidative stress and cellular senescence occur in the anterior segment of APAC patients.

Methods

Study Design and Subjects

Patients were recruited from the Zhongshan Ophthalmic Center of the Sun Yat-sen University (Guangzhou, China) consecutively, from January 2018 to January 2020. The study was approved by the Ethical Review Committee of the Zhongshan Ophthalmic Center. All experimental procedures involving humans were in line with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from all individual participants included in this study.

All patients underwent comprehensive ocular examinations, including IOP measurement by Goldmann applanation tonometry, slit-lamp biomicroscopy, gonioscopy, ultrasound biomicroscopy, fundus examination, and B-scan ocular ultrasonography. IOP was measured repeatedly before operation, and the peak IOP recorded over five days before operation was the observation index in this study. The diagnosis criteria of APAC were similar to those of previous studies[14–18]: (1) the presence of any two of the following symptoms: ocular or periocular pain, nausea and vomiting, and an antecedent history of intermittent blurring of vision with haloes; (2) IOP $\geq$ 22 mm Hg measured by Goldmann applanation tonometry; (3) the presence of conjunctival injection, shallow anterior chamber, and mid-dilated fixed pupil with or without corneal epithelial edema; (4) the presence of an occludable angle in the affected eye verified by gonioscopy; (5) exhibiting signs of acute angle closure attack such as "glaukomflecken" or iris sphincter palsy. The exclusion criteria were (1) secondary acute attack because of lens subluxation, uveitis, iris neovascularization, trauma, tumor, nanophthalmos, or any obvious cataract leading to an intumescent lens; (2) diabetes, systemic hypertension, or other eye disorders; (3) history of laser or intraocular surgery.

Antiglaucomatous medication and surgeries (anterior chamber paracentesis/surgical peripheral iridectomy/trabeculectomy/lens extraction) for APAC eyes were administered according to the American Academy of Ophthalmology Preferred Practice Pattern guidelines on primary angle closure[19] and individual situations. The control group consisted of age-related cataract (ARC) patients without glaucomatous optic neuropathy, history of IOP exceeding 21 mm Hg, or systemic diseases and was scheduled to undergo routine phacoemulsification and intraocular lens placement.

AqH and Anterior Segment Tissues Collection

All surgeries were performed by the same surgeon (J.H.). AqH samples (100 µL) were collected at the onset of surgery and obtained before any conjunctival or intraocular manipulation to avoid breakdown of the blood–aqueous barrier associated with surgical trauma. For determining the levels of SASP-related cytokines in AqH, 25 µL AqH samples were used for each subject; the remaining 75 µL was diluted to 250 µL, with 50 µL being used in each subsequent experiment.

Iris tissue samples were collected during surgical iridectomy or trabeculectomy. As described previously,[20] for iridectomy, a corneal incision was made right in front of the limbus. Applying pressure on the scleral side of the incision to dislodge the iris into the incision. Then, both peripheral stromal and pigment layers of the iris were collected. For trabeculectomy, conjunctive and scleral flaps were first made, followed by a sclerostomy. Peripheral iridectomy was performed, and both peripheral stromal and pigment layers of the iris were collected. Both incisions of iridectomy and trabeculectomy were made in the superior quadrants. Each iris sample obtained was fixed in 4% paraformaldehyde and embedded in optimum cutting temperature compound (Sakura Finetek, Tokyo, Japan). All samples were cut in 10 µm frozen sections for immunofluorescence staining.

Anterior lens capsule tissue samples were collected during cataract surgery as described previously.[21] Briefly, clear corneal main wound and side port incisions were made, then continuous curvilinear capsulorhexis was

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performed using an ocular visco-elastic device and capsulorhexis forceps to collect the central anterior lens capsule. Each anterior lens capsule sample obtained was fixed in fixative solution for further flat mount experiments.

Donors and Preparation of Iris Samples

Age-matched control human donor eyes were obtained from Guangdong Eye Bank. Following the Helsinki declaration, the eyes were obtained with the written consent of the donor or their family for medical research.

Donor demographics are described in Supplementary Table S1. Donors without glaucoma or any history of systemic diseases were included. Only the peripheral iris was used for subsequent detection to match the APAC group.

Estimation of the Content of Oxidative Stress Markers in AqH

ROS,[22] 8-hydroxydeoxyguanosine (8-OHdG),[23] malondialdehyde (MDA),[24] superoxide dismutase (SOD)[25] levels, and reduced and oxidized glutathione (GSH/GSSG) ratio[26] in the AqH samples were detected with the assays provided in Supplementary Table S2.

Assessment of SASP-Related Cytokine Concentration and Senescence-Associated β-Galactosidase (SA-β-Gal) Activity

The concentrations of SASP-related cytokines in the AqH and the activity of SA-β-gal in the anterior lens capsule were determined using the assays described in Supplementary Table S2.

Immunofluorescence

Immunofluorescence of iridal frozen sections and flat mounts of anterior lens capsule was performed as described previously.[27–29] Briefly, the tissues were permeated for 20 minutes and blocked with 5% normal goat serum for two hours at 25°C. Then, the tissues were incubated with primary antibodies overnight at 4°C, followed by a corresponding secondary antibody for two hours (Supplementary Table S3). Images of the stained sections were obtained using a Laser Scanning Confocal Microscope (Carl Zeiss, Oberkochen, Germany).

Quantitative and Statistical Analysis

Positive cell quantitation was conducted by independent masked investigators (P.L. & L.C.) in duplicate. Mean number of labeled cells in human anterior segment samples was calculated from three different fields at magnification × 400 per section, using three sections per sample in each condition (for quantifications on iridal frozen sections), or from one field at magnification × 400 per section and using a minimum of eight different samples per condition (for quantifications on flat mounts of anterior lens capsule). Two-samples independent *t*-, χ^2, and Mann–Whitney *U*-tests were used to compare the two groups. Correlations were assessed using Spearman's rank-order correlation analysis. Statistical analyses were performed with SPSS 25.0 software (IBM, Armonk, NY, USA). Level of significance was set at $P < 0.05$. Data are expressed as mean ± standard deviation.

Results

Demographic and Clinical Characteristics of the Subjects

Characteristics of the study population are described in Table 1. In total, 43 Han Chinese patients were included. APAC patients were younger than ARC patients. The peak IOP recorded over five days before operation of the APAC patients was higher than that in the ARC patients (both $P < 0.001$). There was no significant difference in gender representation between both groups ($P > 0.05$).

Oxidative Stress Markers in the AqH

Increased production of ROS, 8-OHdG, and MDA was observed in the AqH of APAC eyes, compared to that in control samples obtained from ARC patients (Figs. 1A–C). Interestingly, SOD activity and GSH/GSSG ratio (Figs. 1D, 1E), indicating antioxidant indices, were also drastically increased in the APAC group (all $P < 0.0001$).

Correlation of Oxidative Stress Markers With IOP and Age of APAC Patients

IOP was found to positively correlate with ROS, 8-OHdG and MDA (r = 0.494–0.627, all $P < 0.05$), and negatively correlate with GSH/GSSG ratio (r = −0.465, $P < 0.05$) (Table 2). Age was found to be positively correlated with ROS and 8-OHdG levels (r = 0.506–0.827, both $P < 0.05$), and negatively correlated with SOD activity and GSH/GSSG ratio (r = −0.726 to −0.584, both $P < 0.01$) (Table 2).

SASP-Related Cytokines in AqH

The levels of several SASP-related cytokines were increased in the APAC group compared to that of the ARC group, including interleukin (IL)-6, IL-8, tumor necrosis factor–α,

Table 1. Demographic and Clinical Data of APAC and ARC Patients

Characteristics	APAC	ARC	*P* Value
Total No. patients	21	22	—
Age (years), mean ± SD	50.810 ± 12.003	61.500 ± 5.837	<**0.001**[*]
Sex No. (male/total)	8/21	10/22	>0.05[†]
IOP (mm Hg), mean ± SD	37.191 ± 9.245	13.864 ± 2.167	<**0.0001**[‡]
Duration before surgery (days), median (IQ)	4.238 (4.000)	—	—

IOP, the recorded peak intraocular pressure over the five days before operation; IQ, interquartile range; SD, standard deviation. Duration before surgery means the time from onset of symptoms to surgery.
[*] Significance of differences between groups: two-samples independent *t*-test. Bold value represents significance.
[†] Significance of differences between groups: χ^2 test. Bold values represent significance.
[‡] Significance of differences between groups: Mann–Whitney *U*-test. Bold value represents significance.

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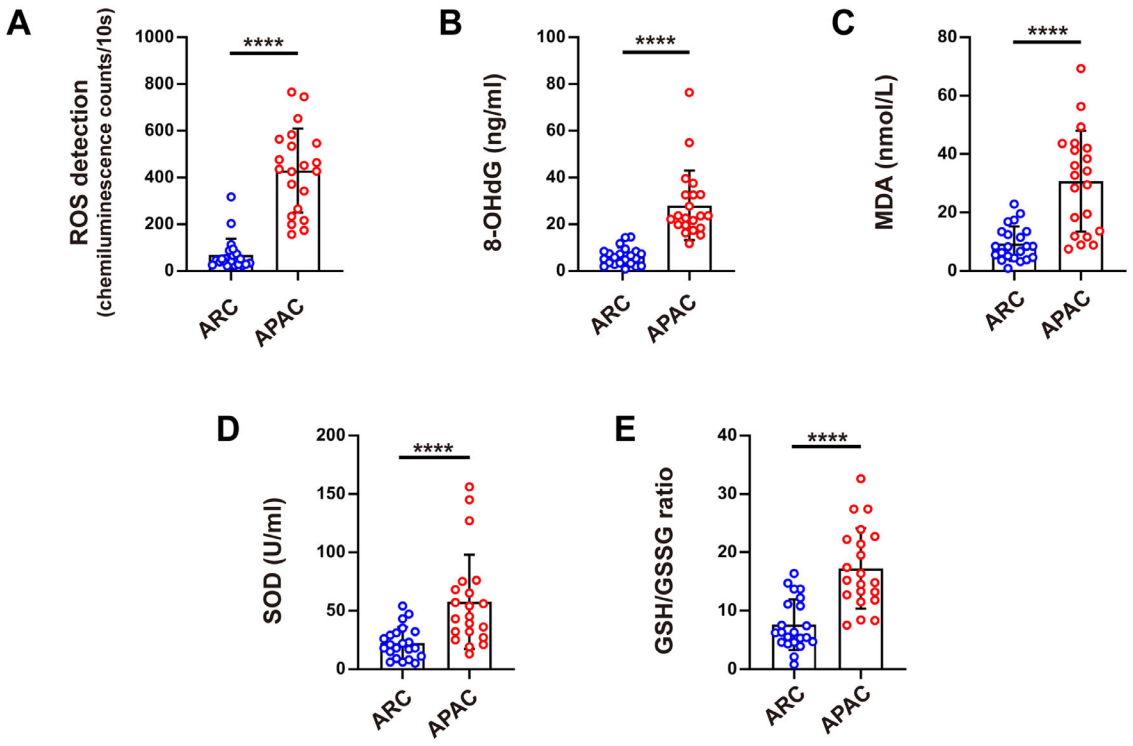

Figure 1. Oxidative stress markers in the aqueous humor (AqH) samples were collected from APAC eyes and ARC eyes (n = 21, 22, respectively). (**A–E**) The levels of ROS, MDA, 8-OHdG, SOD, and GSH/GSSG have been determined as described in the Methods section. Mann-Whitney *U*-test was performed. $P < 0.05$ was defined as significance. ****$P < 0.0001$ versus the ARC group.

Table 2. Correlation of Oxidative Stress Markers With IOP and Age of APAC Patients

	IOP (mm Hg)		Age (y)	
	R	*P* Value*	R	*P* Value*
ROS	0.596	**0.004**	0.827	**<0.0001**
8-OHdG	0.627	**0.002**	0.506	**0.019**
MDA	0.494	**0.023**	0.424	0.055
SOD	–0.301	0.185	–0.726	**<0.0001**
GSH/GSSG ratio	–0.465	**0.034**	–0.584	**0.005**

IOP, the recorded peak intraocular pressure over the five days before operation.

* Significance of differences between groups: Spearman's rank-order correlation analysis. Bold values represent significance.

chemokine (C-C motif) ligand (CCL)-2, chemotactic protein (MCP)–1, growth-related oncogene (GRO) α, macrophage inflammatory protein (MIP)–1α, vascular endothelial growth factor (VEGF)–α, insulin-like growth factor binding protein (IGFBP) 5, IGFBP7, transforming growth factor (TGF)–β1, and matrix metalloproteinase (MMP)–10 (all $P < 0.05$) (Table 3).

Correlation Between Oxidative Stress Markers and SASP Cytokine Levels in APAC Patients

A positive correlation was noted between the levels of ROS and various SASP-related cytokines, viz., IL-6, IL-8, CCL-2, GROα, MIP-1α, IGFBP5, IGFBP7, and TGF-β1 (r = 0.455–0.768, all $P < 0.05$). Additionally, 8-OHdG level was positively correlated with TGF-β1 level (r = 0.481, $P = 0.027$), and MDA level was found to positively correlate with IL-6, IGFBP5, IGFBP7, and TGF-β1 levels (r = 0.449–0.566, all $P < 0.05$).

Conversely, SOD activity was negatively correlated with levels of IL-6, IL-8, CCL-2, IGFBP5, IGFBP7, and TGF-β1 (r = −0.783 to −0.550, all $P \leq 0.01$). GSH/GSSG ratio was negatively related to IL-6, IL-8, CCL-2, GROα, MIP-1α, IGFBP5, IGFBP7, and TGF-β1 levels (r = –0.693 to –0.466, all $P < 0.05$) (Table 4).

Mitochondrial DNA (mtDNA) Oxidative Damage, Apoptosis, and Senescence in the Iris

Fluorescence of 8-OHdG and apoptosis-related factors, such as Bax, B cell lymphoma-2 (Bcl-2), B-cell lymphoma-extra-large (Bcl-xL), cleaved caspase-3, and cAMP response element binding protein (CREB), were enhanced in the iris of the APAC group compared to the control group (Fig. 2A). Staining intensity of p16, p21, and p53 was stronger, and fluorescence intensity of sirtuin1 (SIRT1) declined in the iris of the APAC group (Fig. 2A). Quantitative analysis indicated a marked increment in 8-OHdG-, Bax-, Bcl-2-, Bcl-xL-, cleaved caspase-3-, CREB-, p16-, p21-, and p53-positive cells, and a decline of SIRT1-positive cells in the iris after APAC attack (Figs. 2B–K; $P < 0.05$).

MtDNA Oxidative Damage, Apoptosis, and Senescence in the Anterior Lens Capsule

Staining intensity of 8-OHdG, Bax, Bcl-2, Bcl-xL, cleaved caspase-3, CREB, and p53 was stronger in the anterior lens capsule of the APAC group than that of the ARC group (Fig. 3A). However, SA-β-gal and the fluorescence staining of

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Table 3. Concentrations of SASP-Related Cytokines in the AqH

Cytokine (pg/mL)	APAC	ARC	*P* Value*
IL-1α	44.0476 ± 39.997	20.7273 ± 21.994	0.052
IL-1β	43.176 ± 25.679	44.877 ± 29.714	0.991
IL-6	2880.57 ± 1070.996	101.77 ± 106.973	**<0.0001**
IL-8	788.38 ± 553.431	164.95 ± 146.456	**<0.0001**
IL-13	0	0	1.000
TNF-α	551.4 ± 440.9155	159.6 ± 119.164	**<0.0001**
GM-CSF	8.1429 ± 12.167	7.3636 ± 8.301	0.068
CCL-2	282.81 ± 211.254	145.50 ± 121.791	**0.008**
CCL-20	0	0	1.0
MCP-1	1739.48 ± 1185.390	316.36 ± 328.621	**<0.0001**
MCP-3	0	0	1.0
GROα	75.2381 ± 39.319	33.4545 ± 17.965	**<0.0001**
MIP-1α	14.62 ± 16.566	9.05 ± 10.513	**0.048**
VEGF-α	1305.1905 ± 789.831	260.9091 ± 347.151	**<0.0001**
IGFBP2	78.48 ± 44.539	53.45 ± 30.709	0.061
IGFBP4	0	0	1.000
IGFBP5	3084.95 ± 1303.561	166.09 ± 160.008	**< 0.0001**
IGFBP6	0	0	1.000
IGFBP7	410.5 ± 181.906	132.6 ± 105.121	**<0.0001**
TGF-β1	3057.10 ± 1418.243	302.23 ± 260.093	**<0.0001**
HGF	35.9524 ± 29.889	28.8636 ± 23.526	0.450
MMP-1	6.95 ± 8.488	10.68 ± 11.582	0.340
MMP-3	19.43 ± 20.356	10.55 ± 13.415	0.159
MMP-4	0	0	1.000
MMP-10	81.19 ± 38.798	40.50 ± 21.743	**<0.0001**

HGF, hepatocyte growth factor.
* Significance of differences between groups: Mann–Whitney *U*-test. Bold values represent significance.

Table 4. Correlation Between Oxidative Stress Markers and SASP Cytokine Levels in APAC Patients

	ROS		8-OHdG		MDA		SOD		GSH/GSSG Ratio	
Cytokine (pg/mL)	r	*P* Value*	r	*P* Value*	r	*P* Value*	r	*P* Value*	r	*P* Value*
IL-1α	0.005	0.984	0.314	0.166	–0.101	0.664	0.131	0.571	0.084	0.718
IL-6	0.630	**0.002**	0.369	0.100	0.566	**0.007**	–0.550	**0.010**	–0.562	**0.008**
IL-8	0.768	**<0.0001**	0.348	0.122	0.381	0.089	–0.677	**0.001**	–0.553	**0.009**
TNF-α	–0.061	0.793	0.023	0.922	0.256	0.263	–0.194	0.400	–0.086	0.712
CCL-2	0.560	**0.008**	0.309	0.173	0.291	0.201	–0.558	**0.009**	–0.693	**<0.0001**
MCP-1	0.404	0.069	0.368	0.101	0.262	0.251	–0.081	0.729	–0.328	0.147
GROα	0.455	**0.038**	0.255	0.264	0.408	0.066	–0.407	0.067	–0.467	**0.033**
MIP-1α	0.542	**0.011**	0.118	0.612	0.220	0.338	–0.368	0.101	–0.485	**0.026**
VEGF-α	0.171	0.457	0.109	0.640	0.038	0.871	0.012	0.960	–0.336	0.137
IGFBP5	0.733	**<0.0001**	0.361	0.107	0.484	**0.026**	–0.783	**<0.0001**	–0.639	**0.002**
IGFBP7	0.597	**0.004**	0.246	0.282	0.449	**0.041**	–0.609	**0.003**	–0.466	**0.033**
TGF-β1	0.653	**0.001**	0.481	**0.027**	0.525	**0.015**	–0.576	**0.006**	–0.613	**0.003**
MMP-10	–0.303	0.182	–0.277	0.224	–0.146	0.527	0.056	0.809	0.205	0.373

* Significance of differences between groups: Spearman's rank-order correlation analysis. Bold values represent significance.

senescence-associated markers p16 and p21 were enhanced in the ARC group, along with the decrease of SIRT1 staining intensity (Figs. 3A, 4A). The proportion of DNA lesions, apoptotic cells, and p53-positive cells was significantly increased in the APAC group (Figs. 3B–G, 3J; $P < 0.05$), whereas an increase in the number of p16-, p21- and SA-β-gal–positive cells and a decrease in the number of SIRT1-postive cells were observed in the anterior lens capsule of ARC group over the APAC group (Figs. 3H, 3I, 3K, 4B; $P < 0.0001$).

Discussion

Sudden and dramatic elevation of IOP in APAC can cause pathophysiological changes and functional damage to the anterior segment in the eye. Iris atrophy, mid-dilated pupil, and "glaukomflecken" are strong evidence of the onset of APAC.[2–4] Oxidative stress and cellular senescence have been found in the retina after acute IOP elevation in an animal model of glaucoma.[7,13] However, previous studies on IOP and oxidative stress[2–4,7,13] were limited to experimental animal models, and the pathological changes of the anterior segment after acute ocular hypertension were not observed. In the current study, we found an increase in oxidative stress and premature senescence markers in the anterior segment of APAC eyes. Additionally, the levels of oxidative stress markers were positively correlated with preoperative peak IOP, age and levels of SASP-related cytokines. To the best of our knowledge, this is the first study that analyzed the levels of oxidative stress markers in the anterior segment of APAC

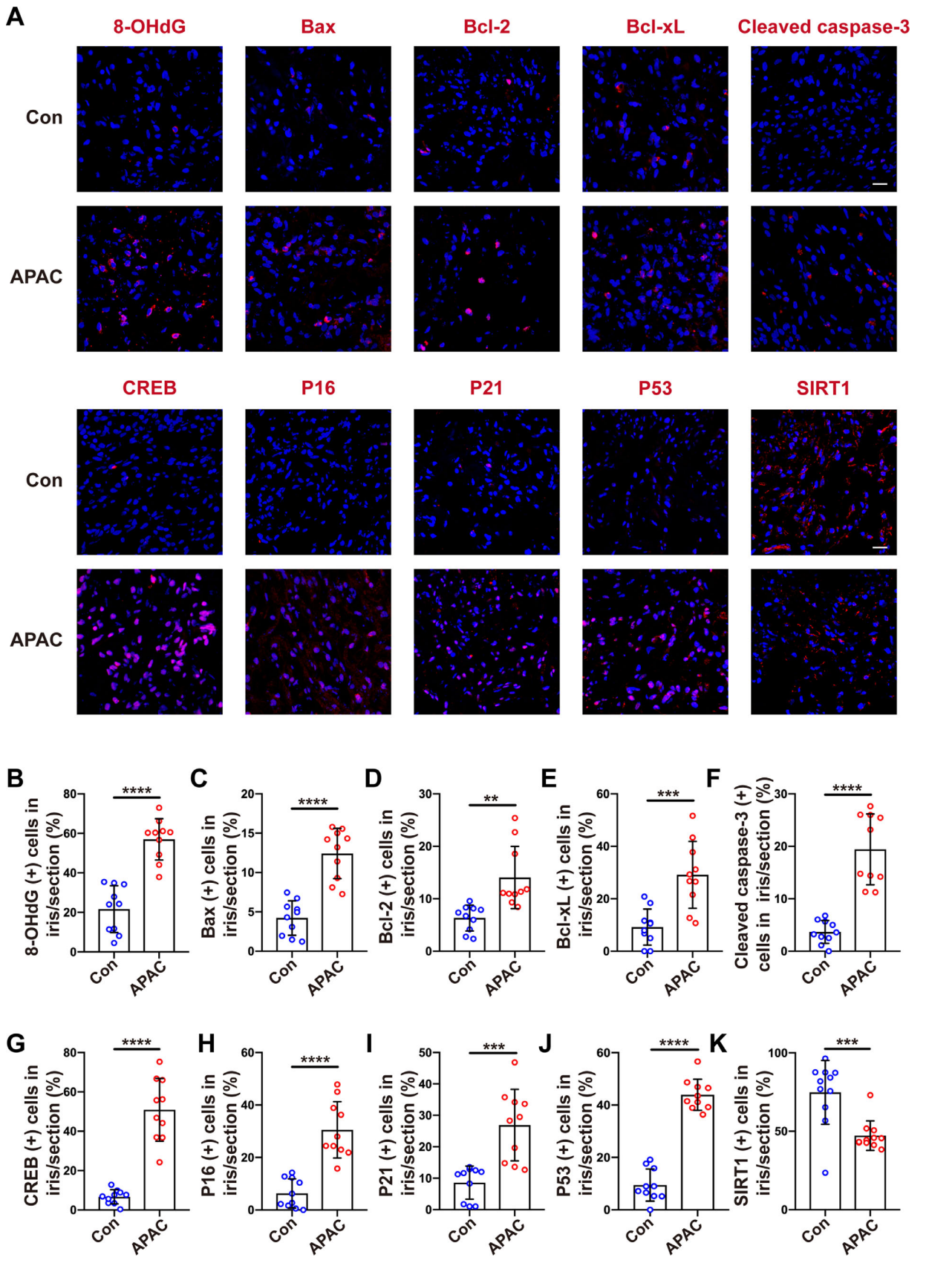

Figure 2. MtDNA oxidative damage, apoptosis, and senescence in iris of APAC eyes and human donor eyes (control). (**A**) Representative immunofluorescent images of iris frozen sections from the APAC group and the control group. The DAPI (*blue*) was used to tag the nucleus. *Scale bar*: 10 µm. (**B–K**) Quantitative analysis of 8-OHdG-, Bax-, Bcl-2-, Bcl-xL-, cleaved caspase-3-, CREB-, p16-, p21-, p53- and SIRT1-positive cells in the iris (n = 10, nine images/sample). Two-samples independent *t*-test was performed. $P < 0.05$ was defined as significance. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ versus the control group.

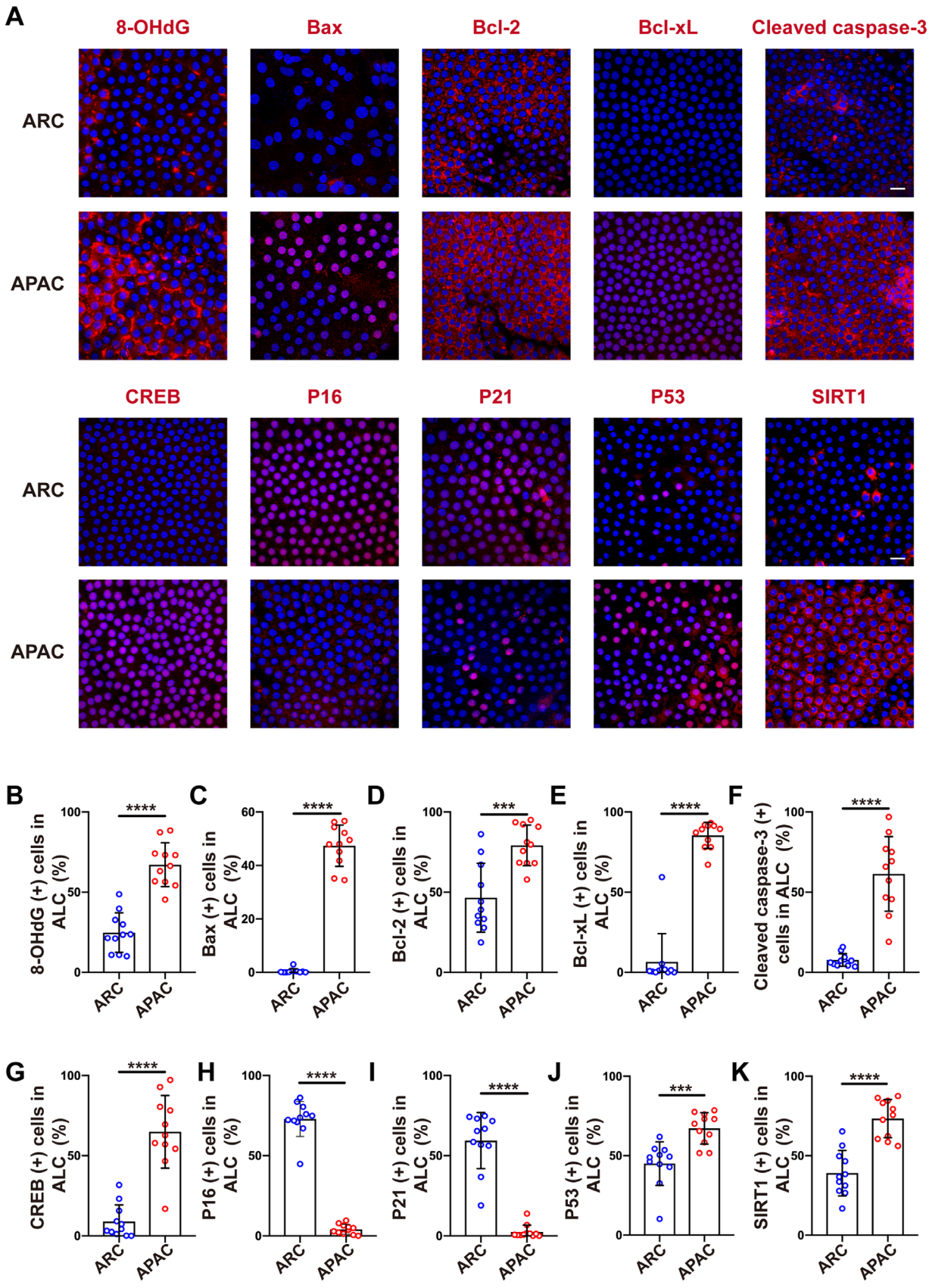

Figure 3. MtDNA oxidative damage, apoptosis, and senescence in ALC of APAC eyes and ARC eyes. (**A**) Representative immunofluorescent images of ALC flat mounts from the APAC group and the ARC group. The DAPI (*blue*) was used to tag the nucleus. *Scale bar*: 10 µm. (**B–K**) Quantitative analysis of 8-OHdG-, Bax-, Bcl-2-, Bcl-xL-, cleaved caspase-3-, CREB-, p16-, p21-, p53- and SIRT1-positive cells in the ALC (n = 11, three images/sample). Two-samples independent *t*-test was performed. $P < 0.05$ was defined as significance. ***$P < 0.001$, ****$P < 0.0001$ versus the ARC group.

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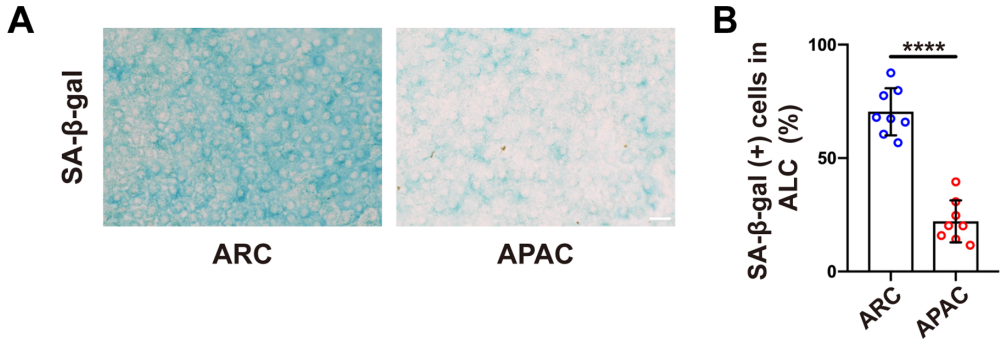

Figure 4. SA-β-gal activity in ALC of APAC eyes and ARC eyes. (**A**) Representative images of ALC flat mounts from the APAC group and the ARC group. *Scale bar*: 10 µm. (**B**) Quantitative analysis of SA-β-gal–positive cells in the ALC (n = 8, three images/sample). Two-samples independent *t*-test was performed. $P < 0.05$ was defined as significance. ****$P < 0.0001$ versus the ARC group.

patients and their relationships with preoperative IOP, age, and SASP-related cytokines. This study provides a theoretical basis for further understanding the pathological changes of the anterior segment induced by acute ocular hypertension and provides new insights for better treatment of APAC patients.

Acute IOP elevation resulting from iridotrabecular contact is a key characteristic of APAC.[1] High IOP leads to the collapse of blood–AqH barrier, and can promote the influx of cytokines generated form anterior segment tissues into the AqH.[30,31] The present study confirmed that the levels of oxidative stress markers, such as ROS, 8-OHdG, and MDA, were considerably increased in the AqH of APAC patients compared to that of the ARC patients. Antioxidant indices were simultaneously and significantly upregulated in the AqH of APAC patients over the ARC group. This may be due to the response of antioxidants to prevent the detrimental effects caused by the excessive release of oxidants.[32–34] Additionally, the number of DNA-lesioned and apoptotic cells was markedly increased in the iris and anterior lens capsule of APAC patients. This indicates that sudden angle closure leads to acute elevation of IOP, and the high pressure compresses the anterior segment tissues intensively, leading to an increase in ROS production from the anterior segment tissues (Fig. 5A). Therefore, oxidative stress may be involved in the pathophysiology of anterior segment changes in APAC.

A correlation analysis between the levels of oxidants and IOP in APAC patients found a clear positive association. This suggested that higher IOP could lead to more severe oxidative stress, which may lead to anterior segment injury in APAC.

Age is an important nonmodifiable risk factor for APAC.[1] Oxidative stress is one of the major causes of age-related diseases.[35] Relationship analysis revealed that age increased progressively with increasing oxidants and decreasing antioxidants in the AqH of APAC patients. This indicates that the oxidative stress in the anterior segment is closely related to age, and the older patients would suffer more severely from APAC. This can be caused by the decline of antioxidant capacity caused by age.[36]

Oxidative stress and ROS trigger telomere shortening and dysfunction, thereby causing cellular senescence, this process called SIPS.[37,38] During senescence, the senescent cells release excessive amounts of secretory proteins into the surrounding extracellular fluid, termed as SASP.[39] SASP-related cytokines are of various types, including inflammatory cytokines, chemokines, growth factors, and MMPs[40] and are important in distinguishing senescent cells from nonsenescent, cell cycle-arrested cells.[39] In this study, we observed that the levels of most of the SASP-related cytokines were substantially increased in the AqH of the APAC group compared with that of the ARC group. Notably, the APAC patients were younger than the ARC patients. Thus premature senescence may have occurred in the anterior segment of the APAC patients. Several studies have demonstrated that many SASP factors have the potential to cause inflammation.[40,41] Meanwhile, transient presence of SASP, especially MMPs and growth factors,[42,43] secreted by senescent cells has also been shown to play a beneficial role in coordinating tissue remodeling and regeneration during acute injury.[44,45] SASP components IL-6, IL-8, and MCP-1 can alert nearby cells and recruit immune cells to promote tissue repair.[46,47] In our study, IL-6, IL-8, MCP-1, MMP-10, and VEGF-α levels were all dramatically increased in the AqH of APAC group, similarly to previous studies.[48,49] Therefore the senescent cells in APAC may be part of a regenerative response to acute injury and play a role in regenerating the tissues.

It was shown that oxidants were positively associated, whereas antioxidants were negatively associated with SASP, indicating that the status of oxidative stress in the anterior segment of APAC patients might result in SIPS, which may be related to the pathophysiological changes in the anterior segment of APAC patients.

Iris atrophy and anterior subcapsular lens opacities ("glaukomflecken") are evident and common signs after high IOP during acute episodes.[1] The number of cells positive for the mtDNA oxidative damage marker 8-OHdG, apoptosis-related markers and senescence-associated markers was considerably increased in APAC irises, indicating that oxidative stress and premature senescence occurred in these irises after acute IOP elevation. SIRT1 is depleted when senescence occurs, upregulating the expression of SASP components like IL-6 and IL-8, through enhancing histone acetylation in the promoter region.[50] We found that the number of SIRT1-postive cells was decreased in the iris of APAC eyes, similar to the results in a previous study where the expression of SIRT1 decreased significantly in the trabecular mesh of patients with high IOP glaucoma.[51] IL-6 and IL-8 levels were also elevated in the AqH of APAC eyes. Therefore oxidative stress-induced premature senescence may be involved in the damage and repair of iris atrophy (Fig. 5B). The number of 8-OHdG-positive and apoptotic cells was significantly increased in the APAC patients over the ARC group. Among all senescence-associated markers, only p53-positive cells were more abundant in the anterior

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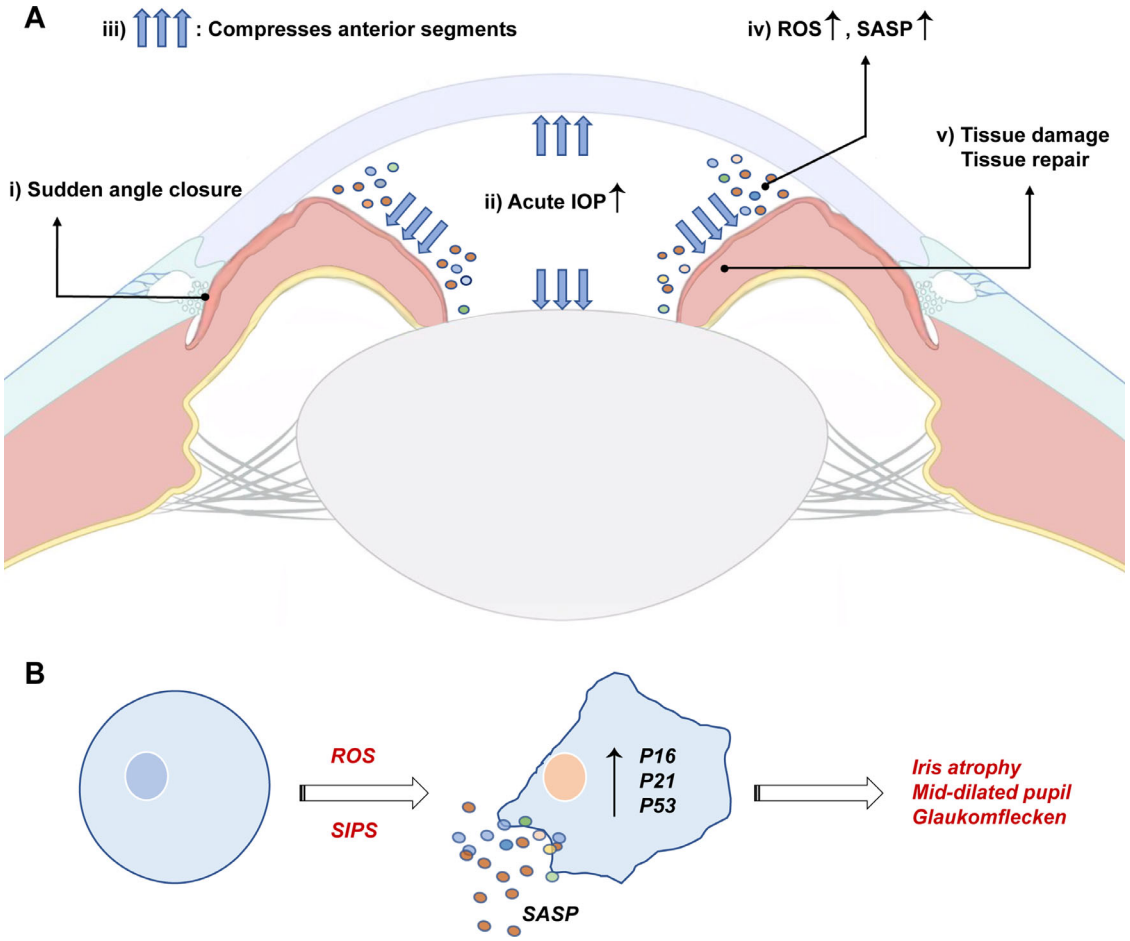

Figure 5. Schematic illustration about oxidative stress and premature senescence occurring in anterior segment of acute primary angle-closure eyes. (**A**) Hypothesis diagram of ROS and SASP producing and entering to the aqueous humor of APAC eyes, in the order of (i), (ii), (iii), (iv), (v). (**B**) Schematic diagram of possible molecular mechanism leading to anterior segment pathological changes of APAC eyes.

lens capsule of the APAC group than in the ARC group, possibly because of the activation by ROS, leading to oxidative stress and apoptosis. Conversely, the remaining results showed that the number of senescent cells increased in the ARC group over the APAC group. Specifically, an increase in the number of p16-, p21- and SA-β-gal-positive cells and a decrease in the number of SIRT1-postive cells were observed in the anterior lens capsule of ARC group over the APAC group, possibly because of the higher average age in the ARC group. Besides, p16 and p21 could promote senescence of lens epithelial cells and eventually form cataracts.[52,53] Therefore oxidative stress markers and SASP-related cytokines in the AqH may be mostly from the iris. Furthermore, the "glaukomflecken" of APAC may be due to the oxidative stress of the anterior subcapsular lens caused by acute IOP attack and the effect of the oxidative stress markers and SASP-related cytokines in the AqH on lens.

Notwithstanding the significance of the present study, there are several limitations. The sample size of the study was relatively small, although the results are statistically significant and immunofluorescent staining of the iris and anterior lens capsule samples corroborates the observations. The iris immunofluorescent staining may be affected by postmortem changes in the control donor tissue, although the death-to-fixation interval was optimum (two to three hours). Thus these results may be sufficient to validate the conclusions of the study. A further study would be conducted in acute ocular hypertension animal models to explore the exact mechanism of pathological changes in the anterior segment of APAC patients.

In conclusion, we propose that oxidative stress and premature senescence are involved in the development of pathological changes in the anterior segment of APAC eyes. Higher preoperative peak IOP and older age cause a more intense oxidative stress resulting in higher levels of SASP, leading to the injury and repair of anterior segment tissues. These results suggest that older APAC patients, or patients suffering a higher IOP, must be treated more proactively and in a timely manner. This study also provides a theoretical basis for further research on drug development for APAC patients.

Acknowledgments

The authors thank the Guangdong Eye Bank (Guangzhou, China) for providing the matched control human donor eyes in this study.

Supported by the Natural Science Foundation of Guangdong Province in China (2021A1515012142), the Young Scientists Fund of the National Natural Science Foundation of China (81900864) and the Research Grant from Guangzhou Municipal Science and Technology Bureau in China (202102010324).

Disclosure: **D. Ye**, None; **Y. Xu**, None; **Y. Shi**, None; **J. Ji**, None; **X. Lu**, None; **H. Chen**, None; **R. Huang**, None; **P. Lu**, None; **Y. Li**, None; **L. Cheng**, None; **Y. Li**, None; **K. Cui**, None; **X. Tang**, None; **L. Luo**, None; **J. Huang**, None

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