



# An Overview of Corneal Topography and Visual Outcomes after Different Pterygium Surgeries: An Interventional Case Series Study

## Farklı Pterijum Cerrahileri Sonrası Kornea Topografisi ve Görsel Sonuçlara Genel Bakış: Girişimsel Vaka Serisi Çalışması

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### Abstract

**Introduction:** To investigate corneal topography and visual outcomes after different pterygium surgical techniques using Pentacam Scheimpflug device

**Materials and Methods:** Ninety-eight unilateral primary nasal pterygium patients underwent surgery under topical anesthesia with either conjunctival autograft (group 1) or anchored conjunctival rotational flap (group 2), both using fibrin tissue adhesive. Baseline and three-month post-operative best-corrected visual acuity (BCVA) and anterior corneal astigmatism (ACA), flattest keratometry (Kf), steepest keratometry (Ks) and posterior corneal astigmatism (PCA) were investigated.

**Results:** In groups 1 and 2, the mean logMAR BCVA increased from  $0.119 \pm 0.113$  to  $0.082 \pm 0.086$  and  $0.169 \pm 0.128$  to  $0.120 \pm 0.121$ , respectively. There were no significant differences between the two groups in pre- and post-operative ACA, PCA, Kf and Ks. Intra-group analysis revealed significant differences in both groups: ACA, with a greater difference in group 1 (2.072), and Kf, with a slightly greater difference in group 2 (1.910). Intra-group analysis revealed no statistically significant PCA changes for either group.

**Conclusions:** Significantly improved anterior corneal topographic changes were found to be highly related to conjunctival autografting. Conjunctival rotational flap, on the other hand, was associated with significantly greater posterior corneal topographic changes.

**Keywords:** Conjunctival autograft; conjunctival rotational flap; corneal topography; pentacam scheimpflug imaging; pterygium

### Özet

**Amaç:** Pentacam Scheimpflug cihazı kullanılarak farklı pterijum cerrahi teknikleri sonrasında korneal topografi ve görsel sonuçların araştırılması.

**Gereç ve Yöntem:** Doksan sekiz tek taraflı, primer, nazal pterijum hastası, konjonktival otogreft (grup 1) veya sabitlenmiş konjonktival rotasyonel flep (grup 2) ile, her ikisi de fibrin doku yapıştırıcısı kullanılarak topikal anestezi altında ameliyat edildi. Başlangıç ve cerrahi sonrası üçüncü ayda en iyi düzeltilmiş görme keskinliği (EİDGK) ve anterior korneal astigmatizma (AKA), en düz (Kf) ve en dik keratometri (Ks) ve posterior korneal astigmatizma (PKA) araştırıldı.

**Bulgular:** Grup 1 ve 2'de ortalama logMAR EİDGK sırasıyla  $0.119 \pm 0.113$ 'ten  $0.082 \pm 0.086$ 'ya ve  $0.169 \pm 0.128$ 'den  $0.120 \pm 0.121$ 'e yükseldi. Cerrahi öncesi ve sonrası AKA, PKA, Kf ve Ks açısından iki grup arasında anlamlı fark yoktu. Grup içi analiz, her iki grupta da önemli farklılıklar ortaya çıkardı; AKA grup 1'de (2.072) daha fazla farklılık gösterirken, Kf'de grup 2'de (1.910) nispeten daha fazla değişiklik gösterdi. Grup içi analizde, PKA değişimi her iki grupta da istatistiksel olarak anlamlı bulunmadı.

**Sonuçlar:** Anterior korneal topografik değişikliklerdeki belirgin iyileşmenin, konjonktival otogreft ile yüksek oranda ilişkili olduğu gözlemlendi. Posterior korneal topografik değişiklikler ise daha çok konjonktival rotasyonel flep ile ilişkilendirildi.

**Anahtar Kelimeler:** Pterijum; konjonktival; otogreft; konjonktival potasyonel flep; korneal topografi; pentacam scheimpflug görüntüleme.

### Introduction

Pterygium is characterized by an encroachment of a wing-shaped fibrovascular bulbar conjunctival connective tissue onto the cornea. It is commonly accompanied by typical dysplasia of conjunctival epithelium and elastoid degeneration of collagen tissue (1). Several hypotheses regarding the mechanism of pterygium-induced corneal changes include pterygium pressure on the cornea, corneal

flattening from tear accumulation around pterygium, and ultrastructural corneal distortion (2). Pterygium surgery may be required prior to any planned refractive surgery because of its impacts on many corneal topographical parameters. Regardless, accurate prediction of refractive changes is occasionally needed. The anterior and posterior corneal surfaces must be

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considered topographically, in order to comprehensively evaluate both corneal astigmatism and other topographical parameters after pterygium surgery. Very few studies, however, have investigated the effect of pterygium surgery on posterior corneal astigmatism (3), including comparison of surgical results based on the type of pterygium excisional surgery. Furthermore, no comparison of commonly performed pterygium surgical techniques in terms of post-operative corneal topographical changes has ever been made. To assess influencing factors for postoperative refractive outcomes, a precise analysis of surgically induced topographical changes following a specific excision technique is required. Pentacam employs a rotating Scheimpflug imaging system and has been used for corneal assessment in a variety of settings. It offers precise measurements of curvature and hence astigmatism on the anterior and posterior corneal surfaces (4). There have been no studies using this device to investigate the effects of various pterygium surgical excision techniques on subsequent visual acuity and corneal topographic parameters, particularly posterior corneal astigmatism. We therefore aimed to investigate changes in corneal topographical parameters and subsequent visual acuity after pterygium surgery with conjunctival autograft and anchored conjunctival rotational flap techniques using the Pentacam Scheimpflug imaging system. A data comparison between the two surgical techniques was also performed.

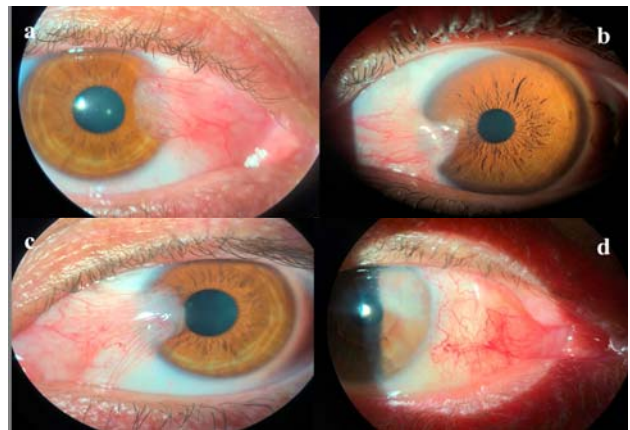
## Material and Method

**Study design:** Ninety-eight patients with unilateral primary nasal pterygium were included in this interventional case series study. All patients underwent pterygium surgery with either a conjunctival autograft (group 1) or an anchored conjunctival rotational flap (group 2), both of which used fibrin tissue adhesive, at the University Faculty of Medicine, Department of Ophthalmology.

**Participant selection:** Patients with unilateral primary pterygium with a corneal extension of  $\leq 4.5$  mm were included in the study. However, those with corneal topography-influencing features, such as prior ocular trauma or intraocular surgery and corneal scarring, contact lens wear, corneal ectasia (keratoconus), bilateral pterygium (ipsilateral temporal and nasal involvement), recurrent pterygium and pseudo-ptyerygium, media opacities such as dense cataract and corneal

opacities or irregularity due to diseases other than pterygium, connective tissue disorders, systemic vasculitis, glaucoma, diabetes mellitus, as well as severe ocular surface disorders such as dry eye and Stevens-Johnson syndrome, were not included in the study. Also, patients with central corneal-involving severe pterygium which could hinder corneal topography reproducibility were not included in the study.

**Preoperative assessment:** Baseline in-depth ophthalmological assessment was performed, including measurements of auto-refraction (Canon R-F10m; Canon Inc., Tokyo, Japan) and best-corrected visual acuity (BCVA) in logarithm of the Minimum Angle of Resolution (logMAR), a slit-lamp biomicroscopy (Haag-Streit BI 900; The USA) of the anterior and posterior segments before and after full pupil dilation. Subjective grading of pterygium by the slit-lamp was performed by a single clinician (HHG).



**Figure 1:** Pterygium grades for which excisional surgery was performed and postoperative view. **(a) Grade-1 pterygium:** This lesion appears to affect the limbus. Papillary reaction is negligible, and there are smooth conjunctival and corneal tissues. **(b) Grade-2 pterygium:** Aside from affecting the limbus, there is moderate vascularity, with only a slight elevation of the conjunctival and corneal tissues. **(c) Grade-3 pterygium:** Wing-shaped fibrovascular tissue encompasses an area between the limbus and pupillary margin, characterized by obvious conjunctival and corneal tissue elevation as well as irregularity. It is possible to see significant vascularity with vessel congestion. In this grade, the visual axis is not affected. **(d)** Appearance of the graft three month after surgery. There is minimal focal corneal opacity on the nasal corneal quadrant, indicating significant pre-operative fibrovascular tissue formation of pterygium.

**Table 1:** Analysis of pterygium surgical techniques in relation to pterygium grade

		Pterygium grade			Total	
		1	2	3		
Surgical technique	Group 1	Number of patients	6	25	15	46
		% within surgical technique	13.0	54.3	32.6	100.0
		% within pterygium grade	37.5	49.0	48.4	46.9
	Group 2	Number of patients	10	26	16	52
		% within surgical technique	19.2	50.0	30.8	100.0
		% within pterygium grade	62.5	51.0	51.6	53.1
Total		Number of patients	16	51	31	98

**Group 1:** Conjunctival autograft technique with fibrin tissue adhesive; **Group 2:** Anchored conjunctival rotational flap technique with fibrin tissue adhesive

Pterygium was initially graded in four stages, predicated on equivalent investigations (5) depending on the existence of fibrovascular tissue, from the nasal limbus to visual axis as follows: grade-1 (<2.0 mm), grade-2 (>2.0 mm and <4.0 mm), grade-3 (>4.00 mm, excluding visual axis) and grade-4 (including visual axis) (Figure 1). Furthermore, the same clinician (HHG) performed baseline and three-month post-operative corneal topography using the Pentacam Scheimpflug imaging system.

**Surgical intervention:** All pterygium surgical procedures were performed by two surgeons (SCI: Conjunctival autografting; and YCY: Anchored conjunctival rotational flap). After application of a 0.5 % proparacaine hydrochloride (Alcaine, Alcon Laboratories, Inc., Forth Worth, TX, USA) for topical anesthesia, a speculum was placed in the eye. An area surrounding the conjunctival component of pterygium was defined with a Gentian violet followed by an injection of 0.5 mL of local anesthetic lidocaine HCL 20 mg/mL + epinephrine 0.0125 mg/mL (Jetokain, Adeka, Turkey) with a 25-gauge needle under the pterygium body. Pterygium was then excised at its base by a pair of Westcott's scissors, and the pterygium apex was scraped off the corneal surface. Any remaining abnormal subconjunctival fibrous tissue was excised completely with a crescent blade. No cauterization was performed.

Either superior temporal conjunctival autograft or anchored conjunctival rotational flap was applied to cover the bare scleral bed (receptor zone).

*Conjunctival Autograft Technique:* In this technique, after measuring the receptor zone with a caliper, ipsilateral superotemporal conjunctival region was marked with Gentian violet approximately >1 mm than the measured receptor zone and a free autograft was dissected as thinly as possible from the Tenon's capsule after subconjunctival injection of 0.2 ml of lidocaine HCl 20 mg/ml and epinephrine HCl 0.0125 mg/ml combination (Jetokain, Adeka, Türkiye). Limbal side of the autograft was extended to 1 mm of the limbal area to incorporate limbal stem cells and the dissected autograft was moved to the receptor zone.

*Anchored Conjunctival Rotational Flap Technique:* In this technique, superior nasal conjunctiva with the pedicle adjacent to the pterygium excision area was marked as >1 mm than the scleral area adjacent to the receptor zone. Subconjunctival administration of 0.2 ml of 20 mg/ml lidocaine HCl and 0.0125 mg/ml epinephrine HCl combination (Jetokain, Adeka, Türkiye) was performed. Blunt dissection of the conjunctival tissue from fornix to 1 mm from limbus and the underlying Tenon was performed using Westcott scissors. The flap was completed by cutting limbal area and rotating at an angle of 90° around the scleral site, maintaining inferior limbal anchoring

**Table 2:** Mean values of corneal topographical parameters for the total and respective study group.

Parameters		Total (N=98)	Group 1 (N=46)	Group 2 (N=52)
		Mean±Standard deviation (Min-Max)		
Anterior corneal astigmatism	Pre-operative	3.65±2.52 (0.30-10.80)	3.71±2.63 (0.60-10.80)	3.60±2.43 (0.30-9.40)
	Post-operative	1.78±1.37 (0.10-5.90)	1.64±1.32 (0.10-5.90)	1.90±1.41 (0.20-5.40)
	p-value*		<0.001	<0.001
Kf	Pre-operative	41.33±1.78 (38.00-45.10)	41.22±1.64 (38.20-45.10)	41.42±1.91 (38.00-44.60)
	Post-operative	43.11±1.17 (40.00-45.60)	42.85±1.14 (40.40-45.20)	43.33±1.17 (40.00-45.60)
	p-value*		<0.001	<0.001
Ks	Pre-operative	44.97±1.80 (41.00-49.20)	44.92±1.97 (41.50-49.10)	45.02±1.65 (41.00-49.20)
	Post-operative	44.97±1.75 (41.10-49.10)	44.87±1.94 (41.40-49.10)	45.06±1.57 (41.10-49.00)
	p-value*		0.091	0.092
Posterior corneal astigmatism	Pre-operative	0.41±0.32 (0.10-1.90)	0.40±0.32 (0.10-1.40)	0.41±0.33 (0.10-1.90)
	Post-operative	0.38±0.27 (0.10-1.70)	0.38±0.27 (0.10-1.30)	0.38±0.28 (0.10-1.70)
	p-value*		0.061	0.061

**Group 1:** Patients undergoing conjunctival autograft technique; **Group 2:** Patients undergoing anchored conjunctival flap technique; **Kf:** Flat keratometry; **Ks:** Steep keratometry; **N:** Number of patients; **\***: The Mann-Whitney U test

point (1 mm pedicle). At this stage, the inferior edge of the flap faces the limbus, whereas the superior edge faces the fornix. Conjunctival tissue was excised 1 mm within the limbus to prevent corneal wound healing by conjunctival epithelium. *Fibrin Tissue Adhesive Application:* After everting the autograft and anchored flap, fibrin tissue adhesive was applied to one of the two respective surfaces. The second component of the fibrin tissue adhesive was applied over the receptor zone after the receptor zone had been dried with Weck-cel. The autograft and anchored flap were delicately moved and positioned over the receptor zone by "bed sheet maneuver." Attention was paid to both sides of the conjunctiva to be properly positioned and to adequately cover the receptor zone. Complete bonding of both tissues was allowed for one minute. The patient was then instructed to

blink several times to verify the graft adherence. All eyes were wrapped in bandages with an antibiotic ointment after surgery. Post-operative treatment, including Zylet (5 mg loteprednol etabonate (0.5%)+3 mg tobramycin (0.3%)) (Bausch & Lomb Inc. Florida, USA) eye drops (4x1), and artificial tear lubricant (Refresh Tears) (8x1) was prescribed for 4 weeks. Routine post-operative follow-up examination was performed on days 1, 7, 30 and 90. Fibrovascular tissue encroachment from the surgical site ≥1 mm onto the cornea was acknowledged as recurrence. Moreover, autograft and/or flap edema, defined as edema greater than one central corneal thickness, and granuloma formation, were assessed by the same surgeon (HHG) using slit-lamp biomicroscopy throughout the study period. The baseline and three-month post-operative data of

BCVA and corneal topographical parameters, including anterior corneal astigmatism, flat keratometry (Kf), steep keratometry (Ks), and posterior corneal astigmatism were collected for statistical analysis.

**Ethical approval:** The study procedure complied with the ethical standards set out in the Helsinki Declaration and received full approval from the Ethics Committee's Institutional Review Boards. (Number: 57212153-000-4577) All participants provided written informed consent prior to the study.

**Statistical analysis:** Categorical data were described using observed frequencies and percentages, and continuous variables were summarized by their means and standard deviations (or medians and interquartile ranges in case of serious deviations from normality) with statistical package (SPSS Inc., version 25.0, Chicago, IL, USA). The assumption of normality in quantitative variables was visually examined with the individual probability charts in the groups and time points to be compared and statistical analysis was performed using the Shapiro Wilk test. The comparison of changes in quantitative variables with time between groups was therefore made using the non-parametric Brunner-Langer model (F1-LD-F1 design), R 3.5.2 software (R software, version 3.5.2, package: nparLD, R Foundation for Statistical Computing, Vienna, Austria; <http://r-project.org>). As a result of the Brunner-Langer model, when the time-dependent change in the groups was not similar (interaction <0.1), the time comparison in each group was performed separately by Brunner Langer (LD-F1 design). The Mann-Whitney U test was used to compare the baseline and the difference between three month and the baseline values for each group.  $P < 0.05$  were considered to be statistically significant.

## Results

All 98 pterygium patients were  $46.94 \pm 10.34$  years old on average. While group 1's mean age was  $47.78 \pm 8.48$  years, group 2's mean age was  $46.19 \pm 11.77$  years ( $p = 0.441$ ). The male-to-female ratios were 24:22 and 35:17 in group 1 and 2, respectively. The overall mean logMAR BCVA increased from  $0.145 \pm 0.123$  to  $0.102 \pm 0.107$ , with statistically significant improvements from  $0.119 \pm 0.113$  to  $0.082 \pm 0.086$  and  $0.169 \pm 0.128$  to  $0.120 \pm 0.121$  in group 1 and 2 ( $p < 0.001$  for both groups). Both surgical techniques had no intra- or post-operative complications.

**Pterygium grade and surgical technique:** There were 16 patients with grade 1, 51 with grade 2, and 31 with grade 3. Analysis of pterygium surgical techniques in relation to pterygium grade is demonstrated in Table 1. Analysis of pterygium surgical techniques in relation to pterygium grade is demonstrated in Table 1.

## Analysis of corneal topographic parameters:

- *Anterior Corneal Astigmatism:* There was no statistically significant difference in pre- and post-operative anterior corneal astigmatism between group 1 and 2 ( $p = 0.686$ ). However, there was a statistically significant intra-group difference in both groups ( $p < 0.001$  for both groups), with a greater anterior corneal astigmatism difference in group 1 (2.072) than group 2 (1.696).
- *Flat K (Kf):* There was no statistically significant difference between groups 1 and 2 in pre- and post-operative Kf ( $p = 0.107$ ). Intra-group difference, on the other hand, was statistically significant in both groups ( $p < 0.001$  for both groups), with group 2 (1.910) displaying a slightly higher change than group 1 (1.630).
- *Steep K (Ks):* There was no statistically significant difference in pre- and post-operative Ks between group 1 and 2 ( $p = 0.592$ ). However, group 1 (0.052) was associated with far more Ks reduction relative to group 2 (-0.044).
- *Posterior Corneal Astigmatism:* No statistically significant difference in pre- and post-operative posterior astigmatism between groups 1 and 2 was identified ( $p = 0.902$ ), and so did intra-group differences in both groups ( $p = 0.061$  for both groups). However, group 1 (-0.020) was associated with slightly less reduction in posterior corneal astigmatism relative to group 2 (-0.030).

## Discussion

Pterygium is common in dry climates (6), with the majority of cases occurring in people between the ages of 20 and 40, based on location, altitude, and ethnicity (7). This condition causes optical irregularities and distortions other than intrusion into the visual axis, as well as corneal topographic changes (1). Patients frequently consult physicians for discomfort, ocular surface inflammation, red eyes, and/or vision impairment. Indications for pterygium surgery include visual impairment due to significant alterations in the refractive state and

corneal curvature prior to entry into the optical zone, ocular motility restriction and diplopia, symblepharon, chronic inflammation and cosmetic effects. Despite the fact that pterygium is known to affect corneal topography, changes in corneal topographic parameters are reversible post-operatively (9,10). The refractive status of the cornea has recently been documented as well, with varying outcomes following pterygium surgery (11,12). Ozdemir et al. (13) reported early significant improvement in pterygium-induced corneal topographical parameters. Normal corneal topography patterns, on the other hand, have been established in the late post-operative period. Moreover, different pterygium surgeries have been associated with post-operative steeper cornea and high mean refractive power (12). It is however uncertain to what extent a particular pterygium surgical technique may have an impact on both anterior and posterior corneal topographic parameters. Significant reductions in pterygium-induced astigmatism have also been reported after various surgical techniques (14). Conjunctival autografting first described by Kenyon et al.(15) in 1985 is considered to be the most effective pterygium treatment procedure (16). Still, compared to this technique, conjunctival rotational flap technique (17,18) has some clinical benefits. The conjunctiva and Tenon's capsule orientation is readily identifiable in this technique. There is also no need for a suture on the pedicle, hence reducing surgery time. Besides, there is a lower incidence of flap edema compared to that of conjunctival autograft, which may be attributed to retention of the vascular network in the limbus around the pedicle. Flap technique demonstrated comparable post-operative changes in corneal topographical parameters and BCVA improvement to conjunctival autograft technique in the current study. Conjunctival rotational flap differs from conjunctival autograft technique due to flap orientation. The comparable post-operative corneal topographic modifications between these two different techniques suggest that flap orientation is not associated with undesirable corneal topographical impacts as long as it is not "inverted." Nonetheless, guaranteeing the potential effect of limbal barrier in the condition of limbal-conjunctival autograft makes flap orientation crucial. The current study investigated and compared post-operative corneal topographic parameters between baseline and post-operative three-month data. Similar to Ozdemir et al. (13) there were early significant changes in pterygium-induced corneal topographical parameters. The

mean age ( $46.94 \pm 10.34$  years) of pterygium patients, most of whom with grade-2 pterygium (51 %), was consistent with prior reports. As regards pterygium surgical techniques, both were comparably associated with significantly improved post-operative BCVA. Despite usually having normal pre-operative BCVA, pterygium patients frequently have subjective visual complaints that improve post-operatively and correlate with corneal topographic parameters. In one study, post-operative assessment of posterior corneal changes using Pentacam Scheimpflug tomography showed no significant posterior corneal changes after different pterygium surgical techniques with sutures, i.e. bare sclera technique (36 eyes), amniotic membrane transplantation (34 eyes), or free conjunctival autograft (26 eyes), using 0.02 % mitomycin C (3). However, there was significantly changed posterior astigmatism orientation from against-the-rule to with-the-rule astigmatism, suggesting a strong influence on the axis of astigmatism. Further, significantly reduced corneal astigmatism in the anterior and posterior corneal surfaces has been documented in another study in which 152 eyes underwent conjunctival autograft with 10-0 absorbable polyglycolic sutures and 11 eyes with amniotic membrane transplantation (19). The same study also found significant corneal steepening, but no significant post-operative change in the posterior corneal surface. Correspondingly, post-operative improvement in corneal astigmatism and increase in corneal steepening have also been reported by Yilmaz et al.(11), and by Razmjoo et al.(20). The current study found similar post-operative changes in corneal topographic parameters, which were accompanied by significant changes, particularly on the anterior corneal surface. There were no significant differences in pre- and post-operative anterior corneal astigmatism or Kf values between conjunctival autograft and anchored conjunctival rotational flap techniques. However, intra-group analysis of these parameters revealed statistically significant differences in both groups, with conjunctival autograft technique being associated with a greater change in anterior corneal astigmatism and slightly less change in Kf than anchored conjunctival rotational flap technique. Despite the obvious differences in Ks and posterior corneal astigmatism between the two techniques, the pre- and post-operative values did not differ significantly. This was also the same for intra-group differences. However, conjunctival autograft technique was associated with a much higher reduction in Ks and slightly lower

reduction in posterior corneal astigmatism than anchored conjunctival rotational flap technique. This suggests that the conjunctival autograft technique is especially effective for correcting pterygium-induced anterior corneal distortions. Conversely, pterygium-induced posterior corneal distortions are likely to improve significantly with the conjunctival rotational flap technique. Horizontal corneal flattening seems to be the most frequent topographical corneal shape associated with pterygium (21). However, this change often tends to decrease after pterygium surgery, as has also been demonstrated in the current study in which significant post-operative changes in Kf and Ks were found. Corneal astigmatism in eyes with pterygium represents the combined effects of naturally occurring astigmatism and pterygium-induced astigmatism (12,22,23). In the current study, both pterygium excision techniques produced significant changes in corneal astigmatism, particularly anterior corneal astigmatism.

**Study limitations:** There are some limitations to this study. Despite the relatively large number of participants, an appropriate randomization method and a control group were lacking. Since conjunctival flap or autograft procedures are most often used in recurrent cases in clinical practice, only primary nasal pterygium investigation may be subject to additional limitation. Although the current study found significant topographical changes even after a relatively short post-operative period, investigating these changes only three months after surgery could be another limitation. After all, cornea has been demonstrated to stabilize one month after pterygium surgery by time-range analysis (24). Long-term prospective studies with more participants incorporating not only different pterygium surgical techniques but also different topographical devices for anterior and posterior corneal topographical assessment may be worthwhile.

## Conclusion

The successful conjunctival autograft technique was associated with significantly greater changes in anterior corneal astigmatism and Ks, implying that this technique may be indicated in pterygium patients with relatively higher pre-operative anterior corneal topographic distortions. The anchored conjunctival rotational flap technique produced significantly greater changes in posterior corneal astigmatism and Kf, suggesting its utility in patients with significant pre-operative posterior corneal topographic changes. To confirm the

clinical relevance of the current study, long-term large-scale prospective studies with various pterygium surgical techniques and topographical devices are still required.

## Declarations:

- **Prepublication or conference presentation disclosure:** This study has not been published, simultaneously submitted, or accepted for publication elsewhere, aside from being posted on preprint servers; <https://doi.org/10.21203/rs.3.rs-280373/v1> with a different title.
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- **Ethics approval and consent to participate:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The local ethics committee approved the study, and the required procedures were applied after the participants' written informed consent.
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