



The Effect of Autografts from the Inferior and Superior Bulbar Conjunctiva on the Ocular Surface in Primary Pterygium Surgery: A Cytology Study

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Abstract

Objectives: This study aimed to evaluate the effect of using an inferior or superior conjunctival autograft in primary pterygium surgery on the postoperative ocular surface.

Materials and Methods: Forty eyes of 40 patients who underwent pterygium surgery with autograft were included in the study. Cytological cell counts were performed on samples taken from the bulbar conjunctiva by impression cytology before and 1 year after the operation. Schirmer 1 test score, lissamine green conjunctival staining score, tear film break-up time (TBUT), and fluorescein corneal staining scores were evaluated. The pain levels of the patients were evaluated with visual analog scale at postoperative 1 day and 1 week.

Results: Corneal and conjunctival staining, TBUT, and Schirmer test results demonstrated significant improvement in all patient groups after surgery, but there was no difference between groups ($p>0.05$). In both preoperative and postoperative impression cytology, the number of goblet cells in the inferior bulbar conjunctiva was higher than in the superior bulbar conjunctiva ($p<0.001$), while there was no such difference in epithelial cell or mucin staining. There were no significant cytological changes postoperatively in either group ($p>0.05$).

Conclusion: Pterygium surgery with autografting improved tear function tests regardless of graft location. Goblet cell count was higher in the inferior bulbar conjunctiva than in the superior bulbar conjunctiva in both postoperative and preoperative impression cytology. However, there

was no significant difference in postoperative epithelial and goblet cell counts or mucin staining between the groups before and after surgery. We think that using the inferior bulbar conjunctiva is an appropriate choice in cases where the superior conjunctiva cannot be used as a graft or when future glaucoma surgery is possible.

Keywords: Pterygium surgery, autograft, superior bulbar conjunctiva, inferior bulbar conjunctiva, impression cytology

Introduction

Pterygium is a common corneal ocular surface disorder caused by fibrovascular tissue that spreads through the limbus from the bulbar conjunctiva to the cornea. Although it is often located in the nasal interpalpebral space, it can also occur on the temporal side.¹ Genetic predisposition plays a role in the etiology, but epidemiological studies support that ultraviolet exposure is the most important environmental factor.^{2,3} Studies have shown that exposure to ultraviolet light during the first years of life has a causal relationship with pterygium development.^{4,5} It is more common in occupations involving outdoor work, such as fishing and farming. Dry, hot air and a dusty environment are also accepted as having a role in the etiology due to chronic irritation.^{2,3,4,5,6}

Pterygium is thought to contribute to the symptoms of irritation, mucoid discharge, and dryness often experienced.³ Abnormal tear film and meibomian gland dysfunction cause dry eye symptoms in patients with pterygium and improve with successful surgical treatment.⁷

Impression cytology of the conjunctival surface is a relatively non-invasive and repeatable procedure. It provides information on cell morphology, cell types, and the topographic cell-cell relationship and is widely used in studies of ocular surface disorders, including dry eye.⁸ Therefore, we planned to use this technique to determine the effect of obtaining grafts from the superior or inferior conjunctiva on cellular changes at the donor site.

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The first record of pterygium surgery is by the Indian ophthalmologist Susruta in 1000 B.C. Since then, surgery has been the primary treatment.⁹ To date, many different methods and surgical techniques have been used, including radiation.¹ The naked sclera technique is among the surgical techniques that has waned in popularity in recent years due to the high recurrence rate, but successful results can be obtained when combined with conjunctival autografting. Although mitomycin C, 5-fluorouracil, and other agents are used as adjuvant therapy to lower recurrence rates, close follow-up is still required for complications.¹⁰ Application of a limbal autograft to the scleral bed after pterygium excision is currently the method that yields the lowest reported recurrence rates.^{11,12,13,14} In studies comparing recurrence between conjunctival autograft and amniotic membrane, the results have been similar or better with conjunctival autograft.^{15,16} However, there is no clear consensus regarding the use of inferior or superior limbal autografts. A few studies in the literature evaluated the effect of obtaining an inferior or superior autograft on surgical success and tear function tests.^{17,18,19} However, these studies have not investigated impression samples. To the best of our knowledge, this is the first cytologic study to evaluate the effect of obtaining autografts from the inferior or superior bulbar conjunctiva on the postoperative ocular surface and the success of primary pterygium surgery.

Materials and Methods

Patients with pterygium who were treated at the University of Health Science Ulucanlar Eye Training and Research Hospital and consented to the planned surgery were included in the study between May 2018 and May 2019. The protocol was approved by the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (decision number E-18-2449, dated 18/04/2018). The study was conducted in accordance with the rules of the Declaration of Helsinki.

The study included patients with no systemic or ocular disease that could cause secondary pterygium by disrupting the ocular surface. Patients who had previous ocular surgery, pseudo-terygium due to ocular trauma or chemical burn, used topical drugs for conditions such as glaucoma or uveitis, or used topical/systemic steroids or non-steroidal anti-inflammatory drugs were not included in the study. All patients in the study voluntarily signed an informed consent form.

Ocular Surface Examination

All participants underwent best corrected visual acuity (BCVA) test with Snellen chart and slit-lamp examination of the cornea, conjunctiva, and eyelids preoperatively and at 1 year postoperatively (Figure 1). Translucency of the pterygium tissue was classified according to the study by Prabhasawat et al.²⁰ Grade 1 (atrophic) is more transparent and the episcleral vessels below can be distinguished, while grade 3 is thick and opaque, and the underlying vessels are not visible. Grade 2 is between these two groups. The preoperative and postoperative tear amounts of the patients were measured by using the Schirmer 1

test. Tear film break-up time (TBUT) and corneal epitheliopathy were evaluated with fluorescein staining, and conjunctival staining was performed with lissamine green.

The Schirmer 1 test was performed without topical anesthetic drops. Standardized Schirmer strips were bent in the notch and carefully placed on the lower lid edge. During the test, the patient was instructed to keep the eyelids closed. The strips remained in place for 5 min or until they were completely saturated with tears. After 5 min, the degree of moistening of the strips was measured using a millimeter scale on each strip. To evaluate TBUT, a fluorescent strip (fluorescein paper, Haag-Streit AG, Köniz, Switzerland) was applied to the inferior conjunctival fornix following a drop of balanced salt solution (Alcon Laboratories, Inc., Fort Worth, TX, USA). After normal blinking for a few seconds to spread the fluorescein, the ocular surface was examined under the cobalt blue-filtered light of the slit-lamp biomicroscope and TBUT was recorded as the time (in seconds) from the last blink to the appearance of the first break in the tear film. The procedure was repeated three times for each eye. After TBUT measurement, corneal staining with fluorescein was evaluated according to the Oxford scheme, which consists of a sequence classified as A-E in order of increasing severity.²¹ Then, a strip with 1.5 mg of lissamine green (Ophthentics, Haryana, India) was placed in the lower lid margin as far temporally as possible, and conjunctival staining was evaluated. Staining scores of the cornea, temporal conjunctiva, and nasal conjunctiva according to the Oxford scheme were recorded for each case.²¹ Staining was graded by comparing the dots on the Oxford chart to the exposed interpalpebral conjunctiva and cornea of the patient (Figure 2).^{21,22} Mean Oxford staining scores were compared between the groups. All ocular surface assessments and impression sampling were performed by the same ophthalmologist.

All surgeries were performed by the same experienced surgeon in the same operating room. Autografts were randomly taken from the superior or inferior conjunctiva, adjacent to the limbus, from an area more than 90° away from the pterygium area.

Impression Cytology Method

Samples for impression cytology were obtained by instilling a single drop of local anesthetic, waiting with the eye closed for

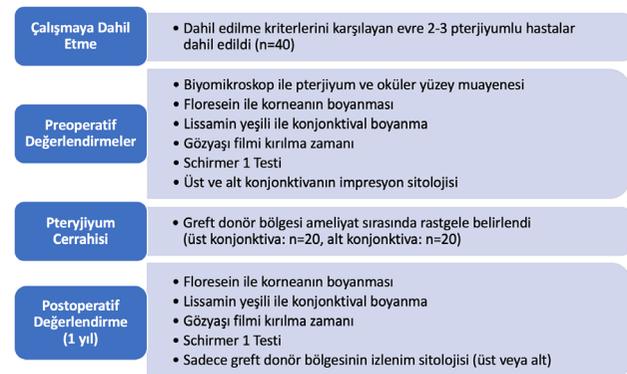


Figure 1. Flow chart of study methodology

		Grade	
	A	0	Absent
	B	I	Minimal
	C	II	Mild
	D	III	Moderate
	E	IV	Marked

Figure 2. Grading of corneal and conjunctival staining (Oxford scheme)

15-20 seconds, then applying a piece of cellulose acetate filter paper to the conjunctival surface. The samples were placed in 96% ethanol and transferred to the cytology laboratory.

The impression samples were stained and visualized as described by Rivas et al.,²³ with a few modifications. In brief, the procedure was as follows: 1) fixation in 96% ethanol; 2) washing in distilled water for 5 min; 3) applying periodic acid for 5 min; 4) washing in distilled water for 5 min; 5) applying Schiff reagent for 5-10 min; 6) rinsing in tap water, followed by staining with Harris hematoxylin for 1 min; 7) rinsing in distilled water, followed by dehydration in increasing alcohol series; 8) clearing the filter paper with xylol; and 9) covering the sample with Entellan new rapid mounting medium (107961; Merck, Germany). Images were obtained using a Zeiss Axio Scope A1 microscope (Carl Zeiss, Oberkochen, Germany). Samples were evaluated for epithelial and goblet cells by a researcher who was blinded to which group the samples belonged to. Impression cytology specimens were graded as normal or abnormal for epithelial cell density, goblet cell density, and mucin spots (goblet cell secretions).^{23,24}

Cells were classified as type 1 epithelial cells with eosinophilic cytoplasm, type 2 goblet cells with basophilic cytoplasm, and type 3 mucin spots that stained eosinophilic.^{24,25,26,27} After the samples were digitally recorded using the Image J processing program (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, <https://imagej.nih.gov/ij/>, 1997-2018), the nuclear-to-cytoplasmic (N/C) ratio and cell density (cells/mm²) were calculated.

Since the graft placement decision was made randomly during the operation, preoperative impression cytology samples were obtained from both the inferior and superior conjunctiva from all patients. At postoperative 1-year follow-up, cytology samples were taken only from the graft site.

Postoperative treatment was the same for both groups. Topical 0.5% moxifloxacin (Moxai, Abdi İbrahim, Türkiye) and 0.5% loteprednol etabonate (Dolte, Abdi İbrahim, Türkiye) were administered 6 times a day for 2 weeks, followed by 1 drop 4 times a day for the next 2 weeks. The patients were

advised to refrain from scratching their eyes after surgery, use sunglasses outdoors, and avoid air-conditioning, dusty, and dirty environments. Follow-up examinations were performed at postoperative 1 day, 1 and 6 months, and 1 year. The patients' ocular pain levels were evaluated using the visual analog scale (VAS) at postoperative 1 day and 1 week.¹⁹ The VAS is a pain measurement tool consisting of a linear scale between 0 and 10 cm, where 0 indicates no pain and 10 indicates the worst pain imaginable. Patients were asked to mark the line with an "X" to indicate pain intensity and the score was determined using a 10.0-point scale. The mean VAS scores of the patients in the inferior and superior graft groups were compared. A flow chart of study methodology is shown in [Figure 1](#).

Surgical Procedure

The surgical procedure was performed under subconjunctival local anesthesia. The pterygium head was lifted and dissected from the corneal surface. The pterygium head and the body tissue were then resected from the underlying sclera 4 to 5 mm from the limbus and after dissection of subconjunctival fibrous tissue, a bare scleral bed was left. The defect area was covered with a free limboconjunctival autograft moved from the superior or inferior bulbar conjunctiva and free from the Tenon capsule. The graft was secured at the limbus and peripherally to the surrounding conjunctiva and episclera using 8-0 Vicryl sutures. Mitomycin C was not used in the surgeries.

Statistical Analysis

The sample size was determined based on a type 1 error rate (α) of 0.05, power of 80%, and effect size (Cohen's d) of 0.8. We determined that at least 20 participants would need to be assigned to each group for a two-tailed t-test analysis.

SPSS software (version 25.0, IBM, Armonk, NY, USA) was used for the statistical analysis. Descriptive statistics, including mean, standard deviation, and range, were calculated for different variables. In all patient groups, TBUT, Schirmer's 1 test, and corneal and conjunctival staining were evaluated using the paired samples t-test. Statistical significance was set at $p < 0.05$. Compliance of the preoperative and postoperative cytology data to normal distribution was investigated with the Kolmogorov-Smirnov test. The results indicated non-normal distribution for all cell types. Therefore, the Wilcoxon test was used to compare pre- and postoperative type 1, type 2, and type 3 cells in the same patient. The Mann-Whitney U test was performed to determine if there was a statistically significant difference between the two groups.

Results

In our study group, the mean age was 53.6±11.2 years (range: 35-74 years) and 65% (n=26) of the patients were male. Pterygium affected the right eye in 18 patients (45%) and the left eye in 22 patients (55%). Twelve patients were grade 2 (30%) and 28 were grade 3 (70%). The distributions of age, gender, and pterygium severity were equal in both groups ([Table 1](#)). The mean preoperative BCVA (in Snellen decimal) was 0.89±0.17 (range: 0.5-1) in the superior graft group and 0.88±0.16 (range: 0.6-1) in the inferior graft group. There was no significant

difference between the two groups (p=0.8). Postoperative BCVA was 0.98±0.04 (range: 0.9-1) in the superior graft group and 0.97±0.06 (range: 0.8-1) in the inferior graft group (p=0.9). The difference between preoperative and postoperative BCVA in the groups was 0.09±0.13 (range: 0-0.4) and 0.05±0.08 (range: 0-0.2), respectively. There was no statistically significant difference between the groups (p=0.56). Mean VAS pain scores on day 1 were 6.2±1.3 (range: 4-8) in the superior graft group and 4.7±1.88 (range: 4-8) in the inferior graft group (p=0.01). On day 7, the scores were 1.5±0.8 (range: 0-3) and 1.3±0.9 (range: 0-3), respectively (p=0.6).

Corneal and conjunctival staining, TBUT, and the Schirmer 1 test showed significant improvement after surgery in both patient groups (Table 2). When the pre-to postoperative changes in these parameters were compared between the superior and inferior graft groups using the Mann-Whitney U test, no significant differences were observed (p>0.05).

Three types of cells were observed in the stained samples. These were epithelial cells with eosinophilic cytoplasm (type 1), goblet cells with basophilic cytoplasm (type 2), and mucin spots with stained eosinophilic cytoplasm (type 3). In between-group comparisons, the preoperative epithelial cell count was 4.13±4.56 in the superior bulbar conjunctiva and 3.53±3.96 in the inferior bulbar conjunctiva, but this difference was not statistically significant (p=0.719). Postoperative values were 4.25±4.79 and 3.06±3.44, respectively (p=0.557). There were significantly more goblet cells in the inferior bulbar conjunctiva

than the superior bulbar conjunctiva both postoperatively and preoperatively (Figure 3, Table 3). No significant differences were observed between the groups in terms of preoperative and postoperative epithelial cell numbers and eosinophilic mucin spots (Table 3). In within-group comparisons, neither group showed any significant cytological changes between the pre- and postoperative 1-year assessments (p>0.05) (Table 4).

Complications such as bleeding and graft necrosis were not observed during the operation or postoperatively. Pterygium size was not measured; autograft size varied in each case but was approximately 4x5 mm. Graft healing occurred in all patients with no redness and discomfort at 1-month follow-up. Pterygium recurrence and unsatisfactory cosmesis was not observed in any of the patients, and no negative feedback was received from the patients at postoperative 6-month follow-up.

Discussion

In this study, the pronounced corneal and conjunctival staining observed before pterygium surgery significantly regressed and the low TBUT and no-anesthesia Schirmer test values significantly increased postoperatively, independent of whether the autograft was taken from the inferior or superior conjunctiva. This may be attributed to the tear instability and dry eye findings caused by the ocular surface irregularity associated with pterygium and the subsequent improvement in the ocular surface after pterygium surgery with autografting.

A variety of results have been reported in the literature regarding the effect of pterygium on tear function tests. Ergin and Bozdoğan²⁸ indicated that pterygium had no abnormal effect on tear function tests in their study of 56 patients. In contrast, a study by Ozsutcu et al.²⁹ including 65 unilateral pterygium patients and their fellow eyes as a control group revealed significant differences in TBUT, Schirmer test, and corneal staining. They concluded that these differences between the two eyes of the same patients were related to pterygium.²⁹ In our study, we demonstrated that pterygium causes deterioration in tear function tests. These discrepancies in the results of ocular surface tests between different studies may be a result of genetic and environmental factors.

Table 1. Demographic distribution of patients in the superior and inferior graft groups

	Superior graft group (n=20)	Inferior graft group (n=20)	p value
Age (years) (mean ± SD)	54.68±10.3	52.52±13.5	0.7*
Gender (male/female) (n)	12/8	14/6	0.410**
Grade 2 pterygium (n)	6	6	0.465**
Grade 3 pterygium (n)	14	14	

*Independent-samples t-test, **Chi-square test, SD: Standard deviation, n: Number of patients

Table 2. Comparison of preoperative and postoperative Oxford staining score (corneal and conjunctival), TBUT, and Schirmer I values in the superior and inferior graft groups

		Preoperative mean ± SD	Postoperative mean ± SD	p*
Superior graft group (n=20)	Corneal Oxford staining score	1.0±0.7	0.24±0.4	0.0001
	Conjunctival Oxford staining score	1.7±0.7	0.18±0.4	0.0001
	TBUT (s)	4.2±1.4	7.0±1.6	0.0001
	Schirmer I (mm)	15.5±5.2	17.0±4.5	0.003
Inferior graft group (n=20)	Corneal Oxford staining score	0.7±0.7	0.3±0.5	0.02
	Conjunctival Oxford staining score	1.5±0.7	0.06±0.3	0.0001
	TBUT (s)	4.0±1.6	6.8±1.9	0.0001
	Schirmer I (mm)	15.1±4.1	17.5±2.7	0.018

*Wilcoxon test. Significant p values (<0.05) shown in bold. TBUT: Tear film break-up time, n: Number of patients, SD: Standard deviation

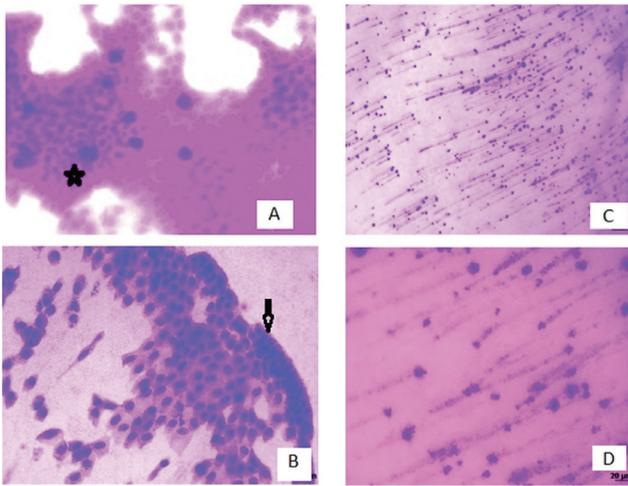


Figure 3. Impression cytology samples obtained preoperatively (A) and postoperatively (B), stained with periodic acid-Schiff and hematoxylin. The arrow (↓) indicates epithelial cells, the star (★) indicates goblet cells. C, D) Goblet cell secretion without goblet and epithelial cells (400X magnification in panels A, B, and D, 100X in panel C)

Table 3. Comparison of impression cytology values between the superior and inferior graft groups

Cell density (count/mm ²)	Superior graft group (n=20) mean ± SD (range)	Inferior graft group (n=20) mean ± SD (range)	P*
Preop type 1 cells	4.13±4.56 (0-15)	3.53±3.96 (0-12)	0.719
Preop type 2 cells	354.9±101.6 (278-698)	476.6±151.6 (310-867)	0.001
Preop type 3 cells	125.9±138.4 (14-450)	79.9±56.5 (11-225)	0.705
Postop type 1 cells	4.25±4.79 (0-14)	3.06±3.44 (0-10)	0.557
Postop type 2 cells	329.3±52.4 (247-440)	480.2±183.3 (312-1015)	0.0001
Postop type 3 cells	119.6±147.1 (3-455)	98.1±86.1 (12-300)	0.801

*Mann-Whitney U test. Significant p values (<0.05) are shown in bold. Preop: Preoperative, Post op: Postoperative, SD: Standard deviation

Li et al.⁷ examined tear film instability, and tear function parameters after pterygium surgery and demonstrated that tear film abnormality and meibomian gland dysfunction improved significantly after surgery. They emphasized that the thickness and size of the pterygium layer were significant in preoperative symptom severity.⁷ Most of our patients (67.7%) had grade 3 pterygium, which is thicker and wider. The marked recovery of tear function at 6-month follow-up was associated with improvement of the ocular surface. A recent systematic review by Linaburg et al.⁶ analyzing 59 studies indicated that abnormal tear function tests improve after pterygium surgery. However, the effect of autograft donor site on tear function and recurrence was not examined in this review. The results of our study showed

Table 4. Comparison of pre- and postoperative impression cytology values within the superior and inferior graft groups

Cell density (count/mm ²)	Cell type	Preoperative mean ± SD (range)	Postoperative mean ± SD (range)	p*
Superior graft group (n=20)	Type 1	4.13±4.56 (0-15)	4.25±4.79 (0-14)	0.672
	Type 2	354.9±101.6 (278-698)	329.3±52.4 (247-440)	0.178
	Type 3	125.9±138.4 (14-450)	119.6±147.1 (3-455)	0.187
Inferior graft group (n=20)	Type 1	3.53±3.96 (0-12)	3.06±3.44 (0-10)	0.06
	Type 2	476.6±151.6 (310-867)	480.2±183.3 (312-1015)	0.46
	Type 3	79.9±56.5 (11-225)	98.1±86.1 (12-300)	0.47

*Wilcoxon test, n: Number of patients, SD: Standard deviation

that tear function tests improved after surgery, but autograft location had no effect on these parameters. However, Linaburg et al.⁶ suggested in their meta-analysis study that the use of an inferior conjunctival autograft may be more advantageous in people with ocular surface disease.

In our study, we did not detect any abnormality in goblet cell density or epithelial morphology at the autograft donor site pre- or postoperatively on impression cytology examination. In addition, we detected extensive mucin spots, as described by Egbert et al.²⁶ These are considered secretions from goblet cells that adhere to the impression paper. No significant difference was demonstrated in these factors pre- or postoperatively according to graft site. This indicates that graft removal does not cause any changes at the donor site. Moreover, no pterygium recurrence was observed in either the inferior or superior autograft group. However, we observed higher goblet cell density the inferior bulbar conjunctiva compared to the superior bulbar conjunctiva. Rivas et al.²³ investigated the topographic distribution of goblet cells with impression cytology and reported densities of 331±148/mm² in the superior bulbar conjunctiva and 427±112/mm² in the inferior bulbar conjunctiva. Although Chan et al.⁸ demonstrated that goblet cell density increased with squamous metaplasia in pterygium tissue, a decrease in goblet cell density was observed in studies by Safarzadeh et al.²⁴ and Julio et al.³⁰ Labbé et al.³¹ explained that the change in the number of goblet cells is related to pterygium activity.

Mucin is secreted by goblet cells and plays an important role in lubrication, ocular surface wetness, and the prevention of microbial infections. Mucin is known to play a role not only in the integrity of the tear film layer but also in the epithelial homeostasis of the ocular surface through its anti-inflammatory and antimicrobial activity.³² Conjunctival autografts are primarily preferred for ocular surface reconstruction. However, nasal mucosal grafts, which also contain goblet cells, can be

used in appropriate cases and have been shown to maintain their effectiveness even in the long term.^{33,34}

Li et al.¹⁷ reported that there was no significant difference in pterygium recurrence with inferior and superior autografts in their recent meta-analysis of randomized controlled studies with a follow-up period of more than 6 months. Our results are consistent with this. However, the inferior bulbar conjunctiva has been preferred over the superior bulbar conjunctiva in patients with superior conjunctival scars, a history of glaucoma surgery, or the possibility of undergoing glaucoma surgery.^{35,36} Similar to the results of our study, other researchers have also reported that less early postoperative pain and discomfort were seen in patients who received inferior autografts.^{18,19} Zloto et al.³⁷ attributed this to the greater range of motion in the upper eyelid than the lower eyelid, which might produce more ocular surface inflammation and delay healing of the corneal epithelium. The superior bulbar conjunctiva cannot be used in glaucoma patients who are candidates for glaucoma filtration surgery or patients who have scarring in the superior bulbar conjunctiva.³² In patients who have already undergone glaucoma filter surgery, the graft donor site should be a suitable distance from the surgical site to avoid impairing bleb function. Undiagnosed and late-recognized glaucoma cases are common worldwide, especially in Africa and Asia.³⁸ Therefore, preserving the superior conjunctiva seems more beneficial to patients in both the short and long term.

Strong points of our study are all surgeries were performed by the same surgeon and in the same environment, and the preoperative and postoperative parameters were evaluated by blinded researchers who did not know which patient was in which group. In addition, to the best of our knowledge, this study is the first cytology study published in the literature to evaluate the effect of using inferior or superior conjunctival autografts on the ocular surface and surgical success in primary pterygium surgery.

Study Limitations

The most important limitation of our study is the small number of patients. We preferred the first year for the last control of patients because pterygium recurrence is frequently observed at around 6 months postoperatively.¹⁷ Although we planned to perform impression cytology at the 1-year visit, some of the patients from whom we took initial samples did not come for follow-up. As a result, the number of patients was lower than we originally planned.

Conclusion

In this study, preoperative impression cytology demonstrated a higher goblet cell density in the inferior conjunctiva than in the superior, and the conjunctiva retained its goblet cell content regardless of whether a superior or inferior conjunctival autograft was used in pterygium surgery. As a result, we think that inferior limboconjunctival grafts should be preferred because they have the goblet cell density to promote surface reconstruction, and this approach preserves the superior conjunctiva for future glaucoma surgery or avoids impairing bleb function in patients

with a history of filtration surgery. Studies with larger patient numbers and longer follow-up may provide more detailed information.

Ethics

Ethics Committee Approval: The protocol was approved by the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (decision number E-18-2449, dated 18/04/2018).

Informed Consent: Obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.K., F.Ç.E., Concept: B.K., E.Ş., Design: B.K., F.Ç.E., İ.İ., B.S., Data Collection or Processing: B.K., F.Ç.E., İ.İ., Analysis or Interpretation: B.K., F.Ç.E., İ.İ., E.Ş., B.S., Literature Search: B.K., F.Ç.E., İ.İ., Writing: B.K., F.Ç.E., İ.İ.

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