Original Article

Observation of Long-term Efficacy of Corneal Limbal Conjunctival Autografts in Microscopy Treatments of Pterygium

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Abstract

Purpose: To compare the long-term efficacy of a corneal limbal conjunctival autograft combined with pterygium excision in the treatment of primary pterygium.

Methods: This random control study included 63 patients (63 eyes) with primary pterygium. These were randomly divided into a group with corneal limbal conjunctival autograft combined with pterygium excision (group A) and a group with pterygium excision with exposed sclera (group B) and subjected to long-term follow up. The surgical outcomes were classified and evaluated (grade Ⅰ-Ⅳ). Several indexes were analyzed, including ocular surface irritation symptom, tear film stability, surgical complication, and recurrence rate.

Results: The follow up ranged from 24 to 38 months, (26.9±3.4) months on average. No recurrence was noted at 6 weeks postoperatively. At 6 months postoperatively, the recurrence rate of pterygium was 5.2% in group A and 8.0% in group B. At 1 year postoperatively, the recurrence rate in group A was 10.5% and 20.0% in group B. At the end of follow up, the recurrence rates were 13.1% in group A and 24.0% in group B. No statistical significance was found between two groups regarding the recurrence rates at each time point (P>0.05). After the follow up (> 2 years), the severity of ocular surface conditions was significantly milder in patients in group A than in group B (P<0.01). No statistically significant differences were noted between the two groups at 6 weeks, 6 months, and 1 year after surgery. No statistically significant differences were observed between the two groups regarding postoperative tear film break-up time. Fewer postoperative complications and milder ocular surface irritation symptoms occurred in patients in group A.

Conclusion: Corneal limbal conjunctival autograft combined with pterygium excision yields sound long-term efficacy and a low recurrence rate and induces only mild damage on the ocular surface when used as a treatment for pterygium. (***Eye Science 2013***; ***28***:***73-78***)

Keywords: microscopic surgery; pterygium; corneal limbus; conjunctival transplant; surgical excision

Introduction

Pterygium is a common external ocular disease that is mainly derived from migrating corneal epithelial cells and fibroblasts[1-3]. The underlying pathogenesis, cell source, and high recurrence rate following excision remain to be explained. Pterygium is mainly treated surgically in clinical practice, with a variety of operations. However, the high recurrence rate after surgery is an unresolved challenge. Successful pterygium surgery is defined as patients who present with no postoperative ocular surface irritation symptoms or topical conjunctival hyperemia, stable tear film, and a recurrence rate of <10%. We previously performed corneal limbal conjunctival autograft (autologous corneal limbal stem cell transplant) in patients with pterygium (152 eyes)[4], and monitored postoperative sequelae in order to find a safer and more efficacious surgical approach. In the present study, we randomly classified 63 patients with pterygium into a c orneal limbal conjunctival autograft group (group A) and a sclerotic exposure pterygium excision group (group B)and observed the long-term efficacy by conducting a follow up for > 2 years. The two groups were then statistically compared for ocular surface conditions, tear film stability, classification of ocular surface irritation, and recurrence rate.

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Subjects and methods

Study design

A random control study

Study subjects

A total of 65 patients (65 eyes) with primary pterygium who were admitted to the Department of Ophthalmology, Zhongshan Hospital, Xiamen University, between January 2004 and September 2008 were enrolled in this study. This included 35 males (35 eyes) and 30 females (30 eyes). Exclusion criteria were acute infectious inflammation on the ocular surface, pingueculae, pseudo-pterygium, systemic connective tissue disease, glaucoma, intraocular pressure>21 mmHg, and undergoing treatment with systemic or topical glucocorticoids.

The affected eyes were categorized as active or inactive pterygium. Active pterygium was characterized by head upheaval, corneal opacity, cellular infiltration into the Bowman's membrane and shallow stroma, rough surface, and thick and expanded blood vessels. Inactive pterygium was characterized by a flat head, absorbed corneal infiltration, no or mild hyperemia in pterygium body, smooth surface, and inactive lesions. The procedures were approved by the Ethical Research Committee at our hospital and informed consent was obtained from each participant. All patients were randomly divided into a corneal limbal conjunctival autograft group or a sclerotic exposure pterygium excision group.

Methods

Preoperative preparation: Patients underwent conventional conjunctival sac irrigation and disinfection, eyelid opening with an eye speculum, alcaine eye drops were administered three times to the operated eyes.

Surgical method: The operation was performed by a single experienced ophthalmologist under microscopy (Zeiss, Germany). Pterygium excision with exposed sclera (group B): topical infiltration anesthesia was performed in the pterygium body using 2% lignocaine. The bulbar conjunctiva covering the neck of pterygium was incised at the corneal limbus, starting from the head of pterygium, along with the body of pterygium as far as the caruncle. The pterygium tissue was sharply separated from the cornea, bulbar conjunctiva, and sclera and removed at 0.5mm anterior to plica semilunaris. After a stanch treatment, the bulbar conjunctiva was fixed approximately 2-2.5 mm after the corneal limbus using a 10-0 nylon suture (Eithicon); the area of the exposed sclera was approximately 2 mm × 5 mm. Limbal conjunctival autograft (group A): a pterygium excision was performed as described above. The bulbar conjunctiva was cut open at 4 mm after the corneal limbus via a subconjunctival injection of 2% lignocaine for infiltration anesthesia. The conjunctiva was separated until 0.5mm inside the transparent cornea, and a conjunctival autograft of 4 mm × 5mm in size was placed on the corneoscleral limbus graft bed, aligning the corneal limbus of graft and graft bed. The 4 corners of the graft were fixed onto the corneal limbus and the shallow layer of sclera and the bulbar conjunctiva was sutured (not including the bulbar conjunctiva adjacent to the graft).

Postoperative handling: The operated eyes were treated with tobramycin and dexamethasone sodium phosphate ophthalmic ointment (Alcon) and deproteinized calf blood extract eye gel (Sinqi Pharmaceutical, China), tightly wrapped with an elastic bandage for 2-3 d, and then treated for two weeks with tobramycin and dexamethasone sodium phosphate eyedrops (qid) and tobramycin, dexamethasone sodium phosphate ophthalmic ointment (qn) and calf blood extract eye gel (qid) after the corneal epithelia were healed. For the patients undergoing pterygium excision with exposed sclera, the stitches were removed on the 7th postoperative day. For those receiving corneal limbal conjunctival autograft, stitches were partially removed on the 7th postoperative day and the remaining stitches were removed on the 10th postoperative day.

Main indexes: Tear film stability and ocular surface were observed at 6 weeks and 6, 12, and 24 months postoperatively. Tear film stability was mainly evaluated by tear film break-up time. Classification criteria of ocular surface conditions were: grade Ⅰ, normal appearance before surgery; grade Ⅱ, hyperemia was noted in shallow layer of sclera, while no fibroproliferation was seen in the surgical area; grade Ⅲ, fibroproliferation was observed in the surgical area but did not invade into the cornea;

grade Ⅳ, recurrent pterygium was seen and subconjunctival tissue proliferation invaded into the cornea[5]. The occurrence of ocular surface irritation symptoms (>6 months), corneal scars, epidermoid cysts, exposed sclera, thickened grafts, postoperative infection, irregular astigmatism, and suture granulomas, etc. was also monitored. During follow up, the administration of medicine to all affected eyes was recorded.

Statistical analysis

The SPSS17.0 software package was adopted for statistical analysis. Measurement data including age, the length of pterygium invasion on the cornea, and tear film break-up time were analyzed by two independent sample *t*-test. The ratio comparison, including sex ratio and recurrence rate, was conducted by a Chi-square test. The ranked categorical data were compared by Ridit analysis. $P<0.05$ was considered to indicate statistical significance.

Results

Among 65 patients (65 eyes) with primary pterygium, 35 (35 eyes) were male and 28 female (28 eyes), aged from 29 to 70 at the time of surgery (54.1 ±9.4 years on average; 54.1 ±9.4 years for males and 55.0±10.0 for females). For the 38 patients (38 eyes) in group A, 19 were male and 19 female(aged 54.5±10.4 years on average), the length of the head of pterygium invasion on the cornea was 4.5±0.8 mm; 31 had active pterygium and 7 inactive pterygium. Group B contained 25 patients (25 eyes), 16 male and 9 female (aged 54.4±8.5 years on average), the length of the head of pterygium invasion on the cornea was 4.7±0.9 mm; 17 had active pterygium and 8 inactive pterygium. No statistical significance was noted between the two groups regarding sex composition, age, the length of the head of pterygium invasion on the cornea, or the preoperative classification of ocular surface conditions ($P>0.05$), as shown in Table 1. The follow up duration was 24-38 months, (26.9±3.4 months on average). Two female patients were lost at the subsequent follow up, one due to keratoconjunctivitis treatment and the other one because of an episode of angle closure glaucoma.

Ocular surface irritation symptoms

A majority of patients in both groups had no complaints about ocular surface irritation symptom at postoperative 6 weeks. One patient (2.6%) in group A and 3 (12.0%) in group B had persistent ocular surface irritation symptoms after operation. Statistical significance was noted between the two groups ($P=0.168$).

Tear film break-up time

In both groups, the tear film break-up time was significantly shorter at postoperative 6 weeks and 6 months than before surgery ($P<0.05$). However, no significant difference was seen in the tear film break-

Table 1 Clinical grouping data of pterygium patients

		Group A: Corneal limbal conjunctival autograft	Group B: Pterygium excision with exposed sclera	Total	*P*
Number of eyes		38	25	63	
Sex	Male	19	16	35	0.274*
	Female	19	9	28	
Age (year)		54.5±10.4	54.4±8.5	54.5±9.6	0.254**
Length of the head of pterygium invasion on cornea (mm)		4.5±0.8	4.7±0.9	4.6±0.8	0.309**
Staging of pterygium	Active pterygium	31	17	48	0.216*
	Inactive pterygium	7	8	15	

* *Chi*-square test; * * Independent samples *t*-test, $\alpha=0.05$.

Table 2 Comparison of postoperative tear film break-up time

	Tear film break-up time x±s (sec)				
	Preoperative	Postoperative 6 weeks	Postoperative 6 months	Postoperative 12 months	After follow-up
Group A: Corneal limbal conjunctival autograft	13.8±4.6	10.8±3.3	11.9±3.6	12.5±4.0	12.5±3.8
Group B: Pterygium excision with exposed sclera	14.9±3.8	10.0±3.1	12.8±3.3	13.8±3.5	13.0±2.8
*P**	0.343	0.312	0.350	0.210	0.552

* Independent samples *t*-test, $\alpha=0.05$.

up time before and after surgery in both groups ($P>0.05$). As shown in Table 2, no statistical difference was noted between the two groups in terms of tear film break-up time measured at any time point ($P>0.05$).

Postoperative recurrence rate of pterygium and classification of ocular surface conditions

The postoperative recurrence of pterygium in two groups is shown in Table 3. No recurrence of pterygium was seen in either group in the early postoperative period (6 weeks postoperatively). The overall recurrence rate at the postoperative 6th month was 6.3%. Two eyes (5.2%) in group A had recurrent pterygium, as did two (8.0%) in group B. The overall recurrence rate at 1 year postoperatively achieved 12.7%, including two eyes (10.5%) in group A and three (20.0%) in group B. At the end of follow up, the overall recurrence rate increased to 14.3%, one eye (13.1%) in group A and one (24.0%) in group B. No statistical significance was observed between the two groups regarding recurrence rate of pterygium at each time point ($P>0.05$). The patients with recurrent pterygium repeatedly underwent pterygium excision during follow up (the results of the 2nd surgery were not included in this study). A comparison of the classification of the ocular surface conditions post-surgery between two groups is shown in Table 4. At 6 weeks, 6 months, and 1 year postoperatively, no significant difference was noted between the two groups in terms of ocular surface conditions ($P>0.05$). After a follow up of more than two years, the status of the ocular surface was significantly better in group A than in group B ($P<0.01$).

Table 3 Comparison of postoperative recurrence rates

	Group A: Corneal limbal conjunctival autograft (number of eyes)			Group B: Pterygium excision with exposed sclera (number of eyes)			Total	P^*
	Nonrecurrent	Recurrent	Total	Nonrecurrent	Recurrent	Total		
Postoperative 6 weeks	38	0	38	25	0	25	63	–
Postoperative 6 months	36	2	38	23	2	25	63	0.522
Postoperative 12 months	34	4	38	20	5	25	63	0.245
After follow-up	33	5	38	19	6	25	63	0.219

* *Chi*-square test, $\alpha = 0.05$.

Table 4 Comparison of postoperative grades of ocular surface conditions

	Group A: Corneal limbal conjunctival autograft (number of eyes)					Group B: Pterygium excision with exposed sclera (number of eyes)					Total	P^*
	Grade Ⅰ	Grade Ⅱ	Grade Ⅲ	Grade Ⅳ	Total	Grade Ⅰ	Grade Ⅱ	Grade Ⅲ	Grade Ⅳ	Total		
Postoperative 6 weeks	24	11	3	0	38	12	5	8	0	25	63	0.087
Postoperative 6 months	16	11	9	2	38	7	8	8	2	25	63	0.246
Postoperative 12 months	15	12	7	4	38	6	7	7	5	25	63	0.106
After follow-up	15	12	6	5	38	3	6	10	6	25	63	0.006**

* Ridit analysis, $\alpha=0.05$.

** At the end of follow-up, the difference of the ocular surface condition grading between two groups was significant.($P<0.01$)。

Other postoperative complications

In group A, 1 case presented postoperatively with scars in the shallow layer of cornea, but no other complications were noted. In group B, 1 patient had remnant fiber tissues in the surgical area, 1 had a scar in the shallow layer of cornea, and 1 had decreased visual acuity after operation induced by irregular astigmatism; no other complications were seen.

Discussion

Surgery serves as the primary treatment of pterygium, but it results in a high postoperative recurrence rate that requires proper resolution. Our previous study that analyzed the surgical efficacy of limbal conjunctival autograft[4] was extended in the present study by categorizing subjects with primary

pterygium into pterygium excision with exposed sclera and limbal conjunctival autograft groups. We then investigated the long-term efficacy of these treatments after >2 years follow-up by statistical comparison of a variety of indexes, including postoperative ocular surface irritation symptom, tear film stability, classification of ocular surface conditions and recurrence rate. Patients who underwent limbal conjunctival autografts had similar tear film stability, less postoperative ocular surface irritation symptoms, significantly higher grade in the classification of ocular surface conditions after the follow up, and lower recurrence rate when compared with patients who underwent pterygium excision with exposed sclera. However, no statistical significance was noted in recurrence rates at each time point.

Most previous studies on postoperative recurrence rates have focused on the effects of surgical skill, adjuvant therapy, and the patients themselves. The pterygium should be removed as completely as possible to prevent its recurrence[6]. Despite the application of microscopic surgery and advances in surgical instruments, the postoperative recurrence rate of pterygium following excision under microscopy and excision with exposed sclera remains high at 11~67%[7]. At present, pterygium excision with uncovered sclera remains the major surgical approach. To reduce the recurrence rate, certain adjuvant therapies, such as intraoperative use of antimetabolites, amniotic membrane, and conjunctival and corneal limbal transplantations have been employed in recent years. Intraoperative use of anti-metabolites (such as mitomycin C) can reduce postoperative recurrence rate of pterygium to approximately 30%. However, the properly designed clinical trials exceeding 4 months that are required for validation of long-term efficacy remain to be conducted[8–10].

Amniotic membrane transplantation during pterygium excision can significantly alleviate postoperative ocular surface irritation symptoms but it fails to reduce the postoperative recurrence rate of pterygium. By contrast, pterygium excision combined with amniotic membrane transplantation yielded a recurrence rate 10% higher than conjunctival flap transplantation[11–13]. A combined treatment of conjunctival flap transfer or transplantation and pterygium excision decreased the recurrence rate to 0–39%[14,15]. On this basis, the use of corneal limbal conjunctival autografts can, in theory, substantially reduce the postoperative recurrence rate of pterygium[16,17]. In our previous study, a total of 152 eyes with pterygium received corneal limbal conjunctival autografts (previously known as autologous corneal limbal stem cell transplantation combined with pterygium excision) and were followed up for 14 months; the 1-year recurrence rate was 11.8%[4]. In the present study, the 1-year recurrence rate in the corneal limbal conjunctival autograft group was 10.5% and this increased to 13.1% at the end of follow up (> 2 years). These values were significantly lower than the 20.0% and 24.0% observed in the pterygium excision with exposed sclera group. The grade of classification of ocular surface conditions was significantly higher after the follow up.

Postoperative ocular surface conditions and tear film changes are also correlated with factors other than primary pterygium, such as intraoperative mechanical injuries to the ocular surface, postoperative tissue inflammation, uneven distribution of tears due to rough ocular surfaces in the surgical area, and postoperative toxicity of antiseptics contained in eyedrops to ocular surface epithelia, *etc*. Corneal limbal conjunctival autografting causes mild damage to healthy corneal limbal stem cells, provides a supply of healthy stem cells to the corneoscleral limbus (the stem cells moderately differentiate into normal corneal limbal epithelia and conjunctival goblet cells), improves the microenvironment of the ocular surface, facilitates the recovery of injured corneal epithelia, reconstructs the injured cornea and corneal limbus, alleviates inflammatory irritation, and improves the tear film break-up time. In this study, a slightly shorter tear film break-up time and fewer ocular surface irritation symptoms were noted at each postoperative time point in the corneal limbal conjunctival autograft group when compared with the pterygium excision with uncovered sclera group. However, the authors cannot safely draw the conclusion that the corneal limbal conjunctival autograft significantly enhances postoperative tear film stability due to the limited sample size of the present study. For the purpose of reducing damage to the

healthy corneal limbus, the sample was collected no deeper than the Bowman's membrane and even the epithelial basement membrane and with an extent of < 1/4 of the circumference. Only the surface layer of epithelia was collected for autologous conjunctival sampling not affecting the Tenon's capsule. The wound in the sampling site required no suturing[18].

A postoperative recurrence of over 90% occurred within 1 year after surgery in patients undergoing corneal limbal conjunctival autograft combined with pterygium excision[19], so that a follow up of 24 to 38 months duration was considered sufficiently long to reflect the postoperative recurrence over a long period in a comprehensive and objective manner. Excluding the subjective influence from patients and surgeons, corneal limbal conjunctival autograft combined with pterygium excision is superior to pterygium excision with exposed sclera in terms of lower postoperative recurrence rate and better long-term efficacy. It is safer than pterygium excision combined with application of antimetabolites and delivers a better cost-performance ratio over pterygium excision in combination with amniotic membrane transplantation. In addition, it can be performed independently by a single ophthalmologist without support from a tissue bank.

To sum up, corneal limbal conjunctival autograft combined with pterygium excision yields long-term efficacy, low recurrence rate, and mild damage to the ocular surface.

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