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ORIGINAL RESEARCH ARTICLE

Amniotic membrane transplantation with narrow-strip conjunctival autograft vs conjunctival autograft for recurrent pterygia

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ABSTRACT

Purpose: To compare amniotic membrane transplantation (AMT) associated with narrow-strip conjunctival autograft vs conjunctival autograft alone for the treatment of recurrent pterygium.

Methods: In this prospective consecutive interventional study, patients with recurrent pterygium were randomly divided into one of 2 groups; group 1: patients undergoing AMT associated with autologous conjunctival graft; and group 2: patients undergoing conjunctival autograft alone.

Results: Of the 80 operated eyes included in this study, 39 (group 1, mean patient age 52.1 ± 11.7 SD years) underwent AMT associated with narrow-strip conjunctival autograft and 41 (group 2, mean patient age 45.8 ± 12.9 SD years) underwent conjunctival autograft alone. In group 1, 6 eyes (15.4%) had grade 1 pterygium, 19 eyes (48.7%) had grade 2 pterygium, and 14 eyes (35.9%) had grade 3 pterygium. In the second group, 5 eyes (12.2%) had grade 1 pterygium, 18 eyes (43.9%) had grade 2 pterygium, and 14 eyes (35.9%) had grade 3 pterygium. No statistically significant difference was found between the 2 groups (p = 0.752). Of the 39 eyes in group 1, recurrent pterygium was observed in 7 cases (17.9%). However, of the 41 eyes in group 2, recurrent pterygium was observed in only 4 cases (9.75%). No statistically significant difference was found between the 2 groups (p = 0.2684).

Conclusions: The results of this study indicate that conjunctival autograft alone might be a better surgical choice for the treatment of recurrent pterygia than combining it with AMT; however, this second option provides a good surgical alternative in cases where little conjunctival donor tissue is available.

Keywords: Conjunctival disease, Cornea, Pterygium

Introduction

Pterygium is a wing-like fibrovascular overgrowth of benign degenerative bulbar conjunctival tissue that extends over the limbus onto the cornea and can cause chronic irritation, impaired cosmesis, and decreased vision (1-3). Treatment options for pterygium include surgical removal, sometimes associated with conjunctival, limbal-conjunctival, or amniotic membrane (AM) grafting, performed in an attempt to reduce recurrence (4). Adjunctive therapies, such as beta irradiation and the use of antimetabolite drugs, are also sometimes employed (5). Recurrent pterygia can induce conjunctival fibrosis and disfigurement, causing considerable discomfort, and can, in general, become increasingly difficult to re-treat.

At present, the conjunctival autograft procedure is defined as the gold standard surgical method for treating both primary and recurrent pterygia, with postoperative recurrence rates reportedly ranging between 0% and 25% (1, 2). However, since the procedure necessitates the removal of a large amount of healthy tissue close to the conjunctival defects post large pterygium removal, there is an increased risk of symblepharon, dellen, and infection. (4) In an attempt to overcome this problem, Dupps and associates (6) reported the use of a narrow-strip (2-mm wide) conjunctival autograft for the treatment of 21 cases of pterygia. In that study, the authors experienced recurrence in only 1 case of recurrent temporal pterygium and stressed the advantages of this technique, including the observation that it is less traumatic for the donor eye. That group's retrospective series, however, included mostly primary pterygia and only 4 recurrent lesions.

Human AM grafting has been advocated for the management of many ocular surface disorders (3), such as persistent corneal epithelial defects with ulceration (7), limbal stem cell deficiency (8, 9), conjunctival neoplasm, scarring (7), and pterygium. The rationale for its use is based on the biochemical properties by which AM transplantation (AMT) inhibits

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pathologic neovascularization (10, 11), scar formation (12), and inflammation (13). Therefore, it is reasonable to hypothesize that with proper management, AM grafts may be used to yield better results for the treatment of recurrent pterygia.

One theoretical advantage of using AM is that there is no limitation on the size of the graft. This allows for the entire defect to be covered, even in cases of large pterygia. The combination of the use of AM plus conjunctival autograft aims to utilize the characteristic properties of AM, as it affects epithelialization and inhibits inflammation, fibrosis, and angiogenesis, as well as providing the advantages of the conjunctival autograft technique, which reportedly has a low rate of recurrence and complications.

Although introducing new guidelines for the treatment of recurrent pterygia, most of the above-mentioned studies were not controlled studies and did not provide good scientific evidence illustrating and supporting the optimal technique for treating this disease. With this background in mind, the purpose of this present study was to compare the use of AM in association with narrow-strip conjunctival autografts versus conjunctival autografts alone for the treatment of recurrent pterygia.

Methods

This prospective consecutive interventional study was approved by the Ethics Committee of the Federal University of São Paulo, Brazil. Eighty eyes of 80 sequential patients with recurrent pterygia without symblepharon or other ocular surface disease treated in the period from July 2005 to December 2008 were randomly assigned to undergo pterygium excision, followed by AM-associated narrow-strip conjunctival autograft (4 × 2 mm) surgery or a conjunctival autograft (approximately 5 × 8 mm) alone. All patients underwent a preoperative clinical evaluation, and written informed consent was obtained from each patient in regards to the treatment that they were going to receive and in accordance with the tenets set forth in the Declaration of Helsinki. The pterygia were graded according to the following classifications reported by Tan and associates (1): T1 (atrophic), a lesion with unobscured and clearly distinguishable episcleral vessels underlying its body; T2 (intermediate); and T3 (fleshy), a thick pterygium in which the episcleral vessels underlie the body and are completely obscured by fibrovascular tissue. All 80 eyes were examined at 1, 7, 30, 90, 180, and 360 days postoperatively and clinical photographs were taken in order to evaluate recurrence. No dropouts were registered. Recurrence was considered any new growth across the limbus.

Preparation and preservation of the amniotic membrane

Human AM was prepared and preserved following the method proposed by Kim and Tseng (10), with some modifications (13). Human placenta was harvested at the time of cesarean section after obtaining informed consent from the donors, each of whom underwent serology tests that were negative for the hepatitis B and C viruses, syphilis, and human immunodeficiency virus. Under sterile conditions, the amnion was separated from the chorion by blunt dissection and was washed with a phosphate-buffered saline solution containing penicillin (1,000 U/mL), streptomycin (20 mg/mL), and amphotericin B (2.5 mg/mL). The AM was then stored at -80°C in a sterile vial containing glycerol and modified Dulbecco's Modified Eagle Medium (1:1; Ophthalmos Industria Farmacêutica Ltda, São Paulo, Brazil).

Surgical technique

All surgeries were all performed by one surgeon (J.B.B.). All patients underwent regional anesthesia with a peribulbar injection of 4 mL of marcaine 0.5% (Marcaína 0.5%; Astra Quiímica E Farmacêutica Ltda, São Paulo, Brazil) and 4 mL of 2% lidocaine without vasoconstrictors (Xilocaiína 2%; Astra Quiímica). Two drops of an adrenaline dilution (1:10,000) were instilled in the patient's eye prior to surgery to induce conjunctival vasoconstriction. A corneal traction suture was then placed at the 12-o'clock position of the limbus to provide good control of the positioning of the globe during the procedure. With the use of forceps and a #15 surgical blade, a cleavage plane was then dissected between the head of the pterygium and the cornea. From this plane, the pterygium was dissected toward its body, up to the limbus. With Westcott scissors and 0.25-mm forceps, the subconjunctival fibrovascular tissue underlying the pterygium was then dissected from the pterygium body and scleral bed and resected by exerting gentle traction with the forceps to improve its exposure. Finally, the pterygium body was resected, along with a thin strip of normal conjunctiva above and below the pterygium. The excised fragments were then fixed in 10% formaldehyde and submitted for histopathologic analysis.

Mitomycin C was not used in any procedure.

Conjunctival autograft

For the conjunctival autograft procedure, the globe was first rotated inferiorly to expose the superior bulbar conjunctiva. With the use of 0.12 forceps and Westcott scissors, a thin, Tenon-free conjunctival graft was then obtained starting 1 mm posterior to the limbus. This tissue was then transferred to the recipient area and fixed to the episclera and conjunctiva with 10-0 nylon continuous or interrupted sutures. The graft size was determined from the measurement of the scleral bed after pterygium excision. We usually oversize the graft by 1 mm to provide a better fit on the recipient scleral bed.

Amniotic membrane with conjunctival autograft

After being thawed, the AM was placed over the exposed area, maintaining the initial orientation, with the epithelium facing upward. The tissue was then fixed to the episclera and adjacent conjunctiva by use of interrupted 10-0 nylon sutures. A small, 4 w 2-mm conjunctival graft harvested as previously described was then placed and secured with 4 10-0 nylon sutures on top of the AM, 1.5 mm from the limbus (Figs. 1 and 2).

Postoperative period

Postoperatively, all patients were treated every 4 hours with 0.1% dexamethasone acetate and 0.3% tobramycin (TobraDex®; Alcon, São Paulo, Brazil). This treatment regimen

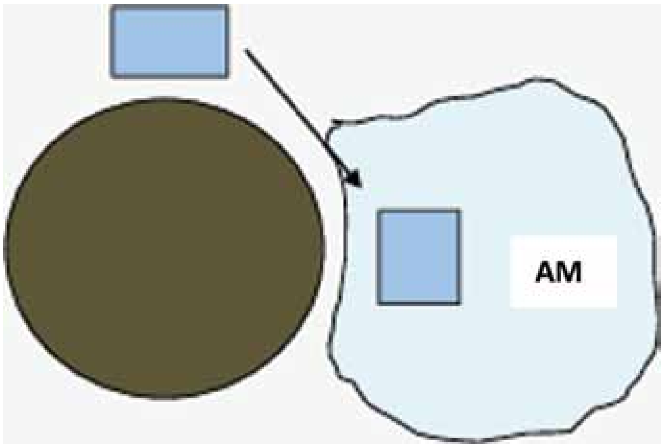

Fig. 1 - Schema shows the technique of amniotic membrane associated with narrow-strip (2 × 4 mm) conjunctival autograft.

Fig. 2 - Preoperative (left) and 1-week (middle) and 6-month (right) postoperative images of amniotic membrane with conjunctival autograft.

was then tapered beginning 7 days postoperatively and discontinued by 60 days postoperatively.

Statistical analysis

For the statistical analysis, data were collected and presented in contingency tables. Continuous and categorical data were compared using the Fisher exact test and the Mann-Whitney test, respectively. To assess the progress of cases over time, the survival curves of the Kaplan-Meier and log-rank tests were used. A p value of <0.05 was considered statistically significant. All data analysis was performed using Stata software v.10 (College Park, Texas, USA).

Results

This study involved 80 eyes of 80 patients with recurrent pterygia. Of those, 41 eyes (51.25%) received conjunctival autografts, while the remaining 39 eyes (48.75%) received AM combined with narrow-strip conjunctival autografts. The characteristics of both groups of patients are shown in Table I. No significant differences were found in regards to age (p = 0.102), number of previous pterygium excisions (p = 0.309), or grade of the pterygium (p = 0.752).

Of the 41 eyes that received conjunctival autografts, 34 eyes (82.9%) had a history of one previous surgery, whereas 7 eyes (17.1%) had a history of more than one previous surgery. Of the 39 eyes in the AM combined with narrow-strip conjunctival autograft group, 29 eyes (74.35%) had a history of one previous surgery, whereas 10 eyes (25.65%) had a history of more than one previous surgery. In regards to the pterygium grade, in the conjunctival autograft group, 5 eyes (12.2%) were pterygium TI, 18 eyes (43.9%) were pterygium TII, and 18 eyes (43.9%) were pterygium TIII. However, in the AM combined with narrow-strip conjunctival autograft group, 6 eyes (15.4%) were pterygium TI, 19 eyes (48.7%) were pterygium TII, and 14 eyes (35.9%) were pterygium TIII.

In regards to postoperative recurrence, it was observed in 4 eyes (9.75%) at the 1-year postoperative follow-up examination in the conjunctival autograft group, yet it was observed in 7 eyes (17.9%) in the AM combined with narrow-strip conjunctival autograft group at that same timepoint. However, this difference was not statistically significant (p = 0.268).

In regards to the grade of pterygium, it was observed that in both groups, the recurrence rate of pterygium grade TI was less than those of pterygium grades TII and TIII. However, no statistically significant differences were observed (p = 0.745 for conjunctival autograft and p = 0.091 for AM combined with narrow-strip conjunctival graft).

In relation to the number of previous surgeries, it was observed that in the conjunctival autograft group, the recurrence rate was higher in eyes with a history of one previous surgery than in eyes with a history of more than one surgery, yet no statistically significant difference was found (p = 0.35). However, in the AM combined with narrow-strip conjunctival graft group, it was observed that eyes with a history of one previous surgery developed significantly fewer recurrences than the eyes with more than one previous surgery (p = 0.001).

In regards to postoperative complications, it was observed that early rupture (occurring 1 day postoperatively) of the suture (interrupted suture) in 1 eye of the group receiving AM combined with narrow-strip conjunctival grafts did not compromise the position of the graft on the scleral bed. In the conjunctival autograft group, the presence of an extensive

TABLE I - Demographic data of the recurrent pterygia patients involved in this study

	Conjunctival autograft	Amniotic membrane + NSCA	p value
Patient age, y, mean ± SD	46.0 ± 13.03	51.7 ± 11.8	0.121
Previous surgery, n (%)			0.3091
1	34 (82.9)	29 (74.35)	
>1	7 (17.1)	10 (25.65)	
Grade of pterygium, n (%)			0.7527
I	5 (12.2)	6 (15.4)	
II	18 (43.9)	19 (48.7)	
III	18 (43.9)	14 (35.9)	

NSCA = narrow strip conjunctival autograft.

conjunctival granuloma was observed in 1 eye at 7 days postoperatively at the donor site area where the conjunctiva had been removed. However, that lesion regressed completely after 3 weeks of being treated with a 4-times-daily administration of 1% prednisolone acetate (Fig. 3).

Fig. 3 - Postoperative conjunctival granuloma in a pterygium patient who underwent conjunctival autograft.

Discussion

Recurrence of fibrovascular lesions on the cornea is the single most common and frustrating complication of pterygium surgery, for both patient and surgeon. Thus, there is an ongoing debate regarding the ideal surgical technique for the removal of those lesions. Recurrent pterygia are characterized by hyperproliferation of subconjunctival fibroblasts that result in fibrosis with a more accelerated growth rate than primary pterygium (14). This fibrosis sometimes involves the medial rectus muscle, causing restriction in ocular movement, symblepharon formation, or both. Therefore, after the excision of the pterygia, it is necessary to use adjunctive therapies, including radiation, chemotherapy, or grafting procedures, such as conjunctival autografts, to reduce the hyperproliferation of fibrovascular tissues and thereby reduce the recurrence rate.

Amniotic membrane transplantation has been used as an alternative to conjunctival grafts to reconstruct conjunctival defects, to restore a normal stroma, and to provide a healthy basement membrane for renewed epithelial proliferation and differentiation (3). Although based on its basement membrane properties that facilitate the migration, adhesion, differentiation, and prevention of apoptosis in epithelial cells, the avascular stromal matrix contains various proteins that suppress tumor growth factor-β signaling, proliferation, and myofibroblast differentiation of normal fibroblasts (12). These mechanisms explain why AMT helps to reduce scarring and fibrosis during conjunctival surface reconstruction and may play a role in preventing fibroblast proliferation.

Earlier reports on the use of AM grafts for recurrent pterygia, either alone (15) or combined with autograft limbal transplantations (16), noted unsatisfactory results, with reported recurrence rates of 37.5% and 25%, respectively. This finding might be partly due to the aggressiveness of the cases described in those studies. Prabhasawat and associates (15) reported a recurrence rate of 37.5% for recurrent pterygia treated by AMT. This rate was significantly higher than in those treated with conjunctival autograft. In another study, the authors observed a lower recurrence rate of 9.5% after modifying the surgical technique by performing an extensive excision of pterygium tissue and subconjunctival injection of corticosteroids (17). In an attempt to further improve on the results, Shimazaki and associates (14) reported a surgical technique in which the AM was associated with a 5 × 5-mm conjunctival autograft for the treatment of recurrent pterygia and obtained a 14.8% recurrence rate. More recently, Taylan Sekeroglu and associates (18) described the association of sutureless AM and narrow-strip conjunctival autograft in 30 eyes with primary pterygia and found that the lesion recurred in only one eye.

Conjunctival autografts have been proven safe and effective for the reduction of pterygium recurrence. However, obtaining a good conjunctival graft requires a skillful conjunctival dissection technique to ensure the optimal handling, harvesting, and preservation of healthy conjunctiva. This may explain why recurrence rates following conjunctival autografts vary from as low as 0% (1, 19) to a wide range of higher recurrence rates in other reports (20).

In a report by Hirst (19) that consisted of 111 patients with recurrent pterygia, all of those patients underwent conjunctival autografts with a modified technique. Interestingly, none of the patients exhibited any recurrence at the 1-year postoperative follow-up. The main change described in that study consisted of a wide resection of the pterygium and the surrounding subconjunctival fibrous tissue, followed by an extensive conjunctival autograft. However, there are some disadvantages associated with that technique that should be highlighted, such as its prolonged surgical time (approximately 90-120 minutes) and the rate of postoperative complications, such as excessive pain and transient diplopia. Furthermore, the need for a large area of viable conjunctival closure defects to be obtained after extensive resection of the lesions calls for conjunctiva that is often not available, especially in cases of extensive pterygia involving the nasal and temporal regions.

A theoretical advantage of using AM tissue is that there is no limitation on the size of the graft, even in cases of double pterygia (nasal and temporal). Thus, a wide resection of fibrous tissue is possible. This allows for the use of AM to cover the entire defect. Suturing AM onto a small piece of conjunctiva allows the membrane to be used as a substrate for the expansion of its cells, which is favored, due to the similarity between their conjunctival basement membranes. The promotion of the growth and differentiation of limbal and conjunctival cells adjacent to the AM reportedly encourages wound healing, reduces inflammation, and encourages eventual restoration of the limbal barrier function (21).

Based on these considerations, we conducted a prospective, randomized study in which we compared a technique proven effective (i.e., conjunctival autograft) in various studies for the treatment of pterygium with a technique that has several theoretical advantages (AMT associated with narrow-strip conjunctival autograft). Until now, and as far as we are aware, there have been no randomized studies proving the effectiveness of this technique against recurrent pterygia.

In the present study, we observed recurrence rates of 9.75% in patients undergoing conjunctival autografts and 17.9% in patients who underwent AMT associated with a narrow-strip conjunctival autograft. Comparison of the recurrence rates among the various studies with pterygium should be carefully evaluated, as many important differences may arise among the studies, including study population, postoperative follow-up periods, number and experience of the surgeons involved, and the definition of recurrence.

Another important aspect in assessing the rates of recurrence following pterygium surgery is the postoperative follow-up period. Studies have shown that it is necessary to follow up the patients for 12 months, during which time 97% of recurrences are observed (22). Hence, any analysis of recurrence that involves a follow-up period of less than 12 months may underestimate the real rate of recurrence. In the present study, all 80 patients were followed up for a minimum period of 12 months.

Ti and associates (23) reported high variability in the rate of recurrence among different surgeons (5% to 82% of recurrences) who performed conjunctival autografts for primary and recurrent pterygia. That study, in which the results of 12 surgeons were investigated, revealed that surgeons with higher numbers of previous surgeries had lower rates of recurrence, demonstrating that experience with the surgical technique is of great importance. In our study, all surgeries were performed by one surgeon, thereby avoiding the variability of results that might have been influenced by the differing experience levels among physicians.

Another aspect that may influence the outcome of pterygium surgery is the degree of inflammation exhibited by the lesion preoperatively. Tan and associates (1) described a new classification for pterygium and determined that the chance of recurrence is related to the degree of inflammation present in the lesion prior to surgery, regardless of the age of the patient. However, in another study that compared the AM with or without the combination of mitomycin C, Ma and associates (5) used the same classification proposed by Tan et al and did not observe any relationship between recurrence and the degree of pterygium.

In the present study, we used the same classification proposed by Tan and associates to analyze if the preoperative degree of inflammation in the lesion represented a possible risk factor for recurrence. In the group of patients who underwent conjunctival autografts, 5 eyes classified as pterygium grade TI experienced no lesion recurrence, while in 36 eyes with pterygium grade TII or TIII, 4 eyes (9.75%) experienced recurrence. However, this difference in the risk of relapse according to the morphology of the pterygium was not statistically significant. In the group that underwent narrow-strip conjunctival autograft combined with AM, the patients with pterygium grade TI experienced no recurrence, while the patients with pterygium grade TII showed better survival than those with grade TIII; however, no significant differences were observed between the latter two. Thus, while no statistically significant differences were observed in our study, the tendency of pterygia with a greater degree of inflammation to experience more recurrences validated the usefulness of the classifications proposed by Tan et al in the preoperative evaluation of patients with pterygia.

In regards to the number of previous surgeries, we noted in our study that in the group undergoing conjunctival autografts, there were no significant differences in the recurrence rate of the patients who underwent one or more previous surgeries. However, in the AM combined with narrow-strip conjunctival autograft group, we observed a higher recurrence rate in patients who had undergone more than one previous surgery.

It should be noted that the recurrence rate measured in our study was 17.9% when using the technique of AMT associated with narrow-strip conjunctival autograft. Thus, this technique might be considered acceptable for patients with recurrent pterygia and may prove to be a valid option for the treatment of recurrent pterygia, even for patients with more than one prior surgery, given that there may be limited conjunctiva available for autotransplantation.

In conclusion, the treatment of recurrent pterygia is a challenging task that frequently results in recurrence and can be complicated by a shortage of conjunctiva available for autograft. The results of this study demonstrate that the use of conjunctival autograft in the treatment of recurrent pterygia may be a better option than combining a narrow-strip conjunctival autograft with AM. However, the latter option provides a good alternative when only a small amount of conjunctival donor tissue is available.

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Disclosures

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