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Limbal Stem Cell Versus Amniotic Membrane Transplantation for Ocular Surface Reconstruction Following Chemical Ocular Burns

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Abstract

Objective: To compare the efficacy, safety, and long term outcomes of both limbal stem cell and amniotic membrane transplantation; being employed for ocular surface reconstruction in cases (patients) with history of ocular chemical burns.

Patients and Methods: 40 eyes of 40 consecutive patients with a diagnoses of partial limbal stem cell deficiency secondary to chemical ocular burn were recruited from the outpatient clinic in Mansoura university, Ophthalmic center and were assigned randomly to two groups; each with 20 eyes of 20 patients. Both groups underwent superficial keratectomy of the conjunctivalised corneal surface, followed by limbal stem cell transplantation and amniotic transplantation in group A, and amniotic membrane transplantation alone in group B. Patients were scheduled for follow-up within the period of one year.

Results: At the end of the follow-up period, all the eyes exhibited a stable and intact corneal epithelial surface. In group A, the mean time for re-epithelialization was 16 ± 4.8 days, while in group B was 22 ± 14.1 days. The overall improvement in visual acuity was observed in 75 % and 60% of the cases in groups A and B respectively.

Conclusions: Limbal stem cell transplantation is a safe and effective procedure for restoring the ocular surface integrity in eyes with partial limbal stem cell deficiency secondary to chemical ocular burn. Amniotic membrane transplantation is also a safe and effective alternative. However, this study shows superiority of the combined use of limbal stem cell transplantation.

Keywords: Stem Cell; Amniotic Membrane; Ocular Burns.

Introduction

The cornea and conjunctiva constitute what is called the ocular surface. This surface is directly exposed to the external environment, and it is being threatened by multiple chemical injuries [1].

Chemical burn is considered as a true ocular emergency that requires immediate and intensive evaluation and care. This type of injury is most common among men with in 20 to 40 years of age, working in industrial chemical laboratories or factories [2]. As a result of common younger age predisposed to ocular chemical injury, the long-term disabilities that followed ocular burns could dramatically affectthe patients' lives. The aim of treatment is toreduce further damage to ocular surface and finally to restore a normal ocular surface anatomy and visual function [3].

The extent of damage and prognosis of a chemically injured eye depend on multiple factors including the type and amount of the agent involved, PH of the chemical substance, and duration of contact. Appearance of the perilimbal area, however, provides the most reliable clues regarding sequelae and prognosis of the injured eyes [4].

Identifying the stage of a chemical eye burn is particularly helpful in predicting the outcome. Most importantly, the relative proportion of surviving limbal tissue has been shown to be a major prognostic factor [5].

Corneal epithelium is renewed continuously by younger cells that migrate inward from the periphery and when older cells are lost from the surface. The constant source of corneal epithelial cell is believed to be the limbus. Corneal epithelial stem cells

have been identified deep within a protected microenvironment or niche at the limbal palisades of Vogt [6].

Regeneration of the corneal surface after an epithelial insult involves division, migration, and maturation of limbal stem cells [7].

When the limbus is intact, corneal epithelial defects heal without delay. But when the limbus is damaged, either because of injury or inflammation, the normal corneal epithelial physiologic features are disrupted [8].

Ocular chemical burns are the most common cause of LSCD which is the main cause of non-healing epithelial defects, stromal inflammation, neovascularization, conjunctivalization and corneal opacification follows chemical burn [9].

In partial LSCD, frequent ocular lubrication and a topical steroid therapy are the adequate treatment. With increased corneal conjunctivalization, and in the presence of some remaining corneal stem cells, Amniotic membrane transplantation (AMT) is an alternative with and has a high success rate [10].

AMT can result in a reduction in ocular surface inflammation and the restoration of stem cell functions during healing of chemical burns [11].

Although, this procedure confers no risk for rejection, the process of creating the graft leaves the contralateral donor eye is at risk for LSCD. This risk is low when fewer than four to six clock-hours of limbal tissue are transplanted [12].

In the present study, efficacy of AMT alone is compared with autologous limbal stem cells transplantation combined with AMT, in cases with limbal stem cell deficiency secondary to ocular chemical injuries.

Patients and Methods

Forty eyes of 40 patients were enrolled to participate in this study, under a protocol approved by the Medical Research Ethics Committee (MREC) of Mansoura University. After adequate explanation of the nature, risks, and possible adverse events of the planned procedure, written informed consent was obtained from each participant. All surgeries were performed at Ophthalmology Center - Mansoura University, between May 2013 and February 2015 and followed up for a year post-operatively.

Forty eyes, of 40 consecutive patients were assigned randomly to two groups; A and B. All cases exhibited limbal stem cell deficiency (LSCD) with circumferential involvement of not less than 180 degrees of the corneal limbus. Demographic data of patients who enrolled into this study is demonstrated in table 1. Patients in both groups underwent superficial keratectomy of the conjunctivalised corneal surface, followed by autologous limbal stem cell transplantation, combined with amniotic membrane transplantation in group A, and amniotic membrane transplantation alone in group B. Cases were scheduled for follow-up over a period of one year after surgery.

In this study, diagnosis of LSCD was made on clinical basis, by demonstrating limbal ischaemia, persistent and recurrent corneal epithelial defects and the loss of limbal palisade of Vogt on the slit lamp. All patients had history of exposure to chemical injury. Other causes of LSCD were excluded by accurate history taking and careful examination excluding cases with multiple previous surgeries or contact lens related keratopathies.

Surgical Techniques

Autologous conjunctival limbal stem cell transplantation

All patients in this study were anaesthetized by retro bulbar block. 20 patients in group A underwent autologous conjunctival limbal stem cell transplantation. The conjunctivalised pannus was removed from the corneaby peritomy followed by superficial keratectomy in the recipient eye(s). The fibrosed subconjunctival tissues and symblepharon were dissected and removed.

The patient's eye was anaesthetized by topical anesthesia, in addition to subconjunctival infiltration anesthesia. Two strips of conjunctival limbal free grafts were harvested by superficial lamellar keratectomy of a 6 mm limbal arc length; 1 mm within the limbus and including 5 mm or more of the adjacent conjunctiva. Conjunctival limbal autografts were harvested from the superior and inferior limbal regions. These two free grafts were sutured to the recipient bed, with the corresponding anatomic sites taken into account and marked by free sutures in the free grafts to mark limbal and conjunctival ends of the free grafts. The free grafts were sutured in the recipient bed by interrupted 10-0 nylon sutures to the limbus and 8-0 vicryl sutures to the sclera.

Amniotic membrane transplantation

20 patients were assigned to group B, who underwent amniotic membrane transplantation. Preserved fresh frozen human amniotic membrane was obtained. Seronegativity for HIV, hepatitis B virus, hepatitis C virus and syphilis was assured.

Conjunctival peritomy was donein the area of LSCD. Subconjunctival fibrosis and symblepharon were dissected and re-

Table 1. Demographic data of patients enrolled into the study.

		Group A	Group B
Total number		20	20
Age range		32 – 42	36 – 40
Mean LSCD degree		270 degrees	
BCVA	Preoperative	1.0 ± 0.36	0.22 ± 0.09
	Post-operative	1.0 ± 1.03	0.6 ± 0.37

moved, and this resulted in a conjunctival recession from the limbus. In the eyes of subtotal LSCD, the peritomy was extended to 360° of the limbus. AM was obtained from its storage medium, peeled from the nitrocellulose packaging to which its stromal surface was adherent, and allocated over the limbus and adjacent cornea with the basement membrane and the stromal side facing down.

The membrane was sutured into place at the limbus with interrupted 8-0 vicryl, also sutures were placed between the AM and the conjunctiva after trimming off the excess membrane.

Following either surgical procedure, bandage contact lens was applied for one week to the recipient eye. Patients in both groups received topical combination of antibiotic and steroid (Dexamethasone 0.1% and Tobramycin 0.3%) four times a day. Topical instillation of eye drops was tapered off over a period of 1-2 months. Patients were followed at an interval of one week for the first month post-operatively, then at an interval of a month till the end of a complete year post-operatively. Ocular inflammation, corneal neovascularization and visual acuities were obtained at each visit.

Results

Group A contained 20 patients; 18 males and 2 females with a mean age of 36.9 (4.6) years,while group B contained 20 patients; 18 males and 2 females with a mean age of 38.1 (2.3 years). The extent of the LSCD in both groups involved from 180° to nearly 360° of the limbal circumference. Surgery was straightforward in all the cases. Neither operative nor early post-operative complications were recorded. Notably, there were no cases of post-operative graft infection or rejection in either group.

Inflammation and corneal vascularization were markedly reduced over the early post-operative period, with gradual significant improvement along the follow-up period. Patients in group A took a mean period of 16 ± 4.8 days to be fully epithelialized. While patients in group B took a longer mean period of 22 ± 14.1 days to obtain a fully epithelialized corneal surface. A single eye (one patient in group A) (5%) suffered from a persistent epithelial defect, which was managed by frequent lubrication and it disappeared within 2 months post-operatively. No case with recurrent epithelial erosion was recorded.

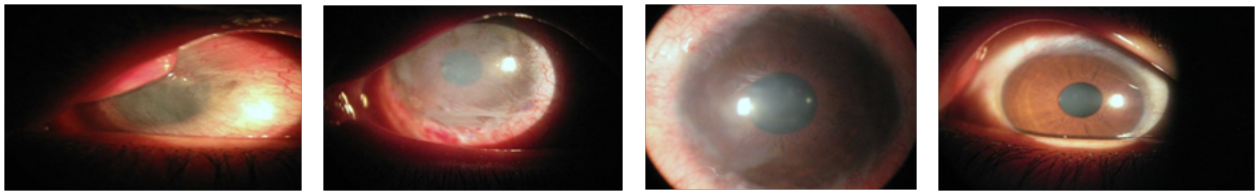
In group B, 2 cases (10%) suffered from a persistent epithelial defect, which needed a 3 month period using aggressive lubrications for healing. 6 cases (30%) developed recurrent epithelial erosions, but improved with time.

At the end of the follow-up period, all eyes, in both groups maintained a smooth and stable corneal epithelial surface; without recurrent erosion or persistent epithelial defect.

Group A (Figure 1 and 2) recorded an improved BCVA in 15 patients, while the remaining five patients remained stable at the same preoperative BCVA. These five cases suffered from corneal stromal fibrosis, which necessitated keratoplasty.

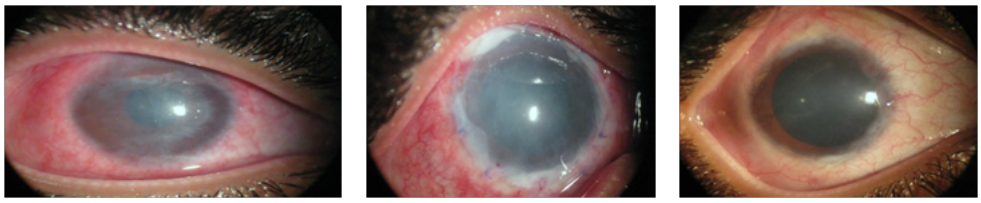
Group B (Figure 3 and 4) recorded an improved BCVA in 12 patients, while worsening of preoperative BCVA was recorded in 4 patients; due to recurrence of corneal stromal vascularization. The remaining 4 patients showed a stable BCVA level

Figure 1. A case of chemical burn with upper symblepharon (a) Preoperative, (b) One week post-operative after limbal stem cell and amniotic membrane transplantation), (c) one month post-operative, and (d) The other eye post-operative.



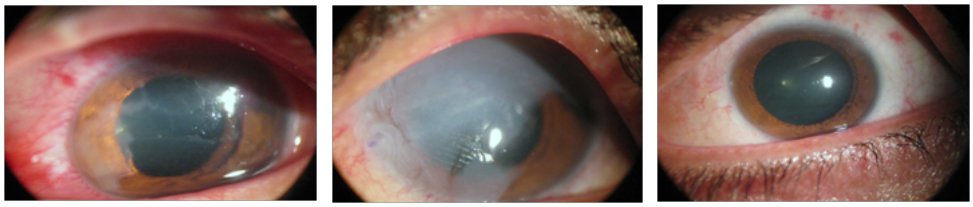
A B C D

Figure 2. A case of chemical burn with diffuse corneal conjunctivalization (a)Preoperative, (b) One week post-operative after limbal stem cell and amniotic membrane transplantation), and (c) one month post-operative.



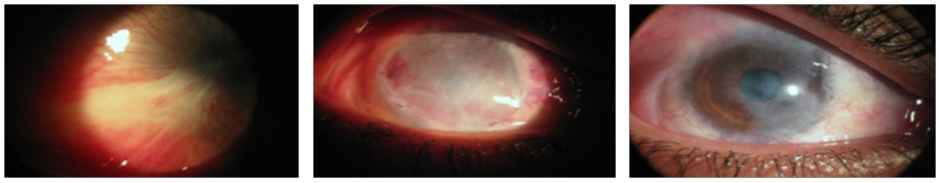
A B C

Figure 3. A case of chemical burn with partial limbal stem cell defeciency: (a)Preoperative, (b) one week post-operative (amniotic membrane transplantation alone), and (c) one month post-operative.



A B C

Figure 4. A case of chemical burn with corneal conjunctivalization and symblepharon: (a) Preoperative, (b) One week post-operative after amniotic membrane transplantation alone), and (c) one month post-operative.



post-operatively, which was due to corneal stromal fibrosis.

Discussion

Apart from the damaging anatomical effects of chemical injury to the eye, loss of limbal stem cells is a major cause of functional diminution of vision after chemical injury. Management of photophobia and ocular discomfort in such cases is difficult, with a limited role of conventional penetrating keratoplasty and limited success [13].

Replenishing of limbal stem cells is vital in such cases. Patients with unilateral LSCD are lucky to have a source for their stem cells to be replenished by using an autologous graft from the other healthy eye. However, bilateral cases, or cases with partial LSCD may benefit from amniotic membrane transplantation [14].

This study compared autologous limbal stem cell transplantation combined with amniotic membrane transplantation to amniotic membrane transplantation alone in patients with LSCD secondary to chemical insult to the eye. Our results showed comparable end results at a year post-operatively, but patients who underwent limbal stem transplantation showed better ocular comfort and more rapid convalescence with less complications.

Patients who participated in this study all suffered from a unilateral LSCD as a result of previous ocular chemical injury. 20 patients in group A received autologous limbal stem cells transplantation combined with amniotic membrane transplantation, and obtained a quiet, non-inflamed ocular surface in a shorter duration than patients in group B; who received amniotic membrane transplantation alone.

Amniotic membrane act by promoting the growth of the residual limbal stem cells. Cases in this study had a mean circumference of lost limbal stem cells of 270 degrees, which may accounts for the longer convalescence period in group B patients [15].

Cases in group B also showed a higher rate of persistent epithelial defects and recurrent erosions. This gives autologous limbal stem cells transplantation superiority in the management of unilateral limbal stem cell deficiency.

Kobayashi et al [16] also reported the usefulness of amniotic membrane patch in ocular chemical burns. Burns were mild with lessthan 1/3 limbal ischaemia in four patients and 1/2 limbalischaemia in one patient. However, the results of their study did not suggest the usefulness of AMT in severe burns.

Meller et al [17] treated 13 eyes of ocular chemical burns with AMT. Seven eyes hadgrade II–III burns and six eyes had grade IV burns. Epithelial defects of all grade II–III burns except two patients were healed within 2–5 weeks. Alleyes with grade IV burns experienced limbal stem cell deficiency. Out of six patients with total limbal ischaemia, three cases required limbal stem cell transplantation.

Similarly, Dua et al [18] reported that in extremely severe burns AMT does not establish the ocular surface orpreserve the integrity of the globe.

Also, Arora et al [19] reported that AMT rapidly restored the ocular surface in Grade II burns. AMT was effective to a certain extent in Grade III burns, but it was not totally effective in preventing symblepharon and corneal vascularization.

These results also suggests that more successful results in the management of ocular chemical burn depend on evaluation of the extent of limbal stem cell deficiency, and that limbal stem cell transplantation is an important option in the management of chemical burns with significant limbal stem cell deficiency.

Conclusions

Autologous limbal stem cell transplantation is an important option for ocular surface reconstruction in cases with unilateral LSCD as a result of chemical ocular injury. This option is proved to be superior to amniotic membrane transplantation alone, with fewer complications and much shorter convalescence period.

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