

Long Term Follow-up of Limbal Transplantation for Unilateral Chemical Injuries: 1997-2014

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Abstract

Purpose: To evaluate the long term results of limbal transplantation (LT) in patients with unilateral total limbal stem cell deficiency (LSCD) after chemical injury.

Methods: The study includes 22 eyes of 22 consecutive patients (20 males and 2 females) who presented with total LSCD after unilateral chemical burns and underwent Limbal transplantation (LT) in the Cornea Service of the Department of Ophthalmology at the Heraklion University Hospital in Crete during the period from 1997 to 2014. All 22 cases underwent Conjunctival Limbal autograft (CLAU) while in 14 surgeries it was combined with amniotic membrane transplantation (AMT). A second stage penetrating keratoplasty (PKP) was performed in 11 cases for visual rehabilitation. The healing time, the changes in VA and the stability of epithelial ocular surface integrity were looked for.

Results: One case failed within 3 months of surgery, while the rest 21 eyes after CLAU maintained ocular surface epithelial integrity during the follow up period (7.8 ± 3.5 years), and showed improvement partially or totally in corneal neovascularization, symblepharon and ocular motility. The mean corneal healing time was 17 days, while visual acuity either showed statistically significant improvement in 18 eyes, with CLAU alone or followed by PKP. No significant difference in the surgical outcome was observed between AMT and none AMT cases.

Conclusions: Conjunctival limbal autografts for limbal deficiency after unilateral chemical burn showed long term success and stability, in 21 out of 22 eyes, whether combined or not with amniotic membrane transplantation.

Keywords: Cornea; Chemical injury; Limbal stem cells; Autograft; Amniotic membrane

Introduction

Chemical injuries range in severity from trivial to potentially blinding with alkali burns to be more common and more dangerous than acid burns. Chemical agent penetration results in necrosis of conjunctival and corneal epithelium, occlusion of the limbal vasculature, precipitation of glycosaminoglycans and corneal stroma opacification [1-4].

Corneal healing process starts from the palisades of Vogt at the periphery of the cornea, where limbal stem cells are located, and migrate to the center of the epithelial defect, according to "XYZ hypothesis" of Thoft [5]. In severe ocular burns when limbal stem cells are destroyed the healing process stops. In such case of LSCD, released cytokines result in abnormal tissue and vessels migration from the conjunctiva to the cornea, a process called conjunctivalization.

Many studies report that limbal transplantation is an effective and safe procedure for the treatment of visual impairment or non-healing corneal epithelium due to LSCD [6-22]. Both acute and chronic stage chemical injuries have been found to benefit from LT. In unilateral cases, autologous conjunctival limbal graft is transplanted from the healthy eye, while in bilateral cases allografts are used from living related donors or cadaver donors. Amniotic membrane transplantation (AMT) [6,7,14] can be used as an adjunct to CLAU or Limbal allograft (LAL) in order to promote epithelialization of the ocular surface, inhibit as much as possible fibrovascular proliferation and improve corneal clarity or to prepare corneal surface for future penetrating keratoplasty (PKP) [15,16,22].

This retrospective study presents data from long-term results of consecutive series of patients with LSCD after unilateral chemical injuries that underwent CLAU in the Cornea Service of the Department of

Ophthalmology at the Heraklion University Hospital in Crete, Greece between October 1997 and June 2014.

Materials and Methods

The data of 22 consecutive patients (22 eyes), who underwent CLAU were studied (Table 1). Inclusion criteria for CLAU surgery included total LSCD due to unilateral chemical burn, diagnosed clinically by the presence of conjunctivalization of the corneal surface, fibrovascular growth, vascularization, and/or persistent corneal epithelial defect and/or symblephara and limitation of ocular motility. All but one case had third degree chemical burn, and in most eyes, the causative agent was lime (Table 1). Limbal graft failure was considered as the study endpoint for each patient. All surgical procedures were performed under general anesthesia by the same surgeon (CSS) at the Cornea Service of the Ophthalmology Department at the Heraklion University Hospital, Crete, Greece, as described by Kenyon and Tseng [8] (CLAU). Briefly, the recipient eye underwent a 360° conjunctival peritomy and all the symblephara were released. The contracted subconjunctival scarred

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CASE	AGE	GENDER	EYE	BURN TO LSCT (m)	DEGREE OF BURN/ AGENT	PREOP BCVA	PROCEDURE	LG HEALING TIME (D)	PREVIOUS/ FOLLOWING PROCEDURES	F/U (m)	LSCT outcome	FINAL VA	CONDITION OF CORNEA/FINAL OUTCOME
1	31	M	OS	2.5	3/Lime	0.6	CLAU	24	- / -	100	S	0.6	DIFFUSE STROMAL OPACITY
2	25	M	OS	2	3/Lime	0.4	CLAU + AMT	32	- / -	76	S	1.0	CLEAR
3	26	M	OD	156	3/Lime	0.05	CLAU + AMT	15	PKP / PKP	84	S	0.4	CENTRAL STROMAL OPACITY
4	41	M	OS	2	3/Lime	0.015	CLAU	21	- / -	192	S	0.9	CLEAR
5	35	M	OS	2	3/Ammonia	0.015	CLAU	17	- / PKP	140	S	0.4	PARACENTRAL GRAFT OPACITY
6	3	M	OD	1.5	3/Lime	0.005	CLAU	13	- / PKP, ECCE+IOL, PV, RD	90	S	NLP	CLEAR/PENETRATING INJURY, PHTHISIS
7	15	M	OD	2	3/Lime	0.4	CLAU + AMT	15	- / PKP	90	S	NLP	CLEAR/TOTAL RD (PHTHISIS)
8	17	M	OS	1.5	3/Lime	0.015	CLAU + AMT	8	- / -	153	S	1.0	CLEAR
9	30	M	OS	2	3/Lime	0.2	CLAU + AMT	11	- / -	107	S	0.9	CLEAR
10	23	M	OS	6	3/Lime	0.1	CLAU + AMT	10	- / PKP	126	S	0.7	CLEAR
11	24	M	OD	36	3/Lime	0.015	CLAU + AMT	11	- / -	84	S	0.7	CLEAR
12	73	F	OD	96	3/Caustic Potash	0.015	CLAU	20	- / -	3*	F	0.015	TOTALLY SCARRED VASCULARIZED CORNEA
13	46	M	OD	228	3/Lime	0.005	CLAU	51	PKP (3) /PKP	135	S	0.6	CLEAR
14	24	M	OD	38	3/Lime	0.005	CLAU + AMT	10	- / PKP	54	S	0.4	PARACENTRAL GRAFT OPACITY
15	47	M	OS	7	3/Lime	0.1	CLAU + AMT	8	- / -	138	S	0.5	CENTRAL STROMAL OPACITY
16	57	F	OS	96	3/Lime	0.005	CLAU + AMT	25	- / -	84	S	0.2	DIFFUSE STROMAL OPACITY
17	24	M	OS	2	3/Lime	0.9	CLAU	9	- / -	72	S	0.9	CLEAR
18	33	F	OS	132	3/Lime	0.015	CLAU + AMT	21	PKP/PKP	26	S	0.4	CLEAR
19	43	M	OD	4	4/Lime	0.015	CLAU + AMT	12	- / PKP-CPC-DSAEK-ECCE+IOL	66	S	0.015	REJECTED CORNEAL GRAFT
20	25	M	OS	22	3/Lime	0.015	CLAU	21	- / -	84	S	0.7	CLEAR
21	45	M	OS	24	3/Lime	0.005	CLAU+AMT	12	OP(11)/PKP-ECCE+IOL	27	S	1.0	CLEAR
22	46	M	OS	12	3/Lime	0.015	CLAU+AMT	12	-/PKP-ECCE+IOL	39	S	0.9	CLEAR

LG: Limbal Graft; CLAU: Conjunctival Limbal Autograft; AMT: Amniotic Membrane Transplantation; ECCE: Extracapsular Cataract Extraction; IOL: Intraocular Lens Implantation; PKP: Penetrating Keratoplasty; PV: Posterior Vitrectomy; RD: Retinal Detachment; CPC: Cyclophotocoagulation; DSAEK: Descemet Stripping Automated Endothelial Keratoplasty; OP: Oculoplastic Surgery; 3*: Failed at 3 Months Postoperatively; S: Successful; F: Failure; VA: Visual Acuity; NLP: No Light Perception.

Table 1: Case History and outcome of 22 patients that underwent Conjunctival Limbal Autograft Transplantation procedure after Corneal chemical injury

tissue was dissected from the sclera under the conjunctival epithelium. Care was taken to preserve healthy conjunctiva as much as possible. Conjunctival edges with scarred tissue were cut circumferentially, and sutured back to the sclera leaving around 3 mm of exposed bare sclera 360° around the limbus. In addition, superficial keratectomy was performed stripping all unhealthy epithelium as well as dissecting fibrovascular tissue invading the cornea. For stromal opacities occupying the superficial 1/3 of the corneal thickness an effort was made to meticulously dissect them, however when deeper, stromal polishing was solely performed. Conjunctival-limbal grafts were harvested from contralateral healthy eyes. Two sectorial autografts of limbal tissue of three clock hours in the upper and 2 clock hours in the lower limbus were excised. The sectorial grafts contained 0.5-1 mm of superficial clear cornea and adjacent bulbar conjunctiva as needed according to the burnt area. The autografts were sutured on the recipient eye and secured in the anatomically appropriate position with Nylon (10-0) sutures to the recipient cornea centrally as well as to the sclera peripherally. When surgeries combined with amniotic membrane transplantation (AMT), the one piece was used basement membrane side up, as a substrate for the conjunctival epithelium, sutured and anchored to the sclera under the conjunctiva with 10/0 Nylon sutures, and a second piece was used as patch over the cornea covering and protecting the grafts, and sutured to the sclera with

one continuous 10/0 Nylon stitch peripheral to the limbus. Finally, in all cases a temporary midtemporal tarsorrhaphy was performed with a single mattress suture 4/0 silk stitch. Tarsorrhaphy was released when cornea healed and/or when a bandage contact lens could be fitted. Sutures were removed after complete healing of the cornea by the limbal grafts, as well as of the rest of the ocular surface, by conjunctival epithelium. Suture removal usually took place one month after surgery.

Postoperatively, all patients received antibiotic/steroid combination eye drops six times per day until reepithelization, and dosage was adjusted according to the degree of surface inflammation. Patients were also instructed to use preservative-free artificial tears frequently for at least three months. Beta blocker eye drops were prescribed in cases of elevated intraocular pressure, either due to inflammation or as response to steroids. A bandage therapeutic contact lens was fitted in the contralateral donor eye, and conjunctival sutures, at the site of donor graft excision, were removed within 3-4 days post operatively.

Eleven out of 22 eyes with deep central stromal scarring obscuring vision, due to the chemical injury, underwent second stage penetrating keratoplasty. In cases with no or less deep corneal opacities, PKP was either not suggested due to improvement of vision with CLAU alone, or rejected by the patients considering their vision to be acceptable, or not

willing to take the risk of another surgical procedure. Outcome evaluation included survival rate of limbal transplants i.e. ocular surface epithelial integrity as well as changes in the visual acuity compared to the preoperative data.

Statistical analysis was performed using SPSS 23. Results are presented as mean \pm standard deviation (SD) and Student paired t-test was used to evaluate the visual acuity results. A *p* value less than 0.05 ($\alpha=5\%$) was regarded statistical significance.

Results

Twenty two patients (22 eyes), 20 males and 2 females, 3 to 73 years old (mean age: 33.3 ± 15.4 years) with total LSCD due to unilateral chemical burn, underwent CLAU 1.5 to 228 months after the injury (mean: 47.6 ± 65.1) with a mean follow up of 7.8 ± 3.5 years (range: 2.2-16 years, (27-192 months, (Table 1)). The mean follow-up period was skewed by 1 eye (case 12, Table 1) that limbal graft failed within 3 months (study end point), even though post-operative examinations continued. Limbal transplantation was combined with AMT in 14 eyes. Eleven eyes required second stage PKP for visual rehabilitation, which was done at least 6 months after CLAU. Three eyes had at least one previously failed PKP, and one patient reported 11 previous oculoplastic surgeries.

No complications were observed during the surgical procedures and contralateral donor eyes epithelialized within 48 h of surgery, and were asymptomatic within 3-4 days. The mean time from surgery to complete healing of the cornea by the limbal grafts as well as ocular surface epithelium was 17.18 ± 9.8 days (range: 8-51 days). Twenty one out of 22 eyes showed stable ocular surface epithelial integrity during the follow up period, which is considered successful Limbal transplant surgery (success rate: 95.4%). Clinically success of limbal graft was combined with partial or total regression of neovascularization, as well as improvement in ocular motility attributed to symblephara excision. In addition, use of amniotic membrane did not seem to affect the surgery outcome (Figures 1a-2d).

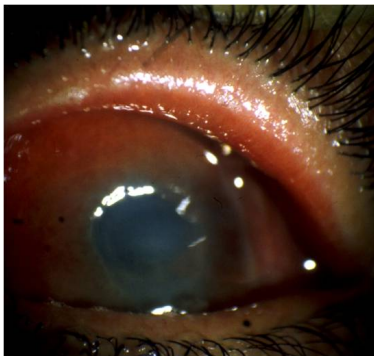


Figure 2a: One and half month after third degree alkali chemical injury OS (case 8). Limbal ischemia and inflammation

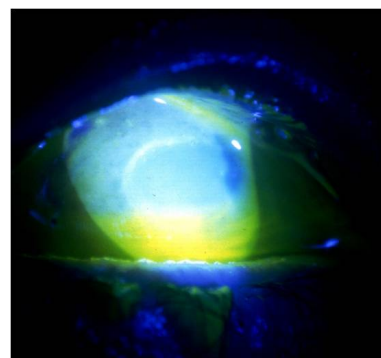


Figure 2b: Same case of Figure 2a stained with fluorescein showing non healing corneal epithelial defect.

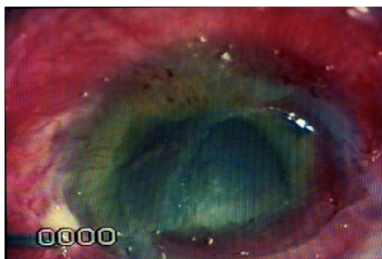


Figure 1a: Two months after third degree alkali chemical injury OS (case 4). Non healing corneal defect, conjunctivalization of the cornea.



Figure 2c: Intraoperative photo of patient in Figure 2b showing symblephara and fibrovascular proliferation causing ocular motility limitation

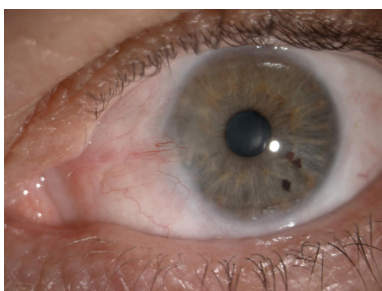


Figure 1b: Sixteen years after CLAU surgery of fig 1 case. Mild pseudopterygium, stable ocular surface epithelial integrity, visual acuity 0.9.



Figure 2d: Twelve years post CLAU and AMT of patient in Figures 2a-2c. Stable ocular surface epithelial integrity, visual acuity 1.0.

Four cases ended up with low or no vision due to early failure (case 12), penetrating trauma (case 6), retinal detachment (case 7) and rejected PKP and DSAEK ((case 19), Table 1). Those cases were exempted from visual acuity statistics. The rest (18 eyes) showed stable (2 eyes) or improved vision (16 eyes). Final corrected visual acuity after LSCT or after secondary successful PKP markedly improved from 1.4 ± 0.74 Logmar (vision range: 0.005-0.9 decimal) to 0.2 ± 0.19 Logmar (vision range: 0.2-1.0 decimal), and this improvement was statistically significant ($p < 0.05$) (Figures 3a-3c).

Discussion

Chemical eye injury requires immediate intervention prior to detailed ocular examination and a full history record. First line treatment consists of copious irrigation, with double eversion of the eyelids and debridement of necrotic areas of corneal epithelium. Chemical injury grading (1-4) depends on the basis of corneal clarity and severity of

limbal ischemia. Mild (grades 1 and 2) injuries have very good prognosis and are treated with a short course of topical steroids, cycloplegics and prophylactic antibiotics for about 7 days. In more severe burns (grades 3 and 4), if topical treatment fails to promote epithelial regeneration and/or prevent corneal ulceration surgical approaches are required [1-4].

Several previous reports have introduced limbal stem cell transplantation as the preferred surgical procedure for total LSCD with very good results after severe chemical injuries [6-22]. Limbal stem cell transplants can be either autograft or allograft (LAL) and LSCT can be combined or followed by amniotic membrane transplantation [6,7,14,20,21] penetrating keratoplasty [6,15,16], division of symblephara, correction of eyelid deformities and keratoprosthesis [1,2,17]. Both approaches (CLAU and LAL) have many limitations. CLAU requires normal ocular surface in the fellow eye and it is less effective in cases of aniridia, Stevens-Johnson syndrome and contact lens-induced keratopathy [6,8,9]. On the other hand, harvesting of limbal tissue within a few hours after death is not always feasible in some countries [7].

We hereby present a large retrospective consecutive case series, focused on Conjunctival limbal autograft transplantation after LSCT for unilateral chemical injury, with a mean follow-up of 7.8 years. Phenotype ocular restoration and visual acuity improvement was significantly high.

In CLAU technique, successful reconstruction of ocular surface is very high [6-8,11,12,14-20] and may reach 100% [20], in contrast to limbal allografts that rejection is more common despite the intensive use of immunosuppressives [9,12,20]. In our study in all cases except one, CLAU was successful. Our experience with limbal allografts is limited (5 cases unpublished) and indeed the results are ultimately discouraging. All published studies conclude that most patients' visual acuity significantly increased after LSCT, corneal clarity improved and eyes experienced a symptomatic relief [14]. Miri et al. [20] reported that VA improved from a mean of 0.121 ± 0.184 preoperatively to 0.313 ± 0.348 postoperatively, while Santos et al. [21] showed that 60.6% of eyes had improvement in postoperative visual acuity. Our study is in accordance with these results since final visual acuity improved from 1.4 ± 0.74 Logmar to 0.2 ± 0.19 Logmar. Two patients totally lost vision from other cause (penetrating injury and retinal detachment), while 2 eyes finally resulted in having the same low vision (a case of failed CLAU and a second due to rejected secondary PKP and DSAEK surgery).

Moreover, when an LAUT is combined with AMT, this seems to provide better visual outcome and decreased risk of graft rejection due to AM anti-inflammatory properties, especially in acute stage chemical injuries [7,14]. This is why it is advisable to do surgery when inflammation subsides, but this is not always possible especially in patients with persistent epithelial defects. In our cases, there was no difference in the results between the AMT and non AMT cases. However, it is necessary to emphasize that each of these surgeries is unique, since there are different parameters involved: severity of burn, degree of inflammation, extent of conjunctival necrosis and fibrovascular proliferation including symblephara, as well as corneal opacity and neovascularization. Therefore, the combination for example of AMT in a case with a large area of conjunctival necrosis could provide a better chance for smooth healing of remaining conjunctival epithelium, and a delay in the regrowth of fibrovascular tissue. Statistics are helpful but this is not a step by step routine procedure. In addition to LSCT, in cases with deep central corneal stromal opacity, PKP is necessary in order to improve patients' visual acuity [7,15,16,20]. PKP can be performed a few months later as a secondary step. Indeed, we performed PKP for visual

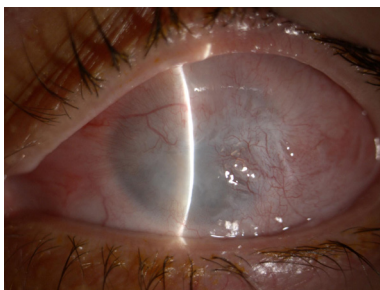


Figure 3a: Third degree alkali chemical burn OS (case 21), 24 months after injury, underwent 11 oculoplastic procedures. Fibrovascular tissue invading all ocular surfaces including the cornea, limitation in ocular motility, deep corneal opacification.



Figure 3b: Six months post CLAU and AMT of case in Figure 3a. Ocular surface epithelium intact, ocular motility markedly improved. Cornea still opaque.

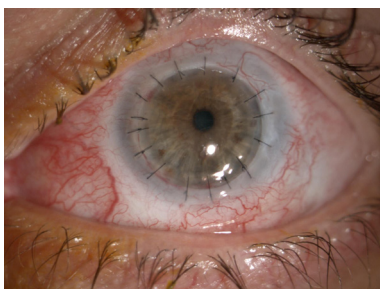


Figure 3c: Post PKP six months after CLAU of case in Figures 3a and 3b. Sutures of PKP removed 1 year after surgery and underwent ECCE + IOL. During the last F/U cornea was clear with intact epithelium and visual acuity of 0.9

rehabilitation in 11 cases at least 6 months after CLAU. Reconstruction of ocular surface reduces the risk of postoperative PKP complications and graft rejection thus improving long-term prognosis [16].

Regarding limbal stem cells transplantation, newer promising surgical approaches have been developed for the treatment of severe chemical burns with total or partial LSCD [23-30]. The main goal of all of these techniques is to reduce the amount of donor limbal tissue required, which minimizes the possibility of damage to the donor eye. Even though many studies [6,8,19,20] indicate that no complications occur in the fellow donor eye when 50% or less of limbal tissue for autograft transplantation is used, there is always concern regarding the eye health of a singled eye patient. The basic idea of these procedures is to culture in AM autologous or harvested limbal stem cells. This procedure was first described by Pellegrini et al. in 1997 and gave encouraging results the following years [24-27]. Moreover, another interesting approach is the *ex vivo* cultured autologous oral mucosal epithelial cells to treat LSCD [27-30]. Recent case studies showed, that it is a feasible technique despite the need of experienced personnel and specialized laboratories, and it can actually restore vision in patients with bilateral severe disorders of the ocular surface up to one year post-operatively. Shortt et al. in a recent study reported an overall success rate of 60% (33% for autografts and 71% for allografts from cadaver eyes) [30].

In conclusion, long term follow up of conjunctival limbal autograft transplantation combined or not with amniotic membrane was an effective procedure, leading to ocular inflammation regression, restoration of corneal surface integrity and improvement of visual acuity, in a series of 22 consecutive eyes of total limbal stem cell deficiency due to unilateral chemical injury.

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