Original article

Long-term Outcome of Limbal Stem Cell Transplantation for Management of Total Limbal Stem Cell Deficiency due to Chemical Burn

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Abstract

Purpose: To evaluate the long-term outcome of limbal stem cell transplantation for management of total limbal stem cell deficiency due to chemical burn.

Patients and Methods: In this retrospective cross sectional study; records of patients with history of severe (grade III to IV) chemical burns who underwent limbal stem cell transplantation in Labbafinejad Medical Center, Tehran, Iran between 2006 and 2016 were reviewed and data including demographic characteristics, visual acuity, surgical interventions and outcomes were reported.

Results: Fifty eyes of fifty patients with a history of conjunctival limbal autograft (N = 24) or keratolimbal allograft (N = 26) with at least 12-months follow-up were included. The overall 1-year and 5-year survival were 100 % and 84.1 % for conjunctival limbal autograft and 80.4 % and 40 % for keratolimbal allograft, respectively (P = 0.037).

The 1-year and 5-year corneal graft survival were 93.3 % and 63.8 % after conjunctival limbal autograft and 92 % and 38.4 % after keratolimbal allograft (P = 0.005 for five year survival). There was a significant improvement in LogMAR BCVA (1.79 versus 2.17, P < 0.001) in all patients with no statistically significant difference between the two groups.

Conclusion: Severe chemical burn is associated with significant ocular morbidity and long-term prognosis is poor. Graft survival rate was significantly better in conjunctival limbal autograft compared to keratolimbal allograft when comparing the long-term outcome of limbal stem cell transplantation for management of total limbal stem cell deficiency due to chemical burn.

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Introduction

Chemical ocular burn is a true medical emergency with serious and potentially blinding acute and chronic complications and is a major cause of limbal stem cell deficiency (LSCD) [1,2]. Damage to the limbal stem cells either by direct damage from the chemical agents or secondary to limbal ischemia caused by damage to the vascular endothelium may result in irreversible loss of limbal stem cells (LSCs) [3]. LSCs are responsible for maintaining ocular surface health by continuous proliferation and differentiation into corneal epithelial cells that are a major part of corneal integrity and normal vision [4]. Hence, damage to the LSCs can lead to disturbance in ocular surface integrity and result in persistent corneal epithelial defect, corneal infection, corneal perforation and even loss of vision. Proper management of LSCD is critical for preventing these serious complications and improving vision. Limbal stem cell transplantation (LSCT) is the standard management of total LSCD with any cause. Gold standard techniques for unilateral and bilateral total LSCD are conjunctival-limbal autograft (CLAU) and keratolimbal allograft (KLAL), respectively [5]. In addition, many patients with chemical burn-induced total LSCD develop significant stromal opacification that necessitates lamellar or penetrating keratoplasty to restore vision [6]. Corneal grafting in these patients should be considered as high-risk due to accompanying ocular surface abnormalities as well as corneal neovascularization [7]. Although CLAU and KLAL are generally successful in short-term, long term visual prognosis depends on the survival of stem cell and/or corneal graft and accompanying ocular complications such as glaucoma [8; 9]. Allograft rejection is the major cause of stem cell failure following KLAL[10]. Although CLAU does not harbor the risk of allograft rejection, chronic inflammation and ocular surface compromise can alter stem cell function and eventually lead to CLAU failure in long term [11]. Furthermore, corneal graft rejection and failure is another major complication in both auto and allograft procedures [12]. Survival of the KLAL in the first two years after LSCT has been reported to be 77 % to 100 % [13-15]. However, the long-term survival of KLAL decreases after 2 years, especially in patients undergoing simultaneous keratoplasty [12; 16; 17]. In one study, KLAL survival rate was 54.4 % at 1 year, 33.3 % at 2 years, and 27.3 % at 3 years [18]. In unilateral LSCD, CLAU resulted in improvement of vision in 35 % to 88 % of cases [19]. Long-term survival of CLAU is reported to be better or equal to allogenic LSCT in different studies [5; 20; 21]. In this retrospective cross-sectional study, we report the long-term outcomes of CLAU and KLAL in a tertiary referral center.

Patients and Methods

Patients

Records of all patients with chemical burn who were referred to the Labbafinejad Medical Center, Tehran, Iran, between 2006 and 2016 and underwent CLAU or KLAL with or without subsequent keratoplasty were reviewed retrospectively.

The study protocol was approved by the ethics committee of the Labbafinejad Medical Center Clinical Research and Development Unit, Tehran, Iran, and adhered to the tenets of declaration of Helsinki. Informed written consent for using their records for future research purposes was obtained from all patients.

Surgical procedures

All procedures were performed by standard techniques described elsewhere [10; 22]. Briefly, for KLAL, fresh globes with intact epithelium and

conjunctiva were obtained from the Eye Bank of the Islamic Republic of Iran. A 360-degree corneoscleral rim including 3-mm sclera and 2-mm cornea was trimmed and thinned from the endothelial side as much as possible to achieve an approximately 100 microns rim containing limbus. In the recipient eye, a 360-peritomy was performed and scar issues were removed. The donor rim was fixed to the sclera using 8-0 Vicryl sutures with a spatulated needle. Then the conjunctiva was sutured using 10-0 nylon. A soft bandage contact lens was placed and lateral tarsorrhaphy was performed. All patients received systemic immunosuppression 2 weeks before until at least 2 years after KLAL. For CLAU, 360-degree conjunctival peritomy was performed in the recipient's affected eye and the scar tissue was removed. Amniotic membrane was placed and fixed to the limbus and sclera using tangential continuous 10-0 nylon sutures. Then, 2 pieces of 60-degree limbal blocks containing enough conjunctiva were harvested from the donor (healthy) eye at 6 and 12 o'clock. The donor limbal blocks were transferred to the recipient eye and fixed at the corresponding superior and inferior locations at the limbus using separate 10-0 nylon sutures. At the end of the procedure, a soft bandage contact lens was fitted and lateral tarsorrhaphy was performed.

Ophthalmic examinations

Relevant data including demographics, date of injury, type of chemical agent (acid versus alkali), date and type of ocular surgeries, acute and chronic complications related to chemical injury or stem cell/corneal graft were extracted from the patients' records. Best-corrected visual acuity (BCVA) was measured by tumbling E-chart at 6 meters before LSCT and at the final examination. Also, slit-lamp examination was performed at each visit to assess the degree of LSCD (defined as the degrees of cornea invaded by conjunctival epithelium), opacification of the crystalline lens (cataract formation), ocular surface staining by fluorescein, tear-film stability (defined by tear-film break-up time and tear meniscus height) and graft-related complications (vascularization, epithelial defect, rejection or failure). Intraocular pressure was monitored using Goldmann applanation tonometer mounted on slit-lamp. Dilated fundus examination was performed to evaluate the optic nerve head cupping or atrophy, retinal detachment and other posterior segment complications. In cases with severe media opacity, ultrasonography B-scan mode was performed for posterior segment evaluation.

The main outcome measures were final BCVA and the survival of the limbal stem cell graft. Successful limbal stem cell graft was defined as stable corneal epithelium without persistent corneal epithelial defect and significant superficial vascularization. Superficial corneal vascularization invading the central 5mm of cornea and/or presence of persistent corneal epithelial defect were considered as signs of stem cell graft failure.

Statistical analysis

All data were analysed using SPSS version 21 (Armonk, NY: IBM Corp) software. BCVA was converted to LogMAR scale for statistical analysis. LogMAR for visual acuities less than 20 / 200 were considered 2.6, 2.7, 2.8 and 2.9 for counting fingers, hand motion, light perception and no light perception, respectively. Mean ± SD of the BCVA LogMAR were calculated and compared between the initial and final visit using paired-samples *t*-test. Chi-Square test was used to compare nonparametric variables. Kaplan-Meier survival

analysis was performed to assess stem cell and corneal graft survivals. P-values less than 0.05 were considered statistically significant.

Results

Records of fifty eyes of fifty patients (44 male and 6 female) including 24 in CLAU and 26 in KLAL group who completed at least 12 months of follow-up were analysed. The mean age of patients was 39.74 ± 12.68 years and the mean follow-up duration was 67.4 ± 47.3 months. The mean initial BCVA was 2.44 ± 0.81 LogMAR. Table 1 shows the demographics in each group of patients. Forty five eyes (20 eyes from CLAU and 25 eyes from KLAL subgroups) underwent optical penetrating keratoplasty (PKP) in a subsequent (20 CLAU and 20 KLAL eyes) or simultaneous (5KLAL eyes) procedure.

Table 1: Demographic characteristics of patients in CLAU and KLAL groups

	CLAU (N = 24)	KLAL (N = 26)	P value
Age (Mean ± SD)	43.42 ± 13.66	36.54 ± 10.94	0.06
Male/Female Ratio	19/5	25/1	0.09
Agent (Acid : Alkali)	5/19	6/20	0.56
Followup (Months) (Mean ± SD)	63.70 ± 55.70	70.82 ± 38.85	0.60
Initial BCVA LogMAR (Mean ± SD)	2.43 ± 0.79	2.44 ± 0.85	0.96

In total, final BCVA improved to 1.85 LogMAR (P < 0.001), which showed significant improvement compared to initial BCVA (2.44 LogMAR).

The final BCVA was not significantly different between CLAU (1.93 LogMAR) and KLAL (1.78 LogMAR) subgroups. It was improved in 28 cases (13 CLAU and 15 KLAL), stayed unchanged in 13 cases (6 CLAU and 7 KLAL) and worsened in 9 cases (5 CLAU and 4 KLAL). We did not find any significant correlation between the type of chemical agent and final visual acuity.

The 1-year and 5-year LSCT survival rate for all cases was 93.4 % and 62.8 %, respectively. It was 100 % and 84.1 % for CLAU and 80.4 % and 40 % for KLAL subgroups, respectively (P = 0.037). Figure 1 shows Kaplan-Meier survival plot of the CLAU and KLAL subgroups.

The overall 1-year and 5-year corneal graft survival rate was 92.7 % and 41.8 %, respectively. It was 93.3 % and 63.8 % for CLAU and 92 % and 30.7 % for KLAL subgroups, respectively (P = 0.005). Figure 2 shows Kaplan-Meier survival plot of the PKP in each subgroup.

Five-year survival rate of LSCT and corneal graft in eyes with simultaneous KLAL and PKP was 37.5 % and 0 %, respectively. The mean interval between PKP and graft failure in simultaneous procedures was 30 ± 19.4 months.

Discussion

Our study showed high success rate of LSCT in patients with total LSCD due to severe chemical injury. Long-term survival of stem cell and corneal graft was significantly better in autograft group as it was expected. Simultaneous KLAL and PKP seems to carry a higher risk of failure of both stem cell and corneal grafts compared to sequential procedure. There was a difference

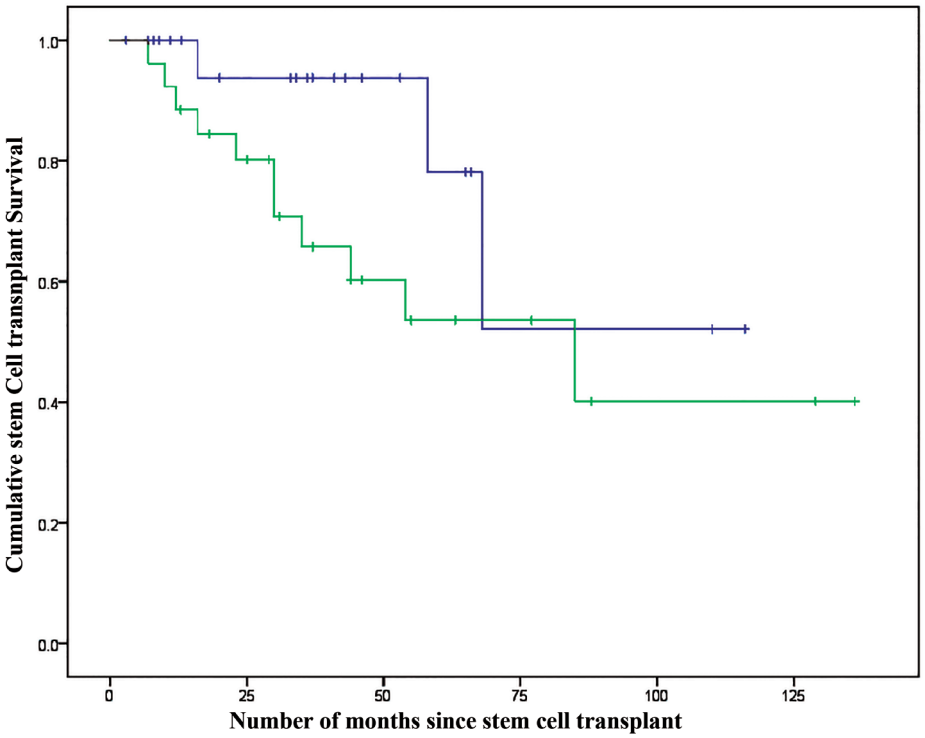

Figure 1: Cumulative survival of the stem cell transplantation in CLAU (blue) and KLAL (green) subgroups

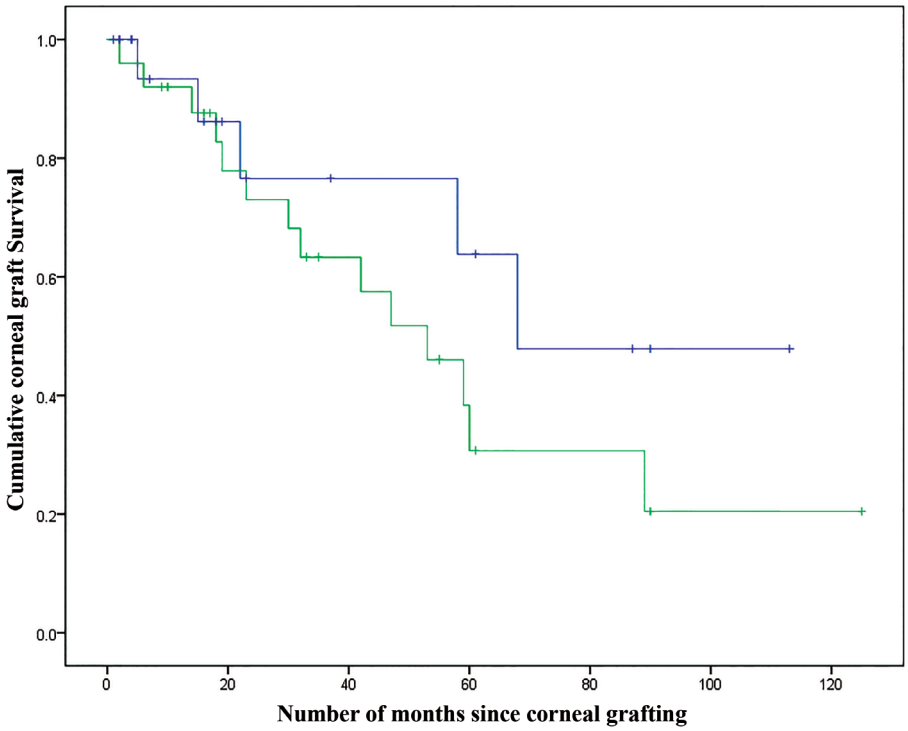

Figure 2: Cumulative survival of the corneal graft in CLAU (blue) and KLAL (green) subgroups

in survival rate between sequential versus simultaneous procedure but our findings might not be reliable because of small sample size.

CLAU has been proved to be a safe and effective procedure for restoration of limbal stem cells in unilateral LSCD [22]. In spite of the development of new epithelial transplant techniques, in unilateral limbal stem-cell deficiency, CLAU from a healthy unaffected fellow eye remains the best option available for restoration of corneal phenotype [23]. Concurrent amniotic membrane transplantation (AMT) has shown to be useful in providing smooth corneal surface for epithelial migration and repairing the adnexal abnormality (i.e. symblepharon) [22]. CLAU with AMT provides long-term symptom relief, improvement in visual acuity and regression of superficial corneal vessels in nearly all cases [22]. However, in a substantial proportion of cases with total LSCD, subsequent keratoplasty is required to restore vision. We did not observe iatrogenic LSCD in the donor eye in our cases. However, donor eye LSCD may develop if the donor eye limbus is already altered [24], hence one should confirm normal fellow eye before considering CLAU. Wylegala et al., [25] compared outcomes of KLAL, living-related conjunctival limbal allograft (lr-CLAL) and CLAU in 43, 26 and 21 eyes, respectively with a mean follow-up time of 31.2 months (range 6-72). They found that graft survival rate and the regularity of the corneal surface differed significantly between the allo and autografts [25]. The 3-year and 6-year graft survival rates were 76.1 % and 61.9 %, for the autologous transplantation group, and 59.4 % and 46.3 %, for the allogeneic transplantation group respectively [25]. However, Barreiro et al., [20] reported that the midterm (approximately 20 months) survival of CLAU was similar to living-related conjunctival limbal allograft (lr-CLAL). Other studies showed that despite the continuous administration of systemic immunosuppression, the success rate of KLAL declines from 75 % to 80 % after 1 year to 50 % after 3 years of followup [13-16]. Miri et al., [21] reported that the long-term survival for CLAU, lr-CLAL and KLAL was 100 %, 89 % and 33 %, respectively. They proposed that this difference might be due to freshness of the donors in CLAU and lr-CLAL and no rejection-related complications in autograft [21]. However the survival rate might be worse in cases with Stevens - Johnson syndrome (SJS), severe dry eye and adnexal abnormality. Preoperative dry eye has been reported as the most important prognostic factor for LSCT survival [11]. In addition, subsequent or simultaneous penetrating keratoplasty might be an additional risk factor for stem cell graft failure, especially in those cases who are known to be high-risk grafts due to significant stromal bed vascularization and concurrent ocular surface compromise [5; 6; 12; 26]. A previous study has reported 92 % graft survival at year 1, 77 % at year 2, 62 % at year 3, 55 % at year 4, and 54 % at year 5 after keratoplasty and LSCT [27].

We found a marked difference in long-term stem cell and corneal graft survival between CLAU and KLAL subgroups. In addition, the prognosis was worst in cases who had simultaneous KLAL and PKP. However, it should be noted that this is a retrospective study, and therefore the surgical procedure was not randomly selected. In addition, the relatively small number of subjects in each subgroup, especially small number of simultaneous procedure makes it difficult to make conclusions. The eyes undergoing KLAL might have more severe injury than those undergoing CLAU. However, the initial and final visual acuity was comparable between the two groups. Also, eyes that had combined limbal and central graft transplantation might have had more severe involvement in the corneal

stroma. Hence, a prospective randomized study is necessary to draw a final conclusion.

Conclusion

Severe chemical burn is associated with significant ocular morbidity and long-term prognosis is poor. Graft survival rate was significantly better in conjunctival limbal autograft compared to keratolimbal allograft when comparing the Long-term outcome of limbal stem cell transplantation for management of total limbal stem cell deficiency due to chemical burn.

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Footnotes and Financial Disclosures

Conflict of Interest:

The Authors have no conflict of interest with the subject matter of the present manuscript.