

## Outcomes of Limbal Stem Cell Transplantation in Limbal Stem Cell Deficiency

Ahmed S. Bayoumy, Mohamed M. Rezk, Amr I. Sharawy, Shereef M. Abdelwahab,  
Mohamed G. Masoud

Ophthalmology Department,  
Faculty of Medicine Benha  
University, Egypt.

**Corresponding to:**

Dr. Mohamed M. Rezk.  
Ophthalmology Department,  
Faculty of Medicine Benha  
University, Egypt.

**Email:**

rezkophth@gmail.com

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### Abstract:

**Purpose:** to evaluate the surgical outcomes and possible complications of limbal stem cell transplantation procedures including keratolimbal autograft (KLAU) transplantation and keratolimbal allograft (KLAL) transplantation. **Methods:** This prospective interventional clinical trial included “17 eyes” of patients (with mean of age of  $56.56 \pm 7.53$  years) with limbal stem cell deficiency (LSCD), at Benha University Hospitals, between May 2022 & May 2024. **Results:** The obtained results of this study showed a statistically significant difference (improvement of VA) between pre-operative and post-operative (after 3 months) visual acuity among studied eyes ( $p$ -value  $< 0.05$ ). It showed a statistically significant difference between surgical outcomes in Keratolimbal Autograft procedures. The obtained results of this study showed that 76.5% of studied eyes showed a complete success, 11.8% of studied eyes showed partial failure, while failure occurred in 11.8% of studied eyes. **Conclusion:** This study reported that limbal stem cell transplantation procedures including keratolimbal autograft (KLAU) transplantation and keratolimbal allograft (KLAL) transplantation- in cases of LSCD- had favorable surgical outcomes. Autologous keratolimbal graft had better outcomes than allografts, with

less costs, fewer complication rates.

**Keywords:** Cornea; Limbal Stem Cells (LSCs); Limbal Stem Cell Deficiency (LSCD); keratolimbal autograft (KLAU); keratolimbal allograft (KLAL).

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## Introduction

The cornea is a transparent tissue which comprises the outermost layer of the eye and acting as a protective barrier against noxious agents and a clear avascular window for optimal visual perception. These structural and functional attributes are reliant on healthy limbal stem cells (LSCs), which constitute a small population of total ocular surface epithelial cells in the basal layer of a highly vascularized and innervated zone between the cornea and conjunctiva known as the limbus <sup>(1)</sup>. Damage to the LSCs leads to limbal stem cell deficiency (LSCD), resulting in instability of the corneal epithelium. LSCD involves replacement of corneal epithelium with conjunctival epithelium, neovascularization, and inflammation <sup>(2)</sup>.

Patients with LSCD often present with pain, photophobia, and decreased vision. Slit-lamp examination shows conjunctival hyperemia, loss of the palisades of Vogt, and a “whorled-like” corneal epithelium. LSCD is also associated with poor epithelial adhesion, resulting in recurrent corneal erosions and persistent epithelial defects. At the chronic stage, the ocular surface is scarred and extensively neovascularized <sup>(3)</sup>. Impression cytology can be used in clinically non-diagnosable cases with high suspicion of LSCD. It detects mucin in the corneal epithelium, indicating presence of conjunctival goblet cells <sup>(4)</sup>.

The choice of procedure for LSCD depends on the extent of the ocular surface involvement (partial vs. total), the laterality (unilateral or bilateral), absence or presence of ongoing inflammation or infection and associated secondary glaucoma <sup>(5)</sup>. In cases with total LSCD, either autologous limbal lenticule from the fellow eye or cadaveric kerato-limbal allograft or allograft- taken from a living family-related donor graft transplantation- can be performed, coupled with topical or systemic immunosuppression <sup>(6)</sup>.

The aim of the study: to evaluate the surgical outcomes and possible complications of limbal stem cell transplantation procedures including keratolimbal autograft (KLAU) transplantation and keratolimbal allograft (KLAL) transplantation.

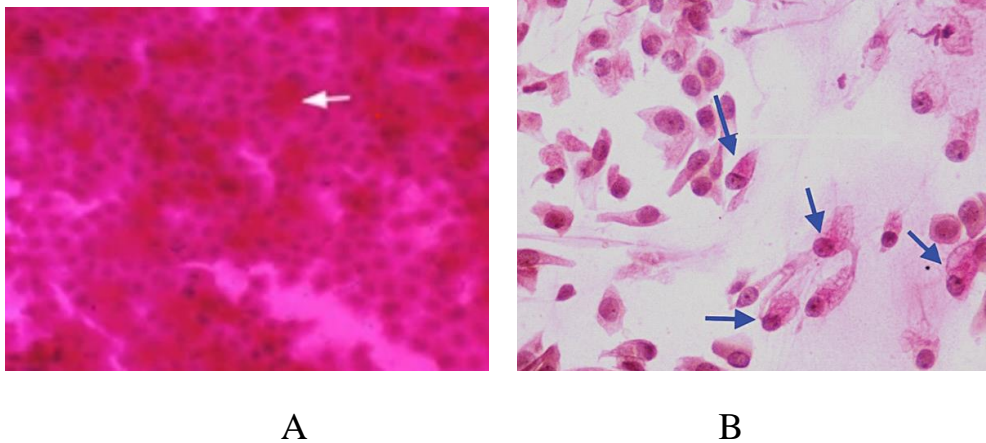
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## Patients & Methods:

This prospective interventional clinical trial included “17 eyes” of patients with limbal stem cell deficiency (LSCD), attending between May 2022 & May 2024, at Cornea subspecialty Clinic of Ophthalmology Department, at Benha University Hospitals. The study was conducted according to protocol approved by The Ethical Research Committee at Benha Faculty of Medicine with approval code: (MD 5-4-2022). Inclusion criteria included patients with LSCD or corneal neovascularization. Exclusion criteria included patients with previous corneal graft rejection, patients with collagen disorders, patients with poor optic nerve function or posterior segment pathology and patients who missed follow up visits. All patients were preoperatively subjected to the following:

**1- History taking, Complete ophthalmic examination of both eyes:** including visual acuity measurement, slit lamp examination & imaging, Ocular surface fluorescein staining, pupil reaction to light, color vision, IOP measurement, fundus examination, in cases of hazy fundus view, B-scan ultrasonography- was done.

**2- Impression cytology:** After instillation of topical anaesthesia, A filter paper of nitrocellulose was applied over the cornea for collection of superficial layers of cells. The presence of goblet cells or conjunctival epithelial cells on the corneal surface indicated invasion of the conjunctival epithelium over the cornea, as shown in Fig (1).



**Fig. 1:** Impression cytology specimens from LSCD cases (A, B):

- A- Impression cytology showing epithelial cells with goblet cells (white arrow)  
 B- Impression cytology showing goblet cells (blue arrows) (periodic acid Schiff stain).

#### Interventional procedures:

- All surgical procedures were performed by the same surgeon under complete aseptic conditions, after peribulbar anesthesia or General anesthesia in uncooperative patients.
- Graft preparation:
- In cases of unilateral LSCD, keratolimbal autograft (KLAU) transplantation from the healthy eye of the same patient- was done. Using a crescent blade, a shallow cut of 3 mm in length was made on the corneal side of the limbus, followed by two radial cuts. The crescent blade was used to create a 2×2 mm strip of limbal tissue by dissecting into the cornea and immediately grafted onto the recipient eye.
- In cases of bilateral LSCD or when patients with unilateral LSCD refused to use the healthy fellow eye as a source of autograft, Keratolimbal allograft transplantation (KLAL) was done, A cadaveric kerato-limbal allograft was obtained from the corneoscleral rim after punching a corneal graft used in another case of keratoplasty. A circular superficial limbal graft was prepared as the following: The graft was first trephined at 8 mm, then a peripheral superficial dissection was performed with a crescent blade, and the scleral tissue was removed with scissors,

and the allograft was immediately grafted onto the recipient eye.

#### - Graft Transplantation:

- A 360° conjunctival circular peritomy was made with scissors. The bulbar conjunctiva was dissected at the limbus 1-2 mm from the clear cornea and the vascular pannus covering the cornea was removed. The affected epithelium and subepithelial fibrosis covering the cornea were removed. Cautery was gently applied to any bleeding vessels.

- The graft was sutured to underlying sclera at the limbus by interrupted 10-0 Nylon sutures.

- Finally, instillation of topical antibiotic followed by application of a bandage contact lens.

#### ➤ Postoperative treatment and Follow-up:

- After surgery, all patients were treated with preservative-free lubricant eye drops every 2 hours, topical preservative-free dexamethasone (0.1%) and moxifloxacin eye drops (0.4%) four times daily- were given with gradual taper over one month.
- Systemic or topical immunosuppressive therapy (e.g., cyclosporine, tacrolimus)-

were used according to the degree of postoperative inflammation.

- Post-operative follow-up visits: after one day, one week, one month, three months.
- AS-OCT and slit-lamp imaging were done at follow-up visits after 3 months.
- The Primary outcomes of this study at the end of the follow-up period included: Relief of symptoms e.g. (pain, discomfort), BCVA improvement & ocular surface stability which consisted of intact corneal epithelium without late fluorescein staining, conjunctivalization, nor inflammation. Ocular surface failure or LSCD recurrence was defined as irregularity of the corneal epithelium, late fluorescein staining, conjunctivalization.
- The secondary outcomes included: complications to the donor eye or the recipient eye; and graft rejection.

#### Statistical Analysis:

The data were tabulated & statistically analyzed by the Statistical Package for

Social Sciences, (SPSS version 26.0 - SPSS Inc., Chicago, IL, USA). Basic descriptive statistics were calculated for all data, and values were reported as the mean  $\pm$  standard deviation (SD). P value less than 0.05 indicated statistical significance.

#### Results:

The obtained results of this study showed that 46.7% of studied patients were males while the remaining 53.3% were females with mean of age of  $56.56 \pm 7.53$  years. The most common cause among the studied eyes was chemical burn representing about 47.1%, while, the least common cause- was ocular trauma representing about 5.9%. The largest percentage of studied eyes were with LSCD stage III representing about 41.2% and corneal vascularization in four quadrants and central zone. Corneal stromal opacities, abnormal central corneal epithelium and late fluorescein- were present among 58.8%, 64.7% and 100% of studied eye,s respectively, as shown in Table (1).

**Table (1):** Pre-operative data among studied eyes (n=17)

| Variable                             | Value                     |    |
|--------------------------------------|---------------------------|----|
|                                      | No. of eyes               | %  |
| Side of affected eye                 | Right                     | 7  |
|                                      | Left                      | 8  |
|                                      | Both eyes                 | 2  |
| Aetiology                            | Chemical burn             | 8  |
|                                      | Contact lens              | 3  |
|                                      | Dry eye syndrome          | 2  |
|                                      | Infectious keratitis      | 3  |
|                                      | Ocular trauma             | 1  |
|                                      | Stage I-A                 | 1  |
| LSCD staging                         | Stage I-B                 | 2  |
|                                      | Stage I-C                 | 2  |
|                                      | Stage II-B                | 3  |
|                                      | Stage II-C                | 2  |
|                                      | Stage III                 | 7  |
| Corneal stromal opacities            | Present                   | 10 |
|                                      | Absent                    | 7  |
| Corneal neovascularization           | 1 quadrant                | 2  |
|                                      | 2 quadrants               | 2  |
|                                      | 3 quadrants               | 3  |
|                                      | 4 quadrants               | 3  |
|                                      | 4 quadrants+ central zone | 7  |
| Abnormal central corneal epithelium  | Present                   | 11 |
|                                      | Absent                    | 6  |
| Late fluorescein epithelial staining | Present                   | 17 |
|                                      | Absent                    | 0  |

LSCD: Limbal stem cell deficiency, HM: Hand movement, CF: Counting fingers.

The obtained results of our study showed a statistically significant difference between pre-operative and post-operative abnormal central corneal epithelium and late fluorescein epithelial staining among studied eyes as they improved post-operatively after 3 months (p-value <0.05), as shown in Table (2).

The obtained results of this study showed a statistically significant difference (improvement of VA) between pre-operative and post-operative (after 3 months) visual acuity among studied eyes (p-value <0.05), as shown in Table (3).

The obtained results of this study showed a statistically significant difference (Improvement of VA) between LSCD stages regarding post-operative (after 3 months) visual acuity (p-value <0.05), as shown in Fig.(2), Table (4).

The obtained results of this study showed that in cases due to chemical burn, contact lens and dry eye syndrome- there was a statistically significant difference between their surgical outcome (p-value <0.05), but in cases due to infectious keratitis and ocular trauma- there was no statistically significant difference between their surgical outcome (p-value >0.05), as shown in Fig.(3), Table (5).

In our study, there were no major intraoperative complications either in donor or recipient eyes. Postoperative subconjunctival haemorrhage occurred in

3 out of 17 eyes, it was self-limited and achieved spontaneous resolution within 2 weeks. Postoperative ocular surface inflammation occurred in 2 out of 17 eyes of KLAL procedures which indicated early rejection, so, topical and systemic cyclosporine were added to the treatment; one case ended in ocular surface failure, the other case partially improved. There was another reported postoperative ocular surface failure occurred in a case of KLAL due to reactivation of necrotizing viral stromal keratitis leading to stromal melting which rapidly progressed to impending corneal perforation. This case was managed by systemic antivirals and amniotic membrane grafting, as shown in Fig.(4).

The obtained results of this study showed that 76.5% of studied eyes showed a complete success, 11.8% of studied eyes showed partial failure, while failure occurred in 11.8% of studied eyes. It showed a statistically significant difference between surgical outcomes in Keratolimbal Autograft procedures, but Keratolimbal Allograft showed no statistically significant difference between surgical outcomes, which indicated a better surgical outcome in Keratolimbal Autograft than in Keratolimbal Allograft procedures, as shown in Fig.(5,6) & Table (6).

**Table (2):** Pre-operative and post-operative abnormal central corneal epithelium and late fluorescein epithelial staining among studied eyes (n=17):

| Variable                             | Pre-operative |      | Post-operative |      | Test of significance | p-value |
|--------------------------------------|---------------|------|----------------|------|----------------------|---------|
|                                      | No.           | %    | No.            | %    |                      |         |
| Abnormal central corneal epithelium  |               |      |                |      |                      |         |
| Present                              | 11            | 35.3 | 4              | 23.5 | Mc=4.00              | 0.046*  |
| Absent                               | 6             | 64.7 | 13             | 76.5 |                      |         |
| Late fluorescein epithelial staining |               |      |                |      |                      |         |
| Present                              | 17            | 100  | 3              | 17.6 | Mc=12.07             | 0.001*  |
| Absent                               | 0             | 0    | 14             | 82.4 |                      |         |

\*: Statistically significant, Mc: Mc Nemar test

**Table (3):** Pre- and post-operative visual acuity among studied eyes (n=17)

| VA      | Pre-operative |      | Post-operative |      | Test of significance | p-value       |
|---------|---------------|------|----------------|------|----------------------|---------------|
|         | No.           | %    | No.            | %    |                      |               |
| HM      | 6             | 35.3 | 5              | 29.4 | MH=3.24              | <b>0.005*</b> |
| CF 50cm | 2             | 11.8 | 1              | 5.9  |                      |               |
| CF 1 m  | 1             | 5.9  | 0              | 0    |                      |               |
| CF 2 m  | 2             | 11.8 | 2              | 11.8 |                      |               |
| 0.05    | 1             | 5.9  | 2              | 11.8 |                      |               |
| 0.1     | 3             | 17.6 | 3              | 17.6 |                      |               |
| 0.16    | 1             | 5.9  | 0              | 0    |                      |               |
| 0.2     | 1             | 5.9  | 2              | 11.8 |                      |               |
| 0.3     | 0             | 0    | 2              | 11.8 |                      |               |

\*: Statistically significant, MH: Marginal homogeneity test, VA: Visual acuity, HM: Hand movement, CF: Counting fingers

**Table (4):** Relation between LSCD stages and post-operative VA among studied eyes (n=17)

| post-operative<br>VA | LSCD stages           |                       |                       |                        |                        |                       | Test of<br>significance | p-value           |
|----------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|-----------------------|-------------------------|-------------------|
|                      | Stage<br>I-A<br>(n=1) | Stage<br>I-B<br>(n=2) | Stage<br>I-C<br>(n=2) | Stage<br>II-B<br>(n=3) | Stage<br>II-C<br>(n=2) | Stage<br>III<br>(n=7) |                         |                   |
| HM                   | 0 (0%)                | 0 (0%)                | 0 (0%)                | 0 (0%)                 | 0 (0%)                 | 5 (71.4%)             | $\chi^2=60.18$          | <b>&lt;0.001*</b> |
| CF 50 cm             | 0 (0%)                | 0 (0%)                | 0 (0%)                | 0 (0%)                 | 0 (0%)                 | 1 (14.3%)             |                         |                   |
| CF 2 m               | 0 (0%)                | 0 (0%)                | 0 (0%)                | 0 (0%)                 | 2 (100%)               | 0 (0%)                |                         |                   |
| 0.05                 | 0 (0%)                | 0 (0%)                | 0 (0%)                | 1(33.3%)               | 0 (0%)                 | 1 (14.3%)             |                         |                   |
| 0.1                  | 0 (0%)                | 0 (0%)                | 2 (100%)              | 1(33.3%)               | 0 (0%)                 | 0(0%)                 |                         |                   |
| 0.2                  | 1 (100%)              | 0 (0%)                | 0 (0%)                | 1(33.3%)               | 0 (0%)                 | 0(0%)                 |                         |                   |
| 0.3                  | 0 (0%)                | 2 (100%)              | 0 (0%)                | 0 (0%)                 | 0 (0%)                 | 0 (0%)                |                         |                   |

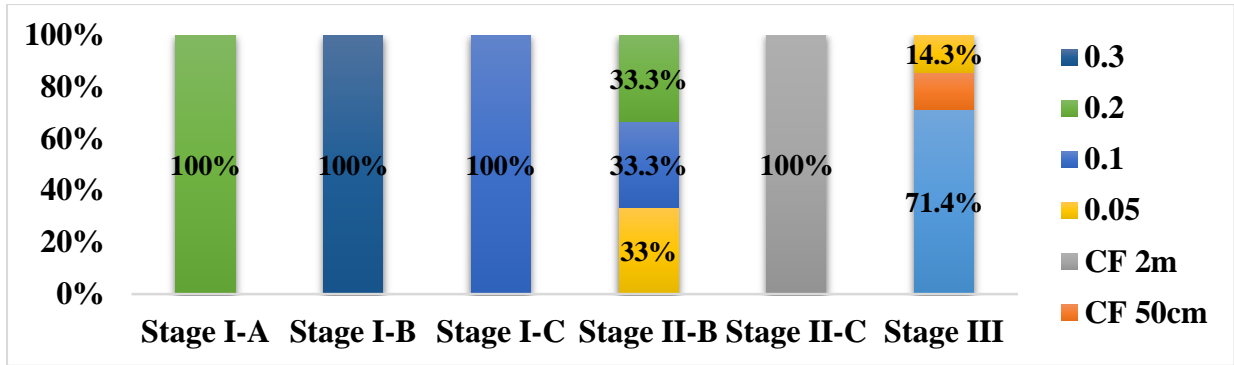
\*: Statistically significant,  $\chi^2$ : Chi-squared test, VA: Visual acuity, HM: Hand movement, CF: Counting fingers.**Table (5):** Relation between aetiology of LSCD and surgical outcomes among studied eyes:

| Aetiology                  | Surgical outcomes |      |                 |      |         |      | $\chi^2$ | p-value |
|----------------------------|-------------------|------|-----------------|------|---------|------|----------|---------|
|                            | Success           |      | Partial failure |      | Failure |      |          |         |
|                            | No.               | %    | No.             | %    | No.     | %    |          |         |
| Chemical burn (n=8)        | 7                 | 87.5 | 0               | 0    | 1       | 12.5 | 16.13    | <0.001* |
| Contact lens (n=3)         | 3                 | 100  | 0               | 0    | 0       | 0    | 9.00     | 0.011*  |
| Dry eye syndrome (n=2)     | 2                 | 100  | 0               | 0    | 0       | 0    | 6.00     | 0.049*  |
| Infectious keratitis (n=3) | 0                 | 0    | 2               | 66.7 | 1       | 33.3 | 3.00     | 0.223   |
| Ocular trauma (n=1)        | 1                 | 100  | 0               | 0    | 0       | 0    | 3.00     | 0.223   |

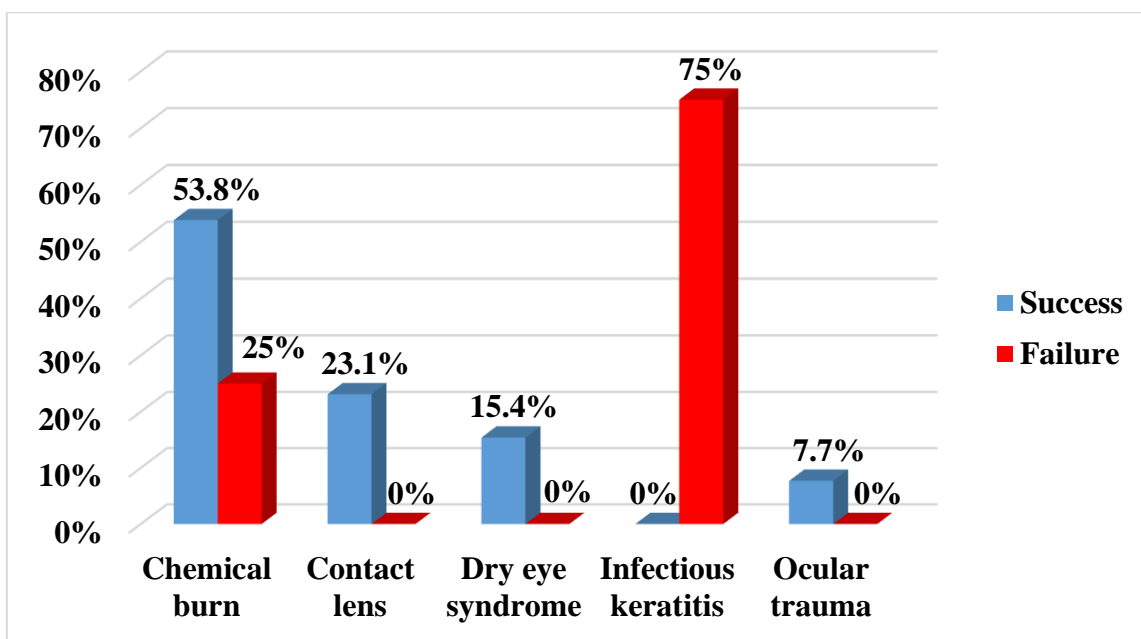
\*: Statistically significant,  $\chi^2$ : Chi-squared test, Percentage calculated by row**Table (6):** Surgical outcomes among studied eyes and their relation to type of surgery:

| Variable                       | Surgical outcomes |      |                 |      |         |      | $\chi^2$ | p-value |
|--------------------------------|-------------------|------|-----------------|------|---------|------|----------|---------|
|                                | Success           |      | Partial failure |      | Failure |      |          |         |
|                                | No.               | %    | No.             | %    | No.     | %    |          |         |
| No. of studied eyes (n=17)     | 13                | 76.5 | 2               | 11.8 | 2       | 11.8 | 21.34    | <0.001* |
| Type of surgery                |                   |      |                 |      |         |      |          |         |
| Keratolimbal Allograft (n=6)   | 3                 | 50   | 1               | 16.7 | 2       | 33.3 | 1.50     | 0.473   |
| Keratolimbal Auto-graft (n=11) | 10                | 90.9 | 1               | 9.1  | 0       | 0    | 24.81    | <0.001* |

\*: Statistically significant,  $\chi^2$ : Chi-squared test, Percentage calculated by row



**Fig. 2:** LSCD stages in relation to post-operative VA among studied eyes



**Fig. 3:** Surgical outcomes in relation to aetiology among studied eyes



(a)

(b)

(c)

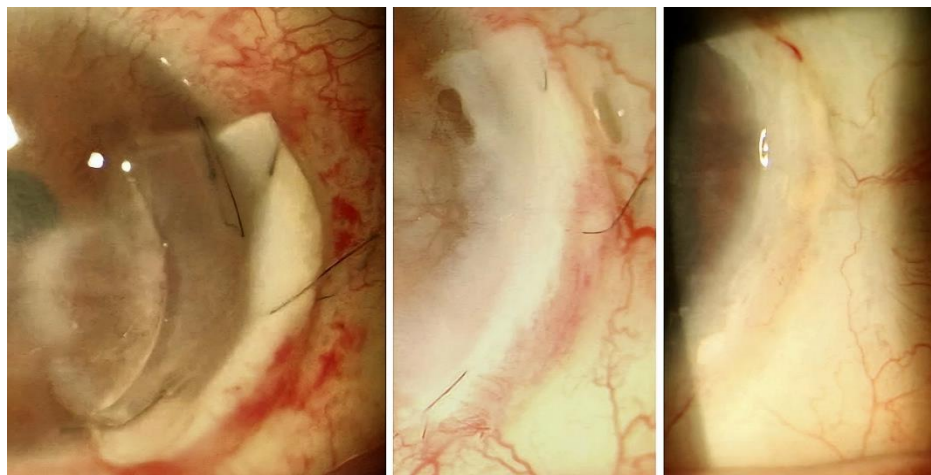
**Fig. 4:** A case example of postoperative ocular surface failure and its management:  
a- Necrotizing Stromal keratitis with epithelial defect and stromal melting.  
b- Descemetocoele and impending corneal perforation.  
c- Amniotic membrane grafting.





(a) (b) (c)

**Fig. 5:** Pre-operative and post-operative photo slit lamp images of Successful Keratolimbal Autograft: Pre-operative (a), after one week (b), and after 3 Months (c).



(a) (b) (c)

**Fig. 6:** Post-operative photo slit lamp images of Successful Keratolimbal Allograft: After one week (a), after 1 month (b) and after 3 Months (c).

## Discussion:

The diagnosis of LSCD depends on both clinical examination and imaging techniques. LSCD is often misdiagnosed, especially at its early stage. Patients may present with non-specific complaints that are associated with other ocular surface disorders, and clinical examination is non-specific as well <sup>(7)</sup>. Precise assessment of the limbal niche before LSC transplantation is essential to predict the therapeutic effect of LSC transplantation and determine which patients can benefit from LSC transplantation <sup>(8)</sup>.

Our study included “17 eyes” of patients with limbal stem cell deficiency (LSCD), with mean of age of  $56.56 \pm 7.53$  years, 46.7% of studied male patients while the remaining 53.3% were females. The most common aetiology among studied eyes in our study was chemical burn representing about 47.1% while the least common cause was ocular trauma representing about 5.9%.

One study included a total of 22 patients underwent Allogeneic limbal transplantation surgery. The mean patient age was 69.5 years. Patient LSCD aetiology was 59% infectious and 41% traumatic <sup>(9)</sup>. Another study included 14



patients (6 females, 8 males). The mean age of patients was 69 years. Five of the patients developed LSCD due to chemical burn. Nine of the patients got LSCD caused by infectious keratitis <sup>(10)</sup>.

Another study showed that eyes with chemical burns fared more favourably than those with ocular pemphigoid and Stevens-Johnson syndrome. The success rate of LSC transplantation may vary according to the original cause of LSCD, preoperative LSCD stage, the presence of other associated factors- inflammatory, immunologic, genetic- that may significantly influence graft survival <sup>(11)</sup>.

Our study also showed that in cases due to chemical burn, contact lens and dry eye syndrome- there was a statistically significant difference between their surgical outcome (p-value <0.05), but in cases due to infectious keratitis and ocular trauma- there was no a statistically significant difference between their surgical outcome (p-value >0.05).

A single-centre analysis of 125 cases of autologous SLET for unilateral LSCD by Sangwan's group demonstrated a 76% success rate and a 75% two-line improvement in visual acuity <sup>(12)</sup>. Similarly, a multi-centre analysis of 68 cases of autologous SLET for unilateral LSCD demonstrated an 84% success rate and a 65% two-line improvement in visual acuity <sup>(13)</sup>.

A systematic review of 22 studies including 1023 eyes, aimed to compare the clinical outcomes of three different techniques of autologous LSC transplantation for treatment of unilateral LSCD. The findings of the review clearly indicated that autologous LSC transplantation- irrespective of the technique used- is a safe and effective therapy for this condition; and that SLET and CLAU have better long-term anatomical and functional success rates in comparison to CLET. The anatomical success rates were almost identical between SLET and CLAU, while the

functional success rates were marginally better with CLAU <sup>(14)</sup>.

Transplantation of autologous LSC was reported to restore a stable corneal surface in 71% of studied eyes with a two-line improvement in visual acuity in 60.5% of eyes <sup>(15)</sup>. The obtained results of our study showed a statistically significant difference (Improvement of VA) between pre-operative and post-operative (after 3 months) visual acuity among studied eyes (p-value <0.05).

The dramatic difference observed between allogeneic grafts and autologous grafts- is likely to be the result of rejection of transplanted allogeneic LSC <sup>(16)</sup>. A difference in LSC impairment could also explain the difference between autologous and allogeneic cases. It was hypothesized that autologous cases could retain buried limbal crypts that are insufficient to maintain limbal function but that could help the transplanted cells to restore limbal function <sup>(17)</sup>.

It was reported an overall success rate of 76% (77% for autografts and 73% for allografts) <sup>(18)</sup>. The obtained results of this study showed that 76.5% of studied eyes showed a complete success, 11.8% of studied eyes showed partial failure, while failure occurred in 11.8% of studied eyes.

### Limitations

One of our study limitations was the shortage of cadaveric keratolimbal allografts due to high cost of corneal graft importing and unavailability of local eye banks. Our study was limited also by the refusal of some patients with unilateral LSCD to harvest Keratolimbal Autograft from the healthy eye. The relatively small sample size and short follow up period- were also considered as study limitations.

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### Conclusion

this study concluded that limbal stem cell transplantation procedures including keratolimbal autograft (KLAU) transplantation from the other eye in unilateral LSCD and keratolimbal allograft (KLAL) transplantation in cases of

bilateral LSCD- had favorable surgical outcomes and low rate of complications. Autologous keratolimbal graft had better outcomes than allografts, with less costs, fewer complication rates.

### Recommendations

- Further prospective studies with larger sample size, with longer follow up duration- are warranted to assess long term surgical outcomes and possible complications of different limbal stem cell transplantation procedures.
- Further studies are recommended to evaluate the surgical outcomes of combined Keratoplasty and LSC transplantation either in single procedure or two separate procedures.

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