

Conjunctivolimbal Autograft versus Cadaveric Keratolimbal Allograft in Ocular Surface Disorder: A Comparison

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Purpose:- To compare the outcomes of conjunctivolimbal autograft (CLAU) and cadaveric keratolimbal allograft (KLAL) in limbal stem cell deficiency (LSCD) with ocular surface disorders.

Materials & Methods:- In this prospective randomized clinical study, 50 eyes of 50 patients with unilateral LSCD were divided into two groups based on the surgical intervention. Group 1 patients (25 eyes) had CLAU, whereas group 2 patients (25 eyes) underwent cadaveric KLAL. The outcome measures were functional vision (best corrected visual acuity $\geq 6/60$), gain of ≥ 2 lines of Snellen's visual acuity, corneal neovascularisation, visually significant corneal opacity (Iris details poorly visible), fluorescein stain, Schirmer's ≥ 10 mm, tear break-up time (TBUT) ≥ 10 seconds.

Abstract

Results:- At 1 year of follow-up, both CLAU and cadaveric KLAL were comparable in terms of visual gain of ≥ 2 lines, functional vision, corneal neovascularisation, Schirmer's ≥ 10 mm, TBUT ≥ 10 seconds. The epithelisation was found to be early with CLAU as compared with cadaveric KLAL ($p = 0.032$). Patients with cadaveric KLAL had more recurrent epithelial defects (40%) as compared to CLAU (24%).

Conclusions:- Both procedures are effective in terms of visual gain and ocular surface restoration. Cadaveric KLAL seems to be a good alternative whenever CLAU is not possible.

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Keywords: limbal stem cell deficiency; ocular surface disorder; conjunctivolimbal autograft; keratolimbal allograft

Introduction

The ocular surface is defined as the tear film and entire epithelial lining bordered by the skin at the superior and inferior eyelid margin. Histologically and physiologically, the ocular surface has two major areas of cornea and conjunctiva separated by the limbus. A smooth corneal surface of epithelial cells supporting an intact tear film is essential for good vision. Ordinarily, a natural turnover of corneal epithelium cells takes place in which superficial cells are shed and replaced by those below. For this system to perpetuate, a constant supply of corneal epithelial cells is required. This constant supply is thought to come from corneal stem cells that reside in the Palisades of Vogt, in the limbal region.^{1,3} The renewal occurs by centripetal and circumferential migration of cells from the limbus.^{2,3} Limbal stem cells deficiency (LSCD) arises in various diseases such as viral keratoconjunctivitis, chronic trachoma, extensive / recurrent pterygium, severe dry eyes, chronic conditions like Stevens Johnson syndrome, radiation keratitis, drug toxicity and ocular cicatricial pemphigoid, congenital abnormalities, such as aniridia, chemical injury. The hallmark of LSCD is a triad of conjunctivalization, neovascularization and chronic inflammation. Ocular reconstruction in LSCD can be achieved by either transplanting conjunctivolimbal autograft (CLAU) from the fellow eye⁴ or conjunctivolimbal allograft from a living related donor (Lr-CLAL)^{5,6} or a keratolimbal allograft (KLAL) from a cadaveric donor.^{7,8} Studies related to cadaveric limbal allograft have been few.^{9,10} Head to head comparison of conjunctival autograft versus cadaveric keratolimbal allograft (KLAL), in terms of visual outcome and ocular surface stability has not been done. Our study aimed to compare

conjunctival limbal stem cell autograft and cadaveric limbal stem cell graft in ocular surface disorders in terms of visual gain and ocular surface restoration.

Material & Methods

This prospective randomized comparative study was conducted at a tertiary-level referral institute. The study comprised of 50 eyes of 50 patients with clinically diagnosed cases of unilateral ocular surface disorders, undergoing limbal stem cells transplantation (LSCT) via CLAU or cadaveric limbal allograft. The study was conducted after approval from institutional ethical committee and an informed written consent was taken with all the tenets of Declaration of Helsinki were followed.

The patients were randomized into two groups by a simple randomization (odd-even) method using computer-generated random numbers: those with even numbers were assigned to CLAU (group 1) and those with odd numbers were assigned to cadaveric limbal allograft (group 2) for LSCT. A detailed clinical history and comprehensive ophthalmic examination was performed in all cases. Uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA), slit lamp examination for ocular surface were recorded. Fluorescein staining to detect breaks in the corneal epithelial barrier and Rose bengal staining to stain the mucus, debris, and devitalized cells of corneal and conjunctival epithelium was performed. Tear film studies, Schirmer tests (1 & 2), Tear film break up time (TBUT) test were used to assess the tear film adequacy. Impression cytology of the perilimbal area with nitrocellulose acetate paper to detect the presence of goblet cells over the cornea was done. Histopathology of the resected pannus was also

done to confirm the diagnosis in selected case in which there was a diagnostic ambiguity. Fundus examination wherever media was clear to rule out posterior segment pathology, was also performed. The lesions included in the study were unilateral ocular surface disorder like post keratitis vascularised corneal scar, chemical or thermal burns, recurrent pterygium, scarring due to any other cause like severe spring catarrhal. Patients with bilateral ocular surface disease were excluded as CLAU is not feasible in bilateral cases and poses a risk of more LSCD. Patients with systemic autoimmune disorders, severe lid deformity, prior ocular surgeries and with any other ocular disease other than ocular surface disorders were also excluded.

Donor Tissue Extraction

Under peribulbar anaesthesia, graft was obtained from the fellow eye with healthy ocular surface. The size of the conjunctival graft required to resurface the exposed scleral surface was determined using Castrovejo calipers in three directions as extent across the limbus, maximum circumferential extent of the bed, and maximum distance from the limbus. This enabled the harvested graft to fit precisely in the bed. The limbal area exposed first and a lamellar dissection 0.5 – 1 mm depth done parallel to the limbus using a surgical blade. Graft of 2 – 3 clock hours in length which extend 1 mm on corneal side and 1- 2 mm on the sclera side was harvested. Dissection carried out with crescent knife bevel up. The lenticule was kept as thin from endothelial side to keep as little stroma in the donor lenticule as possible. The dissected strip placed in sterile Ringer's lactate solution. The donor site was left as such. All patients were operated under peribulbar anesthesia. In the recipient eye, cicatrized tissue over limbus was dissected from the limbus and cornea to have ideal bed for subsequent smooth ocular surface following this procedure. At the end bandage contact lens was applied over the cornea for 7 days. For KLAL, cadaver fresh donor eyes are preferred as the success of the procedure depends on the transplantation of healthy limbal stem cells. Donor graft was taken from eyes enucleated promptly after death preferably within 6 hours and stored for up to 24 hours at 4°C. The globe is wrapped around with a strip of wet gauze and held on a Tudor Thomas stand. A trephine, with a diameter 3 mm smaller than the corneal diameter is used to trephine the donor central cornea into one fourth to one fifth of the stromal depth (approximately 150 µm). A 360 degree peritomy was fashioned to expose limbus. Partial annular keratec-tomy was also performed up to 2 mm from the limbus as preparatory to cadaveric annular graft transplantation. Residual fibrous tissue on the cornea was removed by sharp dissection with the help of crescentic micro surgical blade.

Preparing recipient bed and placing graft. The protocol and initial procedure anaesthesia techniques were essentially identical in both the groups. Under peribulbar anesthesia, cicatrized tissue over limbus and cornea was dissected to have ideal bed for subsequent smooth ocular surface. Residual fibrous tissue over the cornea was also removed by sharp dissection with the help of crescent knife. Peripheral

superficial keratectomy was performed for graft bed using surgical blade. The position of the graft was secured using interrupted 10.0 nylon sutures. The four corners of the graft were an-chored with episcleral bites to maintain position.

All patients received antibiotic steroid ointment along with pad and bandage for the first 24 hrs. Postoperatively, moxifloxacin 0.5%, prednisolone Acetate 1% eye drops and preservative free tear substitute every 2 hourly, for first month were prescribed. Topical cyclosporine 2% was given twice daily for 6 months. The medications were subsequently tapered over the next one month. The follow-up examinations were done at day 1, 1 week, 1 month, 3 months, 6 months, and then at 6-month intervals thereafter. Patients with KLAL (group 2), received systemic immunosuppression. Treatment with oral cyclosporine 5 to 10 mg per kilogram of body weight, was begun one day before surgery and continued for at least one month postoperatively and thereafter if kidney function was normal. Any sign of infection, such as infective keratitis, corneal necrosis, acute allograft rejection, or persistent epithelial defect (PED), was noted.

Outcome Measures

The outcome measures included were functional vision (BCVA \geq 6/60), gain of 2 or more lines of visual acuity, regression of corneal neovascularization, visually significant corneal opacity (iris details poorly visible), Schirmer's test \geq 10 seconds, TBUT \geq 10 seconds. Fluorescein staining and time for complete epithelisation were also assessed. Post-operative complications were noted including graft rejections in both groups.

Statistical analysis was performed using SPSS for Windows software (version 16.0, SPSS, Inc, Chicago, IL). Pearson's chi-square tests were used to compare postoperative outcome at 1 year versus preoperative. Also, it has been used to compare the various outcome measures between group 1 and group 2. P value $<$ 0.05 was considered statistically significant.

Results

Out of 50 eyes of 50 patients included in the study, 25 were allocated into group 1 (CLAU) and another 25 into group 2 (cadaveric KLAL). The mean age was 55.64 ± 6.71 and 58.44 ± 7.82 years in group 1 and group 2, respectively ($p = 0.12$). Sex distribution and laterality is described in Table 1. The mean duration of LSCD in patients of the group 1 was 12.3 ± 3.71 months and the KLAL group was 11.9 ± 3.41 months ($p = 0.7$). All patients in both group 1 and group 2 were followed-up for the minimum period of 1 year. The most common etiology in our study was chemical/thermal burn in both group 1 (64%) and group 2 (60%). Rest of the patients were having post allergic keratoconjunctivitis, keratoconjunctivitis sicca. (Table 2).

Visual Acuity

The number of eyes with BCVA of 6\60 or better (functional vision) were significantly increased from 10 (40%) to 16 (64%) in group 1 ($p = 0.03$), and from 8 (32%) to 16 (64%) eyes

Table 1: Demographic characteristics of patients in Group 1 and Group 2

	Group 1 (Conjunctivolimbal Autograft)	Group 2 (Cadaveric keratolimbal Allograft)
No. of patients	25	25
No. of eyes	25	25
Age, mean \pm SD, years	55.64 \pm 6.71	58.44 \pm 7.82
Age range, years	32 - 64	35 - 62
Men, n (%)	18 (72)	20 (80)
Women, n (%)	7 (28)	5 (20)

Table 2: Aetiology of ocular surface disorder in Group 1 and Group 2

	Group 1 Conjunctivolimbal autograft No. of eyes (%)	Group 2 Cadaveric kerato- limbal allograft No. of eyes (%)	Total number of eyes No. of eyes (%)
Chemical \ Thermal Injuries	16 (64)	15 (60)	31(62)
Post allergic kera- toconjunctivitis	5 (20)	4 (16)	9 (18)
Kerato conjunc- tivitis sicca	4 (16)	6 (24)	10 (20)

in group 2 ($p = 0.02$), postoperatively at 1 year of follow-up. However, there was no statistically significant difference in functional vision was noticed between two groups at the end of 6 months ($p = 0.786$). Two or more lines of visual acuity improvement were noticed in 22 eyes in group 1 ($p = 0.001$), and 20 eyes in group 2 ($p = 0.001$).

Ocular surface

Corneal neovascularisation was found to be significantly less in postoperative period, the incidence of neovascularisation reduced from 23 (92%) to 13 (52%) eyes in group 1 ($p = 0.032$), and from 25 (100%) to 18 (72%) eyes in group 2 ($p = 0.046$), primarily affecting superficial neovascularisation. Regression in corneal neovascularisation was more in group 1 (43.47%) as compared to group 2 (28%), but not statistically significant ($p = 0.056$). Corneal opacity was also seen in 14 (56%) eyes in group 1, and 15 (60%) eyes in group 2 preoperatively which were reduced to 6 (24%) eyes in group 1 ($p = 0.003$), and 7 (28%) eyes in group 2 ($p = 0.002$), respectively (Table 3).

Tear film tests

Various tests were performed to assess the ocular surface stability. The number of eyes with Schirmer's ≥ 10 mm increased from 5 (20%) to 16 (64%) in group 1, postoperatively at 1 year of follow-up. Also, 3 eyes (12%) with Schirmer's < 5 mm showed an improvement to reach 5-10 mm bracket. Among group 2 also, eyes with Schirmer's ≥ 10 mm went up from 5 (20%) preoperatively, to 14 (56%) postoperatively. Two eyes (8%) with reading of < 5 mm improved to the 5 - 10 mm bracket postoperatively. The difference in the Schirmer's values in both the groups following surgery were found to be statistically significant ($p = 0.008$ in group 1; $p = 0.002$ in group 2) (Table 3). There was no statistical significant difference in Schirmer's between both the groups ($p = 0.843$) (Table 3).

Fluorescein staining was used to assess the epithelial integrity. In group 1, number of eyes with positive stain were reduced from 9 (36%) preoperatively to only 3 (12%) postoperatively, showing a marked improvement in the epithelisation. Ten eyes (40%) in group 2 with positive staining were reduced to 3 eyes (12%) following the surgery. Fluorescein staining pattern showed statistically significant difference in both groups ($p = 0.028$ in group 1; $p = 0.02$ in group 2) postoperatively. However, no statistical significant difference was noticed in staining pattern between both the groups ($p = 0.882$) (Table 3).

A significant improvement in tear film status as measured by TBUT was noticed in both the groups. The number of eyes with TBUT ≥ 10 seconds, improved from 10 (40%) preoperatively, to 18 (72%) postoperatively in group 1 ($p = 0.02$). Among group 2 also, there was an improvement in the tear film status with the number of patients with TBUT ≥ 10 seconds increased from 7 (28%) preoperatively, to 14 (56%) postoperatively ($p = 0.008$). No significant difference was noticed in TBUT between two groups ($p = 0.845$) (Table 3).

The epithelisation was found to be better and early with autograft as compared with cadaveric graft. The time for complete epithelisation varied between 7 and 14 days in the autograft group with a mean of 8 days. In the second group the healing time varied from 7 to 18 days with a mean of 12 days. There was statistical significant difference noticed for complete epithelisation time between both the groups ($p = 0.032$). In the KLAL group, 18 patients had healing time more than 10 days.

Table 3

	Group 1 (Conjunctivolimbal autograft)			Group 2 (Cadaveric keratolimbal allograft)		
	Preoperative No. of eyes: n (%)	Postoperative No. of eyes: n (%)	'p' value	Preoperative No. of eyes: n (%)	Postoperative No. of eyes: n (%)	'p' value
Corneal neovascularisation	23 (92)	13 (52)	0.032	25 (100)	18 (72)	0.046
Corneal opacity	14 (56)	6 (24)	0.003	15 (60)	7 (28)	0.002
Schirmer's test ≥ 10 mm	5 (20)	16 (64)	0.008	5 (20)	14 (56)	0.002
Fluorescein staining	9 (36)	3 (12)	0.028	10 (40)	3 (12)	0.02
Tear break-up time \geq 10 seconds	10 (40)	18 (72)	0.02	7 (28)	14 (56)	0.008

Complications

Persistent epithelial defects (defects in epithelium not resolved for > 2 weeks) occurred in 7 (28%) eyes in group 1 and 9 (36%) in group 2. Most such defects were treated successfully either by tarsorrhaphy, frequent use of autologous serum eye drops or transplantation of amniotic membrane. However, 3 eyes in group 2 (cadaveric KLAL) did not healed. These 3 eyes had chemical burn.. Recurrences of epithelial defects were more encountered in cadaveric KLAL (group 2). About 40% eyes in group 2 developed epithelial defects during the follow-up as compared with 24% recurrences in group 1.

Secondary glaucoma developed in 4 (16%) cases of group 1 and 7 (28%) cases of group 2. Of the 11 (22%) eyes with secondary glaucoma, 2 underwent trabeculectomy to reduce intraocular pressure whereas, rest of the other patients were successfully treated with medication. Postoperative uveitis was seen in 2 (8%) cases in group 1, and 7 (28%) cases in group 2. Infectious keratitis was also seen in 2 (8%) cases of group 2 which eventually pro-gressed to rejection episode of the cadaveric KLAL with intense congestion along scleral edge, edema of donor tissue and clouding of tissue and later one of the eye had perforation of cornea.

Discussion

Management of ocular surface disorders with LSCD is always a challenging task for an ophthalmologist. Such disorders cause prolonged inflammation of the ocular surface, damaging the corneal stem cells and epithelial basement membrane. Clinically the corneal surfaces in these disorders carry a common manifestation of poor epithelialization, chronic stromal inflammation, neovascularisation, conjunctival epithelial in growth (conjunctivalization) and decreased vision. In addition matrix metalloproteinase produced by keratocytes, epithelial cells and neutrophils can cause progressive stromal ulceration with risk of corneal perforation. Conjunctival involvement too may lead to scarring, symblepharon formation and aqueous and mucin deficiency. In this study most of the patients had secondary limbal stem cell deficiency.

In our study, the significant improvement of 2 or more lines of visual acuity was no-ticed in both CLAU group ($p = 0.001$) and KLAL group ($p = 0.001$) at 1 year. The success rates for gain of 2 or more lines of visual acuity were comparable between two groups (group 1 = 88%; group 2 = 80%; $p = 0.12$). The success rate for visual gain in patients with CLAU group was comparable to that reported in previous studies.^{4,5,11} The reported success rate for cadaveric KLAL for visual gain of at least 2 lines of visual acuity varied from 40% to 100%.^{7,8,11-13} The visual success rate can't be generalized due to the heterogeneity of patient population, varied techniques and treatment protocols therefore it may be a poor outcome measure to assess the efficacy of either surgical procedure in LSCD.

Ocular surface stability was assessed on the basis of quantification of corneal neovascularisation, visually significant corneal opacity, fluorescein staining, Schirmer's ≥ 10 mm, TBUT ≥ 10 seconds. All parameters were

significantly improved after the both procedures ($p = < 0.05$) and were comparable between the two groups ($p = > 0.05$). The regression of corneal neovascularisation was more in CLAU group (43.47%) as compared to KLAL (28%), but not statistically significant. Titiyal et al¹¹ reported the 50% success rate for reduction of corneal neovascularisation but none of the eye undergoing cadaveric KLAL had corneal clarity sufficient to visualize iris details. However, the visually significant corneal opacity (Iris details poorly visible) regression was 57.14% in CLAU group and 53.33% in KLAL group in our study.

Holland et al¹² reported 72% success rate in stable ocular surface. Turgeon and Thoft¹³ reported 64% improvement in ocular surface and Tsubota et al⁸ reported 67% improvement in ocular surface. Different authors have defined the stable ocular surface differ-ently in their studies. Holland¹² defined ocular surface as stable if there is no or minimal fluorescein staining, and an absence of epithelial defects, neovascularisation, and epithelial opacities. Whereas Titiyal et al¹¹ defined ocular surface stable on the basis on TBUT measurements. We assessed the individual parameters affecting ocular surface stability. Schirmer's ≥ 10 mm improvement was seen in 55% in CLAU group and 45% in KLAL group. TBUT ≥ 10 seconds was achieved in 53.33% in CLAU group and 38.88% in KLAL group. Twenty percent success rate of TBUT ≥ 10 seconds was seen in patients with KLAL in an earlier series by Titiyal et al.¹¹

The mean time of healing for CLAU group was 8 days, whereas KLAL group had 12 days of mean time. CLAU group demonstrated significant early healing as compared to KLAL group. KLAL group experienced more recurrent epithelial defects (40%) as compared to CLAU group (24%) during the follow-up period. Post-operative anterior chamber inflammation was also more encountered in KLAL group. Delayed healing, recurrent epithelial defect and post-operative anterior chamber inflammation might be explained by immunological rejection in KLAL group which could be controlled by immunosuppressive therapy. Only two patients in KLAL group had graft failure due to the immunological rejection.

Allogenic transplant procedure carries a risk of graft rejection. In case of limbal tissue transplant, limbal tissue is rich in vascularity, antigen presenting cells like Langerhan's cells. Kwito and colleague¹⁴ demonstrated the advantages of HLA-typing and cross matching in allogenic conjunctival transplantation from living related donor. Patients with identical HLA matching were less likely to experience the epithelial rejection episodes in their case series. Kenyon and Rapoza¹⁵ did not report any immunological rejection in limbal allograft transplantation in either HLA compatible or non-compatible patients. HLA matching and ABO compatibility is advisable in these allogenic transplant wherever possible. In our study, as there was already shortage of available donor cadaveric graft, so it was not feasible for us to undergo HLA matching. All patients in KLAL group received systemic immunosuppressive agent in the form of oral cyclosporine. Though topical cyclosporine has been proved as effective as systemic cyclosporine in

earlier studies^{16,17} oral cyclosporine was given to all patients undergoing KLAL as it is proven to prolong the graft survival due the low chances of rejections in an earlier study by Ilari, et al.¹⁰

Our study was associated with various limitations. Small sample size and short follow-up period were the major limiting factors. Also the lack of HLA typing and ABO compatibility testing in KLAL group were the limitations. Prospective nature of the study and randomization were the strength. We were able to follow-up all patients without any drop-out for at least 1 year.

In conclusion, though both CLAU and KLAL appear to be effective in restoring the ocular surface in patients with LSCD, CLAU is associated with early ocular surface restoration and with relatively fewer complications. Our study has shown relatively better results with cadaveric KLAL in comparison to earlier series. Cadaveric KLAL is a good alternative in bilateral ocular surface diseases with LSCD.

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