



Original Research Article

Evolution of techniques of limbal stem cell transplantation for restoration of ocular surface in ocular surface disorder with limbal stem cell deficiency

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Amniotic membrane transplant (AMT).

ABSTRACT

Purpose: Evolution of techniques of limbal stem cell transplantation for restoration of ocular surface in ocular surface disorder with limbal stem cell deficiency.

Materials and Methods: It is an observational study in which 32 eyes of LSCD were observed with different surgical techniques. Outcome parameters were evaluated in terms of visual acuity, corneal clarity, symblepharon score, vascularisation. Statistical analysis was done using Anova t-test with significance level $P (<0.05)$. Patients were followed up at the day 7, month 1, and 1-month interval thereafter.

Result: The mean preoperative logMAR best-corrected visual acuity (BCVA) in CLAU, SLET, KLAU, and KLAL were 2.15 ± 0.70 , 2.70 ± 0.20 , 2.75 ± 0.22 and 2.80 ± 0.11 respectively which improved to 1.25 ± 0.50 , 2.00 ± 0.42 , 2.01 ± 0.15 and 2.30 ± 0.18 respectively after 6 months of follow up. Improvement in structural parameter were also seen in different surgical techniques.

Conclusion: Patients in which CLAU and SLET technique were used, had shown better improvement in Visual and structural outcome as compared to those in which KLAU and KLAL technique were used

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1. Introduction

Transparency of cornea is required for a good quality of vision. the cornea contributes the maximum refractive index of the eye so even a slight disturbance in the ocular surface may lead to a major loss to an individual vision in day to day activities and leads to a poor quality of life.

The term "ocular surface" describes the entire mucosal epithelial lining from the mucocutaneous junction of the eyelid margin on to the back of the lids, and over the globe with also covers the cornea. Histologically and physiologically the epithelial surface of cornea and conjunctiva separated by the limbus.¹

The composition of ocular surface includes two distinct types of epithelial cells composed of nonkeratinized, stratified squamous epithelium in cornea and nonkeratinized stratified columnar epithelium in conjunctiva and anatom-

ically continuous with each other at the limbus. The limbal palisades of Vogt and the inter palisade rete ridges are believed to be containing the stem cells. The limbal epithelial stem cells (LESCs) reside in specific regions at the limbus known as the limbal stem cell niches.² The microenvironment of the limbus is responsible for maintaining the stemness of stem cells. The function of Limbal stem cells also includes the formation of a functional "barrier" to conjunctival epithelial cells, thereby preventing them from migrating over the corneal surface. The corneal epithelium is continuously replaced by new epithelium. This cycle of replacement of corneal epithelium is required for maintenance of a healthy ocular surface, corneal clarity and clearer vision.

Stem cells have certain unique characteristics, which include poor differentiation, longevity, short S-phase duration and high capacity of self-renewal with a long cell cycle time, increased potential for error-free proliferation.

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According to X,Y,Z hypothesis X is the proliferation of basal epithelial cells, Y is the centripetal mass movement of peripheral epithelial cell, Z is the loss resulting from death desquamation The corneal epithelium undergoes a constant process of cell renewal and regeneration. Therefore it is endowed with a proliferative reserve in the form of multipotent stem cells located in the basal limbal epithelium. The limbal stem cells probably reside in the basal layer of the palisades of Vogt.³

Various disease processes can potentially deplete the limbal stem cells partially or totally, resulting in stem cell deficiency further compromising the corneal clarity. LSCD may be total or partial. In case of total LSCD, the conjunctival epithelium encroaches the entire cornea as there are no limbal stem cells to stop the process. In partial LSCD, some limbus stem cells are unharmed, and the corresponding areas on the cornea maintain phenotypically normal corneal epithelium.

In a spectrum of ocular disorders such as thermal and chemical burn, autoimmune diseases e.g. Stevens-Johnson syndrome, toxic epidermal necrolysis, ocular cicatricial pemphigoid etc. stem cells may become permanently damaged to a degree which leads to "conjunctivalization" of the cornea with vascularization, appearance of goblet cells, and an irregular and unstable epithelium. This results in compromised ocular surface, irritation, redness and decreased vision due to loss of corneal clarity. To treat this state of limbal stem cell deficiency, limbal stem cell grafts can be harvested from the patient's fellow eye, from a relative, from another living donor with healthy ocular surface, or from a cadaver. Following the procedure, systemic immunosuppression is required. Amniotic membrane transplantation proves to be a useful adjunct to the above procedures in some instances.

2. Materials and Methods

It was a Prospective observational study. It included all cases of ocular surface disorders with limbal stem cell deficiency who underwent limbal stem cell transplantation in the department of Ophthalmology, Gandhi Medical College, Bhopal during the academic session from December 2016 to June 2018. It included all patients through eye OPD, indoor patients, and emergency/casualty and all referred patients from Burn & plastic and other department of Hamidia hospital, Gandhi medical college, Bhopal. The cases were enrolled only after and obtaining the necessary consent for participation.

2.1. The inclusion criteria of the study were

All Patients with ocular surface disorders with limbal stem cell deficiency who attended the eye OPD/emergency /casualty /burn and plastic department Hamidia hospital Bhopal

2.2. Exclusion criteria of the study were

Any Patient with pre-existing ocular diseases other than limbal stem cell deficiency.

Any Patient who will be lost before follow up.

1. Visual acuity was recorded by using Snellen's test type Chart and the best corrected visual acuity obtained. For the purpose of statistical analysis, perception of light and hand movements were taken as +3 in logarithm of minimum angle of resolution (logMAR) and finger counting at 2 m as +2 in logMAR. 6/6 was graded as 0 in logMAR.
2. Detailed history of the patients with demographic and socioeconomic status and history of presenting complains taken in detail regarding name, age, sex, address, occupation, ocular surface disorder with limbal cell deficiency and their presentation to the centre or any first aid taken.

2.3. Presenting complaints about

pain/redness/watering/ itching/ burning/fb sensation/blurring of vision in involved eye etc were noted.

1. External examination with the help of a torch light performed with reference to vision, and to know the condition of ocular adnexa, conjunctiva, cornea, sclera, anterior chamber, iris, and pupil. Intraocular pressure measured digitally.
2. A thorough examination carried out on slit-lamp preoperative and follow up for symblepharon, corneal vascularisation, and corneal clarity was done.
3. For the purpose of statistical analysis symblepharon which resulting in forniceal obliteration due to scar tissue formation was graded from 0 to 4. Score of 1 was given for every 3-clock hour involvement.
4. Corneal vascularization was graded from 0 to 12 clock hours.
5. Corneal clarity was graded from 1 to 4 based on the assessment of iris and pupil on slit lamp examination, with 1 being opaque cornea and no view of underlying iris or pupil and 4 being clear cornea.
6. Direct & indirect ophthalmoscopy was done.
7. Clinical grading done depending upon the extent of involvement of conjunctiva, cornea, limbus and anterior chamber reaction on the basis of Roper Hall Classification for LSCD which is caused by chemical injury.
8. Necessary investigations carried out.
9. Full corneal work up including Schirmer's test, corneal sensation, fluorescein and lissamine staining, TBUT, PAS was done.
10. Blood examination including Hb %, TLC, DLC, ESR, Platelet count, serum blood sugar, serum urea, serum creatinine, serum electrolytes.

11. Urine routine and microscopic.

Prior to any attempt at limbal stem cell transplantation, the ocular surface must be optimized. Conservative first-line measures are based on two general principles: controlling causative factors, and controlling comorbid conditions. Causative factor control includes institution of immunosuppression for autoimmune diseases and/or chronic ocular surface inflammation, eradication of infection with appropriate antibiotic regimen, control of inflammation with corticosteroids, and cessation of iatrogenic insults was done. Comorbid conditions such as aqueous tear deficiency, cicatricial changes of the eyelid and conjunctiva, trichiasis, management was done preoperatively.

2.4. Different surgical technique on the basis of pathology are used they are

1. Auto conjunctival limbal graft with/without amniotic membrane CLAU
2. Simple limbal epithelial transplantation SLET
3. Keratolimbal autograft with/without amniotic membrane grafting KLAU with / without amniotic membrane grafting.
4. Keratolimbal allograft with/without amniotic membrane grafting KLAU

Visual and structural outcome were examined after treatment/surgery follow up of these cases done at day 7, 1 month, followed by 1-month interval thereafter.

Slit lamp examination for corneal vascularisation, symblepharon corneal clarity

Postoperatively. Patients received systemic immunosuppression, because limbal tissue is believed to be more prone to tissue rejection than are conventional corneal transplants. Because the patients with ocular pemphigoid and the Stevens Johnson syndrome had severe dry eye, proper wound healing could not be expected without tear supplementation. Eye drops prepared from autologous serum were used.

2.5. Statistical analysis

Data was recorded on a predesigned proforma, managed on a spread sheet(Excel) later checked for errors. Appropriate statistical tests were applied to analyse the results. Anova t test was used to determine the significance of changes between different groups at each follow-up visit. The chi-square and Z- test was used to compare the categorical variables. Level of significance in statistical test was 0.05. Parameters as the best indicators of improvement in three treatment groups were assessed (C.I 95% 97.5–100; p=0.05).

3. Result and Discussion

The mean age of recipients was 21.16 ± 1.76 SD years. LSCT was performed in various age groups, ranging from 4 to 42 years of age.

maximum patients i.e. 15(46.2%) & 10(31.2%) were in the age group 16-30 years & 6-15 years respectively emphasizing the vulnerability of young adults and school aged children.

With regards to gender, higher prevalence of LSCD was seen in males 80%. Male were at 3:1 times higher risk of LSCD as compared to females. Male: Female ratio was 3:1.

With regards to occupation maximum incidence of chemical ocular injury were found in student 12(37.5%) and labours 9(28%).

Patient's cornea is re-epithelialized with normal epithelium after a successful LSCT, which results in increased clarity, and reduced vascularisation and subconjunctivization. These changes finally improve the patient's vision.

In our study, out of 32 cases of LSCD that underwent different surgical techniques of LSCT and follow up at 7 days, 1 month, followed by 1-months interval thereafter.

We concluded that the mean preoperative logMAR best-corrected visual acuity (BCVA) in CLAU, SLET, KLAU, and KLAL were 2.15 ± 0.70 , 2.70 ± 0.20 , 2.75 ± 0.22 and 2.80 ± 0.11 respectively which improved to 1.25 ± 0.50 , 2.00 ± 0.42 , 2.01 ± 0.15 and 2.30 ± 0.18 respectively after 2 months of follow up.

It is in accordance with the study done by RITU ARORA, PALLAVI et al 2017.⁴ And in Limbal stem cell transplantation: an evidence-based analysis. Ontario Health Technology Assessment Series 2008;⁸ and their results show that vision improvements were affected by graft type that is patients who received a CLAU transplant had a significantly higher rate of improved visual outcome as compared with those who received a lr -CLAL transplant (P = .002).

Similar results seen in the study of KAZUO TSUBOTA et al,⁶ Geetha et al 2017,⁷ Basu et al 2016,⁸ Vazirani J et al 2010.⁹

3.1. Different surgical techniques and structural outcome

Structural outcome as defined by improved corneal surface which includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.

Structural outcome with different surgical techniques, The mean preoperative symblepharon score CLAU, SLET, KLAU, and KLAL were 2.50 ± 0.33 , 2.75 ± 0.76 , 3.05 ± 0.30 , and 3.10 ± 0.27 respectively and reduction in this score from preoperative value to 0.15 ± 0.25 , 0.49 ± 0.20 , 0.09 ± 0.30 , and 0.21 ± 0.09 respectively, The mean preoperative corneal clarity in CLAU, SLET, KLAU, and KLAL were 2.25 ± 0.50 , 1.75 ± 0.70 , 1.25 ± 0.22 And 1.20 ± 0.15

respectively which improve to 3.0 ± 0.08 , 2.95 ± 0.10 , 2.25 ± 0.15 and 1.85 ± 0.16 respectively after 6 month of follow up. And Corneal vascularization was graded from 0 to 12 clock hours. The preoperative mean clock hour vascularization in CLAU, SLET, KLAU, and KLAL were 8.50 ± 1.75 , 10.05 ± 0.90 , 9.15 ± 0.75 , 10.25 ± 0.95 respectively which reduce to 2.30 ± 0.45 , 4.40 ± 0.50 , 3.01 ± 0.25 , and 4.85 ± 0.39 respectively. Similar result seen in study Limbal stem cell transplantation: an evidence-based analysis. Ontario Health Technology Assessment Series 2008;8(5) in which they determined the impact of graft type on transplantation success, stratified analyses of the outcome measures by graft type were conducted. The results were short term corneal surface improvement in CLAU, Ir -CLAL, Thus, CLAU transplantation is the treatment of choice.

Similar result seen in Wylegala et al,¹⁰ Miri et al 2010¹¹ analysed graft survival rate and the regularity of the corneal surface differed significantly between the Allograft and autografts.

4. Conclusion

In our study, chemical injuries were found to be the most common cause of LSCD and the incidence was more among younger age group, with majority of injuries seen in the age group 16-30yrs. & 6-15yrs. Males were more commonly involved. The prevalence of LSCD were higher among the lower socioeconomic group.

Patients in which CLAU and SLET technique were used, had shown better improvement in Visual and structural outcome as compared to those in which KLAU and KLAL technique were used. This concludes that significantly better outcomes were seen with auto transplantation of the limbus as compared to allogeneic limbal grafts.

However, there are few limitations in our study. The sample size was small with limited follow up of 6 months limits the use of statistical test. For increasing accuracy of result more no. of cases need to be studied.

4.1. List of Abbreviation

SC-Stem cell

LSCD -limbal stem cell deficiency

LSCT-limbal stem cell transplantation

OSD -ocular surface disorder

CLAU -Conjunctival limbal autograft

KLAU - Keratolimbal autograft

KLAL -Keratolimbal allograft

CLET-cultivated limbal epithelial Transplantation

SLET-Simple limbal epithelial Transplantation

AMT- Amniotic membrane transplantation

SJS-Steven Johnson Syndrome

CL-Contact lens

VA-visual acuity

BCVA-best-corrected visual acuity

F/U -Follow up

H/O- History of

5. For all author source of support

None.

6. Prior presentation /publication

None.

7. Source of Funding

None.

8. Conflict of Interest

None.

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