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Amniotic membrane transplantation with or without autologous cultivated limbal stem cell transplantation for the management of partial limbal stem cell deficiency

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Purpose: To compare the outcomes of amniotic membrane transplantation (AMT) vs cultivated limbal stem cell transplantation (LSCT) in eyes with partial limbal stem cell deficiency (LSCD) following chemical burns.

Methods: Eyes with unilateral partial LSCD ($\leq 180^\circ$ involvement) were randomized in two groups to undergo either pannus resection combined with AMT or pannus resection combined with LSCT in a tertiary eye care hospital. Primary outcome measures were time to corneal epithelialization and absence of conjunctivalization of the cornea. Patients were followed up at 1 week, 1, 3, 6, and 12 months after the surgical procedure.

Results: There was no difference between mean age (30.85 ± 5.8 vs 28.64 ± 6.4 years, $P=0.40$) and sex distribution of patients between the two groups at baseline. Mean time to corneal epithelialization was 10.45 ± 5.8 days in the AMT group and 11 ± 3.9 days in the LSCT group ($P=0.43$). At the end of 1 year, there was no significant difference between the degree of conjunctivalization of cornea, ($P=0.06$) corneal vascularization, ($P=0.08$), and clarity ($P=0.07$) in both groups.

Conclusion: Our study showed that AMT alone is a useful therapeutic modality in cases with partial LSCD due to ocular chemical injury. Stem cell transplantation may not be required in these cases.

Keywords: partial LSCD, amniotic membrane transplantation, limbal stem cell transplantation

Introduction

Limbal stem cell dysfunction (LSCD) or limbal deficiency can lead to ocular surface abnormalities, which are characterized by chronic epithelial defects, stromal inflammation, corneal vascularization, conjunctivalization, and corneal opacification.^{1,2} For patients with unilateral limbal deficiency, the contralateral eye can provide healthy limbal tissue for limbal autograft transplantation,^{3,4} whereas for patients with severe bilateral limbal deficiency, homologous limbal tissue can be used for keratolimbal allograft transplantation.^{5,6} Unfortunately, the graft rejection rate is high in allograft transplantation, and long-term systemic immunosuppressive treatment is required for these patients.⁷ Stem cell-based therapy has been performed for over a decade for LSCD with good outcomes.⁸ However, there is a concern of inducing LSCD in the donor eye; therefore leading to its modification involving a smaller source tissue in conjunction with in vivo expansion. In addition to limbal stem cell transplantation (LSCT), amniotic membrane transplantation (AMT) has also been employed in cases with partial LSCD. Gomes et al⁹ and Anderson et al¹⁰ proposed AMT as an

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alternative to limbal transplantation for partial LSCT. The anti-inflammatory, antiangiogenic and antifibroblastic effects of amniotic membrane justify its use in patients with ocular surface disease.^{11,12} In the present study, we compared the outcomes of LSCT and AMT in patients with partial LSCT secondary to ocular chemical injury.

Methods

This was a prospective comparative study. The study included 20 eyes in each group with a diagnosis of partial LSCT, (involvement of 180° or less) secondary to alkali burns. The patients were recruited from the cornea clinic of a tertiary eye care center. The diagnosis of partial LSCT was based on clinical findings of focal loss of limbal palisades along with conjunctivalization. Patients were randomized in two group to undergo either pannus resection with AMT or pannus resection combined with LSCT.

The study protocol was approved by the ethics committee of All India Institute of Medical Sciences, New Delhi. The study complied with the tenets of the Declaration of Helsinki. A written informed consent form was obtained from all patients after explaining the nature and consequences of the procedure. Patients with prior ocular surgery, other ocular comorbidities, and bilateral involvement, were excluded. Uncorrected visual acuity (UCVA) and best-corrected visual acuity were noted. Slit-lamp examination was performed to assess the grade of chemical injury according to Dua's classification, corneal clarity, epithelial defect diameter, and the extent of limbal involvement. Intraocular pressure and any other anterior chamber or fundus abnormalities were noted.

The statistical analysis was performed using SPSS statistical software for Windows (Microsoft Corporation, Redmond, WA, USA). Quantitative variables were analyzed using paired and unpaired independent *t*-tests. A *P*-value of <0.05 was considered statistically significant.

Technique of limbal biopsy

Limbal biopsy was performed on the healthy contralateral eye. A limbus-based conjunctival flap was raised, the dissection was started in the subconjunctival plane and advanced over toward the limbus, extending 1 mm into clear cornea. Conjunctiva posterior to the pigmented palisades was incised, about 2 clock hours of pigmented limbus tissue was obtained. The limbal biopsy tissue was transported in human corneal epithelium medium to the laboratory, divided into small pieces, and then placed on the basement membrane side of a de-epithelialized human amniotic membrane (HAM).

A feeder cell-free submerged explant culture system was used to obtain a confluent monolayer of epithelial cells at the end of 2 weeks.

Ex vivo cultivation of limbal stem cells

HAM was obtained from voluntary seronegative donors (HIV, hepatitis B surface antigen, hepatitis C virus, syphilis) undergoing elective cesarean section after obtaining a written and informed consent explaining the nature and consequences of the procedure. Cryopreserved HAM was thawed, washed with PBS, and incubated with trypsin (0.25%) and ethylenediaminetetraacetic acid (0.1%) at 37°C for 20 minutes. The epithelium was then removed by gentle scraping.

The harvested limbal tissue pieces were allowed to adhere on the surface of de-epithelialized HAM for 10 minutes, after which few drops of growth medium were added and incubated at 37°C with 5% CO₂ for 6–7 hours. After incubation, 2 mL of growth medium was again added and incubation was done for 21 days. The medium was changed twice weekly, and growth was monitored using an inverted microscope until a monolayer of cells was identified. The culture medium consisted of growth factors and supplements Dulbecco's Modified Eagle's Medium/Ham F12 (3:1), fetal bovine serum (10%), insulin (5 mg/mL), hydrocortisone (0.5 mg/mL), glutamine (2 mmol/L), penicillin and streptomycin (100 U/mL), and human epidermal growth factor (20 ng/mL).

The phenotype of the cultivated limbal epithelial stem cells was evaluated by reverse transcriptase PCR and using immunocytochemistry. Reverse transcriptase PCR was performed to look for the expression of stem cell markers (p63, ABCG2, and K19). Immunocytochemistry was performed to look for expression of cornea specific cytokeratins (K3).

Surgical technique

All surgeries were performed under peribulbar block. After excising the pannus tissue, amniotic membrane with or without cultured stem cells was transplanted as per the treatment group. Excised pannus tissue was submitted for histopathological evaluation to evaluate cellular phenotype. The nitrocellulose paper holding the amniotic membrane was peeled off and the membrane was applied over the entire ocular surface, with its stromal side facing towards the cornea. Interrupted 8-0 vicryl sutures were then applied to anchor it with the underlying episcleral tissue. Similar surgical technique was used for transplantation of limbal stem cells.

The human amniotic membrane with the monolayer of cultivated epithelial cells was transferred to the ocular surface with the epithelial side facing up, and was secured to the surface using interrupted 8-0 vicryl sutures.

Postoperatively, topical broad-spectrum was used four times a day along with corticosteroids and preservative-free artificial tears every 2 hours. Topical corticosteroids were gradually tapered weekly and were continued for a period of 8 weeks. Patients were followed up on week 1, and months 1, 3, 6, and 12 after surgery. Time to complete epithelialization, corneal clarity, epithelial defect status, corneal vascularization, and complications were recorded.

Results

During the study period, 20 patients (20 eyes; 14 males, 6 females) received AMT alone and 20 patients (20 eyes; 13 males, 7 females) received AMT with cultivated LSCT from fellow eye. The age and sex distribution was comparable among the two groups. Mean age of patients was 30 ± 5.8 years in AMT group and 28 ± 6.4 years in the LSCT group ($P=0.40$). The average time from injury to transplantation was 6 months, which was comparable among the groups. There was no significant difference in the demographic profile of the patients in both patients. All surgeries were performed successfully with no intraoperative complications. Histopathology of resected pannus tissues revealed cells with conjunctival phenotype along with goblet cells.

The mean time to epithelialization was 10 ± 4.5 days in AMT group and 11 ± 3.9 days in LSCT group ($P=0.43$). At the end of 1 year, there was no statistically significant difference between both groups in regard to corneal conjunctivalization ($P=0.06$), residual corneal vascularization ($P=0.08$), and corneal clarity ($P=0.07$) (Table 1). Best-corrected visual acuity of $\geq 20/40$ was achieved in 65% of the cases in AMT group and 68% of the cases in LSCT group (Table 1). Two cases in AMT group and one case in LSCT group developed pyogenic granuloma in the postoperative period. A repeat AMT was successfully performed in these cases.

Table 1 Results at 1-year follow-up

Parameters	AMT group	LSCT group	P-value
Epithelialization	10 ± 4.5 days	11 ± 3.9 days	0.43
Conjunctivalization (%)	164 ± 47.4	171 ± 35.2	0.06
Vascularization (%)	158 ± 68.3	159 ± 58.21	0.08
BCVA	0.37 ± 0.17	0.36 ± 0.09	0.8
BCVA $\geq 20/40$	65%	68%	

Abbreviations: AMT, amniotic membrane transplantation; BCVA, best-corrected visual acuity; LSCT, limbal stem cell transplantation.

Discussion

Chen and Tseng¹³ first defined the concept of partial LSCD. It was hypothesized that such cases could achieve corneal epithelialization because of the existing residual stem cell function, and therefore the corneal surface could be reconstructed by debridement of conjunctivalized epithelium with AMT.¹³ Several studies have shown that amniotic membrane promotes expansion of residual limbal epithelial stem cells in eyes with partial LSCD.^{9,10,14} Kheirkhah et al¹⁵ performed superficial keratectomy followed by AMT using fibrin glue in eleven eyes of nine patients with partial to complete LSCD. During a mean follow-up of more than 1 year, all eyes maintained a smooth and stable corneal epithelial surface without recurrent erosion or persistent epithelial defect. Another study from Japan¹⁶ reported long-term results (mean follow-up, 52 months) of AMT in 16 eyes with partial LSCD. Although an improvement in the visual acuity postoperatively was seen, overall more than half of the cases developed recurrent LSCD, and nearly half of the recurrences occurred within the first postoperative year. The authors concluded that AMT by itself may be insufficient to achieve and sustain good outcomes, and the addition of LSCT along with other modalities may be beneficial. In contrast to these study results, the recurrence rate in our study was very low, with only case requiring a repeat AMT. This difference can be attributed to the varied etiology of partial LSCD in the study from Japan.¹⁶ Cell-based therapy using LSCT for restoration of the ocular surface in LSCD has been advocated as an alternative to AMT. Although previous studies have shown that cultivated LSCT is an excellent treatment for eyes with partial LSCD of different etiologies, this method relies on dedicated laboratory support, good yield of cultivated limbal stem cells, and a viable donor site.

Conclusion

We believed that in partial LSCD, the excision of conjunctival scar tissue at the site of deficiency alone can help in restoration of ocular surface, as stem cells present in undamaged area help in restoration of ocular surface. To test this concept, we compared the outcomes of AMT and LSCT in cases with partial stem cell deficiency secondary to ocular chemical injury. To our knowledge, this is the first comparative study using these two commonly used treatment modalities. We observed that AMT alone is a useful therapeutic modality in cases with partial LSCD secondary to chemical injury.

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Author contributions

RBV was involved with the concept and design of the study; NS and VJ aided in analysis and interpretation; NS, SM, VJ, and RBV were involved in writing the article; RBV critically revised the article; NS, SM, and RBV gave final approval of the article; NS and SM assisted in data collection and provision of materials, patients or resources; NS aided in statistical methodologies; VJ and RBV were involved in the literature research; and NS, SM, and RBV provided administrative, technical or logistic support. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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