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ORIGINAL ARTICLE

A CLINICAL STUDY OF LIMBAL STEM CELL TRANSPLANTATION IN VARIOUS OCULAR SURFACE DISORDERS

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ABSTRACT: AIMS: The purpose of this study is to evaluate the various causes of limbal stem cell deficiency, to assess signs and symptoms in ocular surface disorder and improvement following stem cell transplantation, to carry out limbal stem cell transplantation in various ocular surface disorders and to assess various intraoperative and postoperative complications following stem cell transplantation. **METHODS:** This study included 40 cases of ocular surface disorders treated with limbal autograft transplantation. Patients were recorded in a predesigned proforma, including the detailed history and complete ophthalmological examination. All cases of ocular surface disorders were treated with conjunctivo-limbal autograft transplantation under peribulbar and facial anaesthesia. **RESULT:** Maximum number of patients belong to age group 30-40 years. Males were more commonly affected by ocular surface disorders than females. (1.8:1). various causes of limbal stem cell deficiency were reviewed. The most common etiology associated with limbal stem cell deficiency was pterygia (60%) followed by vascularised leucomatous corneal opacity (20%), chemical burns (15%) and epitheliopathy (5%). Most common symptoms reported by patients were redness (100%), foreign body sensation (90%), watering (85%) and photophobia (50%). Most common signs found were conjunctival congestion (90%), corneal vascularization (70%), conjunctivalisation (70%) and epithelial defects (30%). 72.5% of patients reported increased ocular comfort. Postoperative incidence of redness was reduced from 90% to 20%, watering from 85% to 15% and photophobia from 50% to 25%. Improvement in signs was noted as decrease in conjunctival congestion from 90% to 22.2%, vascularisation from 70% to 35%, conjunctivalisation from 70% to 21.5% and epithelial defects from 30% to 16.6%. **CONCLUSIONS:** Use of autologous limbal transplantation with corneal and conjunctival tissue as a vehicle was useful for ocular surface reconstruction in patients with stem cell deficiency.

KEYWORDS: Ocular Surface, Limbal Autograft, Stem Cell, Vascularised, Leucomatous, Epitheliopathy, Conjunctivalisation.

INTRODUCTION: The ocular surface is a complex biological continuum responsible for maintenance of corneal clarity, elaboration of a stable tear film for clear vision, as well as protection of eye against microbial and mechanical insults. The ocular surface epithelium comprises corneal, limbal and conjunctival epithelia, of which the conjunctiva extends from the corneal limbus up to the mucocutaneous junction at the lid margin. All three are covered by a stratified, squamous, non-keratinizing epithelium at the surface of the eye that functions in innate defense at the ocular surface.

These epithelia sit on a basement membrane and are connected through an identical anchoring complex to an underlying connective tissue stroma. Functionally, all three regions of the epithelium serve as the most important barrier to fluid loss and pathogen entrance and they support the tear film by synthesizing membrane associated and secreted mucins.

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Normal corneal epithelium is maintained in the state of homeostasis by balancing epithelial shedding from the surface with centripetal movement of stem cell-derived epithelial cells from the limbus and proliferation of the basal cells.[1]

Stem cells are defined by their capacity of unlimited or prolonged self-renewal that can produce at least one type of highly differentiated progeny.[2] Cotsarelis et al.[3] confirmed the presence of a small subpopulation of slow-cycling limbal basal stem cells that had a significant reserve capacity and proliferative response to wounding. The stem cells have a capacity to self-renew and generate transient amplifying cells that are positioned in the basal cell layer of the corneal epithelium. Limbal stem cell deficiency can present with clinical signs of persistent corneal epithelial defect, neovascularization, "Conjunctivalization," scarring, stromal ulcer, calcification, and band keratopathy.[4] The most common symptoms of limbal stem cell deficiency are decreased vision, photophobia, tearing, blepharospasm, recurrent pain, and redness.

Chronic inflammation of the conjunctiva, conjunctival scarring, symblepharon formation, trichiasis, distichiasis, meibomian gland dysfunction, and lacrimal gland inflammation are all factors that can lead to tear film abnormalities and ultimately to damage to limbal stem cells and corneal epithelium that can present as persistent epithelial defect, corneal scarring, and neovascularization.

These factors can compromise limbal stem cells and exacerbate limbal stem cell insufficiency. Therefore, they need to be identified and eliminated to the maximum possible extent before limbal stem cell grafting to increase the graft survival time.

Limbal stem-cell deficiency can be primary, related to an insufficient stromal microenvironment to support stem cell function, such as aniridia, congenital erythrokerato-dermia, keratitis associated with multiple endocrine deficiencies, neurotrophic (Neural and ischaemic) keratopathy and chronic limbitis; or secondary (More common) related to external factors that destroy limbal stem cells such as chemical (Most common) or thermal injuries, SJS, ocular cicatricial pemphigoid (OCP), multiple surgeries or cryotherapies, contact lens wear, or extensive microbial infection.[5,6]

In patients with total limbal stem cell deficiency, limbal auto- or allo-transplantation is indicated for corneal surface reconstruction. This may be combined with or followed by keratoplasty. Several modifications have been described of the technique proposed by Kenyon_and_Tseng.[7] All these procedures aim to transplant a new source of epithelium for a diseased ocular surface and the removal of the host's altered corneal epithelium and pannus. After successful transplantation, the host cornea will be permanently covered by epithelium from the donor limbus. Although all techniques used in stem cell transplantation are in principle similar, the source of donor stem cells can vary. Donor tissue can be obtained from the fellow eye (Limbal autograft) in cases of unilateral disease or from a living related donor (Usually gives a better tissue match) or from a cadaver donor (Limbal allograft) when both eyes are affected. Limbal transplantation procedures also vary depending on the carrier tissue used for the transfer of limbal stem cells. Carrier tissue is needed in limbal transplants because it is not possible to transfer limbal stem cells alone. Limbal transplant procedures have used either conjunctiva (Conjunctival limbal graft) or cornea (Kerato limbal graft) as a carrier tissue for limbal stem cells.[8]

Tseng et al.[9] in1998 used amniotic membrane transplantation associated with limbal transplantation in cases with total stem cell deficiency. Conjunctival transplantation is, however, useful in other conditions, for example, to reconstruct the conjunctival surface in cases of symblepharon and to treat primary and recurrent pterygia.

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Keratoepithelioplasty was proposed by Thoft as another alternative to reconstruct the ocular surface in patients with conjunctivalisation of the corneal surface.[8] In this technique, lenticules of peripheral corneal epithelium with superficial stroma were grafted. A few years later the same author modified the technique to include limbal tissue, acknowledging the importance of stem cell transplantation in these conditions.[8]

Successful limbal transplantation can achieve rapid surface healing, stable ocular surface without recurrent erosions or persistent epithelial defects, regression of corneal vascularisation and restoration of a smooth and optically improved ocular surface, resulting in improved visual acuity and, probably, increased success for subsequent keratoplasty.

In patients with unilateral total stem cell deficiency a limbal autograft transplantation is recommended. Partial removal of the limbus from the fellow eye is believed to be relatively safe, although some cases may have compromised donor surface after partial removal of the limbal zone.

The risk of epithelial problems in the donor eye is low when less than four to six clock hours of limbal tissue and a moderate amount of conjunctiva are removed. When patients have bilateral total ocular surface disease, allograft transplantation becomes necessary. If living relatives are potential donors, an HLA-matched tissue is preferred. When cadaver donor tissue is used, "fresh" eyes are preferred because the success of the procedure depends on transplantation of healthy limbal stem cells. Whole eyes are convenient because they provide better stabilisation during dissection of the limbal sclerocorneal rim.

In limbal allografts the surface disorder can recur if there is immunological destruction of the transplanted limbal stem cells. A high rate of immune reactions can be expected because of the high immunogenic stimulus of the limbal transplant, related to the relative abundance of Langerhans cells and HLA-DR antigens. They play an important role in the afferent arm of allograft rejection, and effective immunosuppression is considered essential for at least 12 months after surgery when non-HLA matched limbal allografts are used. In some instances permanent systemic immunosuppression may be needed. Oral cyclosporine A is the most commonly used agent.[10] In addition to oral cyclosporine, Tsubota et al also used topical cyclosporine (0.05%) and high dose intravenous dexamethasone in their patients.[11] The use of FK 506, a new immunosuppressive agent from the fermentation broth of Streptomyces tsukabaensis, for immunosuppression in limbal or corneal allografts has been recently reported by Dua and Blanco.[12] Limbal rejection can be suspected with the development of inflammation and/or acute or chronic severe surface abnormalities. Daya et al.[13] in 1999 have reported the clinical features of limbal allograft rejection.

In their study, the clinical features of limbal allograft rejection varied according to the presentation, whether acute or chronic, of the rejection episode. Acute rejection was associated with intense sector injection at the limbus, edema, and infiltration of the lenticule, punctate keratopathy and epithelial defects. In low-grade rejection there was mild diffuse or perilimbal injection, elevated perilimbal area, punctate epithelial keratopathy and epithelial irregularity.[13]

AIIMS AND OBECTIVES:

1. To evaluate the various causes of limbal stem cell deficiency.
2. To assess signs and symptoms in ocular surface disorder and improvement following stem cell transplantation.
3. To carry out limbal stem cell transplantation in various ocular surface disorders.

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4. To assess various intraoperative and postoperative complications following stem cell transplantation.

MATERIALS AND METHODS: This prospective study was undertaken in the Department of Ophthalmology, S. S. Medical College and Gandhi Memorial Hospital, Rewa. The study included 40 cases of ocular surface disorders treated with limbal autograft transplantation. Patients having bilateral disorders, dry eye and collagen vascular disorders were excluded from this study. This research work was executed after approval from the Ethical Committee. Patients were recorded in a predesigned proforma, including the detailed history and complete ophthalmological examination including visual acuity and anterior segment examination using slit lamp examination.

Intraocular pressure was recorded using Schiotz Tonometer. Special investigations were performed for diagnosing ocular surface disorders including Schirmer's test I and II, tear film break up time, fluoroscein and rose Bengal staining. Under aseptic precautions, all cases of ocular surface disorders were treated with conjunctivo-limbal autograft transplantation under peribulbar and facial anaesthesia. The surgical procedure of conjuctivo-limbal autograft transplantation involved, transferring of a free graft of limbal tissue along with bulbar conjunctiva to the affected area.

Postoperatively all the patients were given topical antibiotic steroid combination 6 times a day for the first week and then tapered over next 5-6 weeks and preservative free lubricant ointment for fifteen days. Patients were followed daily till epithelialisation was complete, then weekly for two weeks, then fortnighthly for one month, then monthly for three months and quarterly for one year.

OBSERVATIONS AND RESULTS:

Table 1: Shows distribution of cases according to age of patients. A total of 40 cases were studied. The mean age of population was 50±11.5 years.

Age Group (Years)	No. of Cases
30-40	16
41-50	10
51-60	06
61-70	08
Mean age	50±11.5 years

Table 1: Age distribution of Patients

Table 2: shows sex distribution. In our series of patients, 65% were males and 35% were females. Male to female ratio was 1.8:1.

Total no. of Patients	Male	Female
40	26(65%)	14(35%)

Table 2: Sex Distribution

Various causes of limbal stem cell deficiency were reviewed in present study. The most common etiology associated with limbal stem cell deficiency was pterygia (60%) followed by vascularised leucomatous corneal opacity (20%), chemical burns (15%) and epitheliopathy (5%). (Table 3)

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Sl. No.	Disease	No. of Cases	Percentage (%)
1	Pterygia	24	60
2	V.L.O.	8	20
3	Chemical burn	6	15
4	Epitheliopathy	2	5

Table 3: Various causes of ocular surface disorders

Table 4 shows most common signs found were conjunctival congestion (90%), corneal vascularization (70%), conjunctivalisation (70%) and epithelial defects (30%). Improvement in signs was noted as decrease in conjunctival congestion from 90% to 22.2%, vascularisation from 70% to 35%, conjunctivalisation from 70% to 21.5% and epithelial defects from 30% to 16.6%

Sl. No.	Signs	Pre-operative	Post-operative
1	Conjunctival congestion	36(90%)	8(22.2%)
2	Conjunctivalisation	28(70%)	6(21.5%)
3	Corneal vascularisation	28(70%)	10(35%)
4	Epithelial defect	12(30%)	2(16.6%)

Table 4: Post-operative improvement in signs

Most common symptoms reported by patients were redness (100%), foreign body sensation (90%), watering (85%) and photophobia (50%). Postoperative incidence of redness was reduced from 100% to 20%, foreign body sensation from 90% to 20%, watering from 85% to 15% and photophobia from 50% to 25%. (Table 5)

Sl. No.	Symptoms	Pre-operative	Post-operative
1	Redness	40(100%)	8(20%)
2	Foreign body sensation	36(90%)	8(20%)
3	Watering	34(85%)	6(15%)
4	Photophobia	20(50%)	10(25%)

Table 5: Post-operative improvement in symptoms

DISCUSSION: The present study comprised 40 patients of which 26(65%) were males and 14(35%) were females. The age distribution of patients varied between 30 years and 70 years, with a mean age of 50±11.5 years. These results were consistent with similar studies carried out by Srinivas et al.[14] and Tsubota et al.[15] With mean age 42.7±14.5 years and 49±14.7 years respectively. In our study, the largest causative group of ocular surface disorders comprised pterygia (60%) followed by leucomatous corneal opacities (20%), chemical burns (15%) and epitheliopathies (5%).

The most common symptoms associated with ocular surface disorders in our study included redness (100%), foreign body sensation (90%), watering (85%), and photophobia (50%).

The signs noted were conjunctival congestion (90%), vascularization (70%), conjunctivalisation (70%) and epithelial defects (20%).

These findings were consistent with Joseph Frucht et al(1998).[16] and Wong et al (2000).[17] which showed that long standing signs and symptoms characteristic of chronic and diffuse stem cell deficiency include decreased vision, photophobia, discomfort, redness, vascularisation, scarring and recurrent epithelial defects. Following limbal stem cell transplantation, we noted a significant reduction in severity of symptoms. Post-operative incidence of redness was reduced from 100% to 20%, watering from 85% to 15% and photophobia from 50% to 25%. Improvement in signs was noted as decrease in conjunctival congestion from 90% to 22.2%, vascularisation from 70% to 35%, conjunctivalisation from 70% to 21.5% and epithelial defects from 30% to 16.6%. These findings were consistent with J. F. Ronk et al.[18] (1994) and Tsai et al.[19] (2000) which showed similar results.

We noted a 72.5% incidence of ocular comfort in our series of patients after limbal stem cell transplantation. This compares well with study of Srinivas et al.[14] Following conjunctival limbal transplantation, no major intraoperative complications were noted except button-holing in 5% of cases while placing the graft on recipient bed. In present study, post-operatively graft edema occurred in 100% of cases, which resolved within 10-15 days, subconjunctival hemorrhage in 2.5% of cases due to inadvertent trauma to conjunctival vessels and inadequate hemostasis, tenon's granuloma in 5% of patients due to inadvertent removal of tenon's along with graft and suturing of graft only to adjacent conjunctiva not with episclera and graft rejection in 5% of cases. These findings were concordant with those of Murat Guler et al.[20] and Mutlu et al.[21] In our study, Graft retraction was found in 5% of cases and papillary conjunctivitis in 2.5% of cases and these findings were consistent with Srinivas et al.[14] In present study, persistent epithelial defects was found in 16.5% of cases and recurrence in 8.3% of cases after mean follow up of 9±3 months. These results were similar to those of Kenyon and Tseng et al.[7] and Tsubota et al.[11]

CONCLUSIONS: Maximum number of patients belong to age group 30-40 years. Males were more commonly affected by ocular surface disorders than females. (1.8:1). various causes of limbal stem cell deficiency were reviewed in present study. The most common etiology associated with limbal stem cell deficiency was pterygia (60%) followed by vascularised leucomatous corneal opacity (20%), chemical burns (15%) and epitheliopathy (5%). Most common symptoms reported by patients were redness (100%), foreign body sensation (90%), watering (85%) and photophobia (50%). Most common signs found were conjunctival congestion (90%), corneal vascularization (70%), conjunctivalisation (70%) and epithelial defects (30%). 72.5% of patients reported increased ocular comfort. Postoperative incidence of watering was reduced from 85% to 15%, foreign body sensation from 90% to 20%, and photophobia from 50% to 25%. Improvement in signs was noted as decrease in conjunctival congestion from 90% to 22.2%, vascularisation from 70% to 35%, conjunctivalisation from 70% to 21.5% and epithelial defects from 30% to 16.6%.

Following conjunctival limbal transplantation, no major intraoperative complications were noted except button-holing in 5% of cases while placing the graft on recipient bed. Postoperatively graft edema occurred in 100% of cases, subconjunctival hemorrhage in 2.5% of cases, tenon's granuloma in 5%, graft rejection in 5% of cases, graft retraction was found in 5% of cases and papillary conjunctivitis in 2.5% of cases.

Thus, use of autologous limbal transplantation with corneal and conjunctival tissue as a vehicle was useful for ocular surface reconstruction in patients with stem cell deficiency.

The importance of recognizing and preventing the migration of conjunctival epithelium onto corneal surface as highlighted by this study, will significantly improve outcome of autologous limbal transplantation.

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