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Review Article

Ophthalmology Section

Limbal Stem Cell Deficiency: A Review Focusing on Staging, Diagnostic and Treatment Modalities

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BY-NC-ND

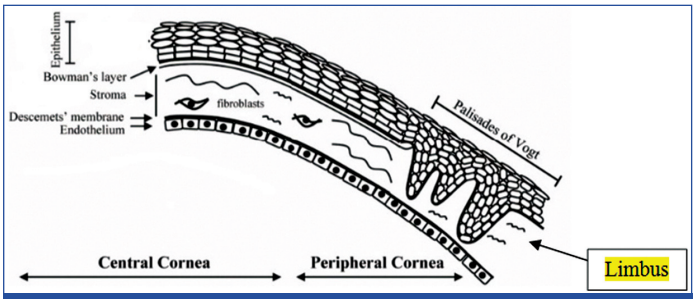
ABSTRACT

The cornea being a transparent tissue acts as a refractive surface as well as a protective barrier of the eye. The corneal epithelium is being continuously replaced and renewed by the Limbal Stem Cells (LSC). The turnover of corneal epithelium is brought about by asymmetrical differentiation and self-renewal of LSCs, their migration to the cornea and desquamation of current corneal epithelium. LSC are undifferentiated cells that acts as progenitors of corneal epithelium and maintains corneal homeostasis. LSC resides in its own microenvironment known as LSC niche, which are capable of sustaining these tissue regenerating cells. Any intrinsic factors (e.g., toxic epidermal necrolysis or mucous membrane pemphigoid), extrinsic factors (e.g., thermal burns, radiations, chemical burns) or genetic defects (e.g., aniridia) cause impairment of LSC or its niche leading to Limbal Stem Cell Deficiency (LSCD). LSCD is associated with invasion of cornea by conjunctival epithelium, corneal opacity, and visual impairment. The diagnostic modality of LSCD includes fluorescein staining and slit lamp examination, impression cytology, confocal scanning and anterior chamber optical coherence tomography. The staging of LSCD is important for deciding the treatment modalities. In LSCD, the most common treatment modality includes LSC transplant from a healthy eye. Non LSC transplantation techniques are being used to prevent allograft rejection. In this review article, the authors aim to summarise the existing knowledge of the aetiology, staging and treatment modalities of LSCD.

Keywords: Conjunctival limbal autograft, Cultivated oral mucosal epithelial transplant, Limbal stem cell niche, Non limbal epithelial cell transplant

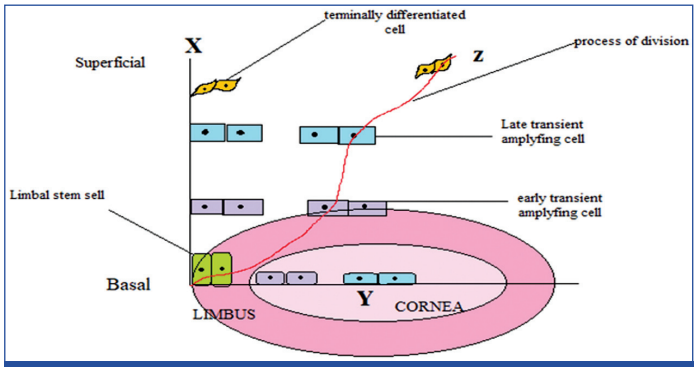
INTRODUCTION

Cornea is the anteriormost part of the eye, which acts as a refractive surface and forms a protective barrier from the outside environment. The conjunctival epithelium, covering the sclera, is contiguous with the corneal epithelium. At the junction of the corneal and conjunctival epithelium lies the limbus [Table/Fig-1]. The LSC are present at the basal limbal epithelium surrounded by a specialised microenvironment called the LSC niche. These LSC niche are found in limbal crypts between stromal projections such as palisade of Vogt [1,2].



[Table/Fig-1]: Limbal Stem Cell (LSC) niche.
(Image courtesy: https://doi.org/10.1002/stem.794)

The corneal epithelium because of its barrier function and maintenance of its transparency has to renewed by LSCs asymmetric division, migration and proliferation. The LCS is thus responsible for maintaining the corneal homeostasis, which can be explained by the X-Y-Z hypothesis of corneal regeneration represented in the [Table/Fig-2]. According to Thoft RA and Friend J the X represents the asymmetric differentiation of LSC into Transient Amplifying Cells (TAC) and stem like daughter cell. The Y represents the centripetal migration of TAC and proliferation into fully differentiated corneal epithelial cells. The Z in the X-Y-Z hypothesis represents the desquamation of the superficial cell of the cornea [3]. The rate of turnover of corneal epithelium increases in case of corneal wound or diseases [4].



[Table/Fig-2]: X-Y-Z Hypothesis for corneal regeneration.
(Image courtesy: International Journal of Molecular Sciences, Doi: 10.3390/ijms19071982)

Deficiency or dysfunction of LSCs or their niche can lead to a pathological condition called LSC Deficiency (LSCD). LSCD is characterised by corneal neovascularisation, delayed or absent healing, and corneal scarring. The limbus is the barrier between the conjunctival and corneal epithelium, hence loss or dysfunction of LSCs or their niche can lead to invasion of corneal epithelium by conjunctival epithelium known as conjunctivalisation [5,6]. LSCD can be bilateral, however, unilateral LSCD is usually more common. There are different aetiologies for unilateral and bilateral LSCD, which will be discussed further. The most common cause of both bilateral and unilateral LSCD is burn injury of the eye [5]. The management of LSCD can be done by LSC transplantation. However, there are certain limitations, such as lack of donors of limbal tissue. It therefore becomes really important for development of novel therapeutic strategies [7].

Definition of Limbal Stem Cell Deficiency

An ocular surface disorder caused due to loss in either number or function of LSCs is known as LSCD. There is loss of corneal homeostasis, leading to clinical features of LSCD [6]. According to Deng SX et al., there will be invasion by conjunctival epithelium

of the cornea, termed as conjunctivisation along with corneal neovascularisation [8]. The clinical features will include corneal epithelial erosions, recurrent inflammation, corneal scarring and opacification, recurrent corneal ulcers, chronic pain, and partial or complete visual impairment [5]. It is believed that pterygium is secondary to partial LSCD, however, it can occur without LSC dysfunction [9].

AETIOLOGY OF LIMBAL STEM CELL DEFICIENCY

Most of the research regarding LSCD was focused on the therapeutic approaches. However, in 2018, a 10-year-old descriptive study was published by Vazirani J et al., to focus on the demography and the underlying causes of LSCD. The outcomes of the study depicted that unilateral LSCD occurred more commonly than bilateral. The most common cause of both, however were ocular surface burns, lime being the most common agent responsible [10]. The aetiological factors of unilateral and bilateral LSCD are summarised in [Table/Fig-3].

Unilateral LSCD	Bilateral LSCD
Ocular surface burns (84%)	Ocular surface burns (30%)
Stevens-Johnson syndrome (4%)	Allergic conjunctivitis (29%)
Atopic keratoconjunctivitis (3%)	Stevens-Johnson syndrome (23%)
Mucous membrane pemphigoid (3%)	Aniridia (9.43%)
Trauma (2% each)	Mucous membrane pemphigoid (3.54%)

[Table/Fig-3]: Aetiology of unilateral and bilateral LSCD [10].

Aetiological classification can also be based on whether the cause is either acquired or genetic. Genetic or congenital causes of LSCD include aniridia, Ectrodactyly-Ectodermal dysplasia-Clefting (EEC) syndrome, Peter's anomaly, Keratitis-Ichthyosis-Deafness (KID) syndrome and xeroderma pigmentosa. Acquired aetiology can be (I) immune related like Stevens-Johnson syndrome, mucous membrane pemphigoid and atopic keratoconjunctivitis (II) non-immune related like trauma, bullous keratopathy, severe infections or iatrogenic [5,10]. The aetiological classification is demonstrated in [Table/Fig-4].

Genetic or congenital	Acquired	
1) Aniridia	Immunological causes	Non-immunological causes
2) Xeroderma pigmentosa	1) Mucous membrane pemphigoid	1) Chemical and thermal burns
3) Peter's anomaly	2) Stevens-Johnson-syndrome	2) Severe infection
4) Ectrodactyly-Ectodermal dysplasia-Clefting (EEC) syndrome	3) Severe atopic keratoconjunctivitis	3) Iatrogenic (e.g., contact lens wearing and ocular surgery involving limbus)
5) Keratitis-Ichthyosis-Deafness (KID) syndrome	4) Severe vernal keratoconjunctivitis	4) Toxicity from medications like mitomycin
6) Epidermolysis bullosa	5) Graft-versus-host disease	5) Bullous keratopathy

[Table/Fig-4]: Aetiology of LSCD [5,10].

DIAGNOSTIC MODALITIES OF LIMBAL STEM CELL DEFICIENCY

Signs and Symptoms

In the early stages, the patient may not show any symptoms. Patients with symptoms usually present with pain, ocular discomfort, photophobia, blepharospasm, impaired vision or blindness. There will be chronic pain due to recurrent inflammation. There may be history of recurrent atopic or allergic conjunctivitis. Sometimes non healing of corneal erosions even after therapy can indicate towards the of diagnosis of LSCD [8].

The cornea will become translucent, hazy and scarring might be present. The corneal epithelium is thick and uniform and will become thinner and irregular in LSCD. There may or may not be neovascularisation along with conjunctival epithelial invasion of cornea [5]. The diagnosis is greatly helped by fluorescein staining examined by slit lamp under cobalt blue light, which will show whorled pattern. Since conjunctival epithelium lacks tight junctions, there may be stippling or granular appearance [8]. There will be epithelial irregularities and fluorescein stain will be pooled in areas with thinning of epithelium. There may be a difference in staining patterns in different stages of LSCD. There are few limitations like non-specific patterns of fluorescein staining or very indistinct changes of corneal epithelium. Cytological studies further help in confirmatory diagnosis of LSCD [11].

Impression Cytology

It is a non invasive cell collecting method where cells are collected from the superficial layers of the epithelium. Impression cytology is used to depict conjunctivalisation of cornea. The conjunctival epithelium consists of goblet cells, which are absent in corneal epithelium. In histopathology, the cells collected from impression cytology, are stained using periodic acid Schiff, Haematoxylin and Eosin (H&E) staining or Giemsa shows presence of goblet cells, indicative of LSCD [8,12]. Immunohistochemistry detects markers like cytokeratin, which are intracellular proteins present in the epithelial cells. Cytokeratin 7, 13 and 19 are specific for conjunctival epithelium. Cytokeratin 12 is specific for corneal epithelium. Cytokeratin 3 is present in both corneal and conjunctival epithelium [12,13]. The marker for goblet cells is MUC5AC, however due to less number of goblet cells, absence of this marker does not rule out of LSCD [14]. It is considered as the gold standard method for the diagnosis of LSCD [8].

In-vivo Confocal Scanning Microscopy

It is a non invasive method of microscopy for visualising significant changes in the microstructure of corneal and limbal epithelium. It examines the presence of goblet cells, the basal epithelial thickness, the sub-basal nerve plexus and the density of cells in the basal epithelium. There will be significant decrease in thickness in the basal epithelium and sub-basal plexus of nerves or there will be absence of palisades of Vogt. In vivo confocal microscopy also helps in staging of LSCD [5,13].

Anterior Chamber Optical Coherence Tomography (AC-OCT)

It is a non invasive imaging technique of the anterior chamber. In LSCD, there will be thinning of limbal and corneal epithelium, which can be observed by the AC-OCT. There will be 20-30% of thinning in the epithelium. The thinning of the limbal epithelium is attributed to the loss of palisades of Vogt, which can be visualised by AC-OCT. Severity of the disease can be determined by the AC-OCT scans. Hyper-reflectivity of the corneal epithelium along with decreased light penetration depicts conjunctivalisation [8,12]. The AC-OCT scans also help determine the epithelial and stromal reflectivity. The ration of both (ES ratio) >1.29 points towards a diagnosis of LSCD [15].

Optical Coherence Tomography- Angiography (OCT-A)

It is a non invasive imaging modality for the microvasculature of the eye. The changes in limbal vascularisation as well as neovascularisation of the cornea can be determined by OCT-A. An increase in severity of LSCD is characterised by increased density of limbal vascularisation as well as corneal neovascularisation. The most significant characteristic of OCT-A is its ability to differentiate between true LSCD and its similar conditions showing corneal vascularisation. On segmentation of the superficial layers, the vascular density does not show a lot of change. However, in non-LSCD cases because of deep vascularisation, there will be significant decrease in the limbal vascular density on segmentation of superficial layers. The limitation of OCT-A is that it cannot be used in grading the severity of the disease [12,15].

STAGING OF LIMBAL STEM CELL DEFICIENCY

An objective grading system was evolved by international consensus. Accordingly, there are three stages based on the corneal and limbal involvement, which increases in severity. In stage I, the central cornea, that is the central 5mm of the cornea are not involved. The limbal involvement in this can be subdivided into (A) where there is <50% limbal involvement, (B) where there is >50% but <100% limbal involvement and (C) where there is 100% limbal involvement. Stage II, the central 5 mm of the cornea is affected. It can again be subdivided based on the limbal involvement into (A) where there is <50% limbal involvement and (B) where there is >50% but <100% limbal involvement. The third stage, stage III, involves the entire cornea [5,8].

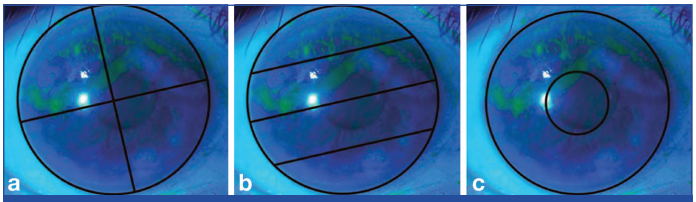
Another more precise grading system was developed by Aravena C et al., allotting scores based on limbal involvement, corneal involvement and visual axis involvement [16]. The limbal scoring is depicted in [Table/Fig-5].

Scoring	Limbal involvement in clock hours
1	1-3 clock hours
2	4-6 clock hours
3	7-9 clock hours
4	10-12 clock hours

[Table/Fig-5]: Scoring for limbal involvement [16].

The corneal surface is divided into four regions by drawing three parallel lines, perpendicular to the axis of greatest corneal involvement.

Involvement of each region was awarded one point. If the visual axis, that is the central 4 mm of the cornea, was involved, 2 points were assigned. If there was no visual axis involvement, no points were allotted [8,16]. Accordingly, the total score was obtained by adding all the scores of these three parameters, the lowest score being 2 and the highest being 10. LSCD was divided into mild, moderate and severe based on this clinical grading depicted in [Table/Fig-6,7].



[Table/Fig-6]: Diagram of the LSCD clinical grading system. Limbus involvement in clock hours (a); corneal surface area (b); and visual axis involvement (c) [16].

Grading	Score
Mild	2-4
Moderate	5-7
Severe	8-10

[Table/Fig-7]: Depicting clinical grading of LSCD [16].

MEDICAL MANAGEMENT OF LIMBAL STEM CELL DEFICIENCY

Medical management is indicated in limbal distress and early stages of LSCD. Limbal distress is when there is not a clear dysfunction or deficiency of LSCs, rather distress of LSCs where they are unable to proliferate due to an acute injury [17]. The main aim of the therapy is to basically control the aetiological factors, optimisation of ocular surface and further stop the progress of the disease. The autoimmune diseases and inflammation of the ocular surface should be managed [5]. In contact lens wearers, the conservative management was to suspend the use of contact lens. Other methods included excessive use of artificial tear drops, maintaining lid hygiene [18]. LSCD and contact lens use is associated with tear film dysfunction. Tear film plays a major role in maintaining the proper functioning of the corneal epithelium. Dry eye is often associated with LSCD and hence its management plays an important role in conservative treatment. Artificial tear use, warm compresses, doxycycline as well as supplementation with omega-3 fatty acids shows significant improvement in dry eye symptoms [19,20]. To combat chronic inflammation, which is a clinical feature of LSCD, short-term corticosteroids can be administered, like prednisolone or methylprednisolone. Corticosteroids also help in regression of conjunctival haze from the cornea. Vitamin A ointment is also considered to be effective [18]. Scleral lens, called Prosthetic Replacement of the Ocular Surface Ecosystem (PROSE), have been found to be effective when used before surgical management in improving visual acuity and optimising ocular surface [21].

SURGICAL MANAGEMENT OF LIMBAL STEM CELL DEFICIENCY

After optimisation of the ocular surface by medical management, surgical as well as cell based therapeutic management is done depending upon the staging and the laterality of the disease [5,22]. Stages I and IIA are medically managed along with sequential sectorial conjunctival epitheliectomy along with amniotic membrane transplantation and pannus removal in progressive cases [22,23]. In stages IIB and III with unilateral involvement, autologous LSC transplantation is the treatment of choice. In bilateral involvement of stages IIB and III, allogenic LSC transplantation, keratoprosthesis and Cultivated Oral Mucosal Epithelial Transplantation (COMET) can be done [22].

Limbal Epithelial Cell Transplants

Conjunctival Limbal Autograft (CLAU)

CLAU is used in unilateral LSCD. It is the removal and transfer of two grafts of the limbal tissue from the healthy eye of the patient. There is a risk of iatrogenic LSCD in the donor eye due to excessive removal of LSCs [24,25]. In the donor eye, 3-4mm from the limbus, an incision is given on the conjunctiva, followed by peritomy in 6 clock hours parallel to the limbus. The conjunctival flap is reflected 1mm beyond the vascular arcades and then excised. The graft is then sutured to the recipient eye in its appropriate anatomical position [26]. A study conducted by Eslani M et al., investigated long term results of CLAU involving 27 subjects, demonstrated ocular surface stability in 77.8% of the subjects [27].

Living-related Conjunctival Limbal Allograft (lr-CLAL)

In patients with bilateral LSCD, conjunctival limbal allograft is harvested from the living relatives of the patients. This is beneficial in having greater chance of HLA compatibility and reduces the risk of graft rejection. The patient still needs to be on immunosuppressive drugs [28]. Under local anaesthesia, the dissection of the donor eye spans from 3mm outside the limbus and 1mm into the limbus. Usually, two tissues are harvested superiorly and inferiorly in 3 clock hours. The harvested limbal tissue is then sutured into its suitable anatomical position in the recipient eye along with the conjunctival tissue and amniotic membrane [29].

Keratolimbal Allograft (KLAL)

It is usually preferred in cases of bilateral LSCD due to contact lens or Stevens-Johnson syndrome, because of their lack of conjunctival involvement. Here, cadaveric limbal tissue are harvested along with cornea as a carrier [30]. In the donor eye, the central cornea is separated from corneoscleral rim using corneal trephine and the peripheral corneo-limbal tissue is dissected without much stromal involvement. In the recipient, the graft should cover 360 degrees, one end succeeding the other. Amniotic membrane is used to cover cornea and adjoining conjunctiva and the grafts are placed over them in their appropriate anatomical position. The disadvantage of KLAL is the long-term immunosuppressive therapy and increased risk of graft rejection [26,29].

Cultivated Limbal Epithelial Transplantation (CLET)

It was first demonstrated by Pellegrini G et al., for the management of unilateral LSCD. It is the cultivation of autologous limbal tissue, harvested by limbal biopsy, on the amniotic membrane with explant tissue culture techniques. The limbal tissue sheets along with amniotic membrane are then transplanted in the diseased eye [31]. Autologous CLET shows long-term survival of the graft as well as improvement in vision without any significant complications [32]. Confocal microscopy one year after CLET showed, no presence of palisades of Vogt, 31% patients showed mixed type of conjunctival and corneal epithelium whereas, 46% patients showed corneal epithelium in the cornea [33].

Simple Limbal Epithelial Transplant (SLET)

The corneal epithelium affected by LSCD is scraped off, which is known as de-epithelisation or superficial keratectomy. A fresh amniotic membrane is placed on the de-epithelised cornea with the help of fibrin glue, a small limbal graft obtained by limbal biopsy of approximately 1 clock hour or less is taken from the unaffected eye and divided into 4-6 pieces. These divided pieces of limbal graft are then put on the amniotic membrane, leading to the cultivation of limbal tissue in vivo. This technique was discovered by Sangwan V et al., is found to be successful. The long-term outcome by a study by Basu S et al., found that 76% patients showed positive outcome [7,34,35].

Non Limbal Epithelial Cell Transplants

Cultivated Oral Mucosal Epithelial Transplant (COMET)

The oral mucosal epithelial cells have been transplanted in bilateral LSCD to prevent allograft rejection. A stratified sheet is formed by cultivated oral mucosal epithelial cells. The cultivated sheets in the basal layers demonstrated proliferation markers like p63 as well as k3 and k19 which are specific for corneal epithelium [36]. The COMET have shown 43% to 67% positive results, however, due to the oral mucosa being thicker, the visual outcomes were dissatisfactory [37].

Mesenchymal Stromal Stem Cells (MSC)

These are multipotent stem cells derived from different tissues such as bone marrow, dermis, periosteum, and fat cells. With the transplantation of MSC, there is a likelihood of the redevelopment of LSC niche, which can prolong the therapeutic outcome. These cells are anti-inflammatory in nature and can control angiogenesis, hence, can be potentially used for the LSCD. The lack of clinical date is a hinderance in establishing whether MSCs will fulfil the assumption of showing positive and prolonged therapeutic outcome [38,39].

Recommendation

It is important to educate the patients about the disease and its complications as it can lead to visual impairment and can also lead to blindness if left untreated. This will affect the ability, self-esteem and self-care and often leads to psychological impairment, especially in younger and middle age group. It is also very important to educate about usage of topical drugs as few medications can lead to structural changes of epithelium, tear film dysfunction and dry eye which can lead to LSCD.

CONCLUSION(S)

The LSC play a significant role in maintaining the vision. Various aetiological factors can lead to the dysfunction or deficiency of these cells. Corneal diseases are more commonly the reason of blindness worldwide, LSCD being one of them. LSCD is loss of corneal homeostasis due to loss or dysfunction of LSC or their niche. LSCD is a fairly new entity and still requires extensive study for proper diagnosis and management. The diagnostic tools for LSCD have been useful for not only diagnosis but also follow-up after treatment. Non invasive imaging techniques have vastly improved the sensitivity and specificity of diagnosis and should be used to confirm all cases of LSCD. There are newer treatment modalities, which have significantly reduced the use of allographic transplantation, which is accompanied by severe complications. However, the interactions and signalling pathways between the LSCs and their niche are yet to be fully understood. There is still scope for development of pharmacological advancements when it comes to therapeutic management. Some researchers believe that LSCD can be reversible and manageable by conservative and medical treatments. Understanding the signalling pathways and interactions between LSC and their niche may help us in reversing LSCD.

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