# Management of Limbal Stem Cell Deficiency

Essay

Submitted for the partial fulfillment of The master degree **in ophthalmology** 

By:

Mohamed Yossry Mohamed M.B.B, Ch

Supervised by

Prof. Dr. Zafer F. Ismail

Professor of ophthalmology Faculty of medicine Ain-Shams University

## ASS. Prof. Dr. Thanaa Helmy Mohamed

Assist. Prof. of Ophthalmology Faculty of medicine Ain-Shams University

Faculty of Medicine Ain-Shams University

## **ACKNOWLEDGEMENT**

I am greatly honored to express my deepest gratitude to **DR.ZAFER FAHIM ISMAEL**, Professor of Ophthalmology, Ain Shams University for giving much of his valuable experience and advice, and for his kind support and encouragement throughout this work.

I would like also to thank **Dr. THANA HELMY MOHAMED,** assistant professor of Ophthalmology, Faculty of medicine, Ain Shams University, for his kind assistance, beneficial ideas, and encouraging comments to finish this work.

Many thanks go to all the staff members of Ophthalmology

## LIST OF CONTENTS

Chap	ter Page
ACKN	NOWLEDGMENTi
LIST	OF CONTENTii
LIST	OF TABLESiii
LIST	OF FIGURESiv
LIST	OF ABBREVIATIONix
1.	Anatomy and Physiology of Limbal Stem cells Limbus and Stem Cell Anatomy and Function1
2.	Etiology of Limbal Stem Cells Deficiency29
3.	Clinical Picture and Diagnosis of Limbal Stem Deficiency
4.	Staging of Limbal Stem Cell Deficiency62
5.	Managements of Limbal Stem Cells Deficiency70
6.	Postoperative Management of Limbal Stem Cells Transplant
7.	Corneal Transplantation and Keratoprosthesis In Ocular Surface Disease
	SUMMARY143
	REFERENCES146
	ARABIC SUMMARY

## LIST OF TABLES

Table		Page
(1.1)	Differences between epithelial cells of the limbus and central cornea (CK, cytokeratin; CX, connexin; EGFR, epithelial growth factor receptor)	21
(4.1)	Staging of ocular surface disease based on number of lost stem cells and presence or absence of conjunctival inflammation.	62
(5.1)	Classification of epithelial transplantation procedures for severe ocular surface disease.	71
(5.2)	Algorithm for an approach to treat patients with severe OSD	73
(6.1)	Immunosuppressive regimen after keratolimbal allograft transplantation	114
<b>(6.2)</b>	Side effects of systemic corticosteroid therapy	122
(7.1)	General indications for use of individual keratoprosthesis devices	139
(7.2)	Prognostic categories with the Boston keratoprosthesis	139

## LIST OF FIGURES

Figure		Page
(1.1)	The location of corneal epithelial stem cells at the corneoscleral limbus.	5
<b>(1.2)</b>	Illustration of stem cell control by the niche.	7
(1.3)	A Healing of corneal epithelial wound involving the limbus showing a preferential circumferential migration of tongue-shaped sheets of limbal epithelial cells arising from either end of the remaining intact epithelium. (Slit lamp anterior segment photograph with fluorescein dye). B 'Columnar keratopathy' is the name given by the author to this presentation of alternating columns of fluorescein stained epithelium and normal corneal epithelium. These correspond to the limbal palisades and represent an early sign of limbal stem cell deficiency	18
(1.4)	Slit lamp photograph of the limbus showing the palisade (ofVogt) structure with: A pigment columns migrating into peripheral cornea and B fluorescein staining of columnar migration in response to a central abrasion	19
(1.5)	A Impression cytology specimen of the human limbus from an eye bank donor eye. The limbal cells are smaller, tightly packed and show a greater nuclear-cytoplasmic ratio. B Montage of the human limbus, peripheral cornea and conjunctiva	22
(1.6)	Limbal epithelial crypt: representing a solid cord of cells extending from the undersurface of a limbal palisade. These cells are positive for the putative stem cell marker ABCG2. Haematoxylin stained cryo section.	23

Figure		Page
(2.1)	Aniridic keratopathy. Conjunctivalization of the peripheral cornea. No stromal disease at this stage	30
(2.2)	Autosomal dominant keratitis. A 27-year-old man with central fibrovascular ingrowth and fibrotic nodules	33
(2.3)	Keratitis-ichthyosis-deafness (KID) syndrome. Failed penetrating keratoplasty due to total limbal deficiency and corneal surface failure.	35
(2.4)	Cicatricial Pemphigoid	45
(2.5)	Complete kiratinization of ocular surface in Stevens–Johnson syndrome	48
(2.6)	Vascularized trachomatous pannus	48
(3.1)	A Signs of mild limbal stem cell deficiency – peripheral conjunctivalisation highlighted with fluorescein staining. The junction of corneal and conjunctival phenotypes of epithelia is marked with arrows. B Peripheral vascularisation with loss of limbal architecture	51
(3.2)	Peripheral conjunctivalisation with pooling of dye and stippled staining of the abnormal epithelium, between 12 and 3 o'clock	52
(3.3)	Conjunctivalisation of the cornea involving the visual axis following chemical injury. The demarcation between the two phenotypes of cells is clearly visible.	52
(3.4)	Superficial and deep vascularisation with a fibrovascular pannus encroaching on the cornea following chemical burn in which 9.5 clock hours of the limbus and 60% of the conjunctiva were involved	54

Figure		Page
(3.5)	Persistent epithelial defect and fibrovascular pannus on cornea related to total stem cell deficiency following unilateral alkali (cement) burn	55
(3.6)	A Right eye of patient with 10 clock hours of limbus and 70% conjunctival involvement following a chemical (alkali) burn. B Left eye of same patient with 12 clock hours of limbs and 90% conjunctival involvement. Scarring, vascularisation, adhesions and some keratinisation are present. The lids on both sides were also severely damaged	56
(3.7)	A Impression cytology from surface of cornea with stem cell deficiency and a fibrovascular pannus showing goblet cells. PAS stain. B Biopsy of fibrovascular pannus showing multilayered epithelium, vascularisation and intraepithelial lymphocytes along the basal layers	59
(3.8)	Limbal palisades of Vogt. Trabecular extensions of the conjunctiva growing from outside (in this case from below) in a radial pattern toward the cornea	61
(4.1)	Ocular surface disease stage Ia. Sectoral stem cell deficiency due to mitomycin-C.	65
<b>(4.2)</b>	Ocular surface disease stage IIa. Aniridic keratopathy	65
(4.3)	Ocular surface disease stage Ib. Partial limbal deficiency from previous acid injury	66
(4.4)	Ocular surface disease stage IIb. Previous alkali injury with total limbal deficiency and uninflamed conjunctiva.	67
(4.5)	Ocular surface disease stage Ic. Partial limbal deficiency and chronic conjunctivitis.	68

Figure		Page
(4.6)	Ocular surface disease stage IIc. Total ocular surface failure and persistent conjunctival inflammation due to Stevens–Johnson syndrome.	69
(5.1)	Technique for conjunctival limbal autograft (CLAU). A, Recipient eye preparation.	80
<b>(5.2)</b>	Conjunctival limbal autografting.	81
(5.3)	Technique for living related conjunctival limbal allograft transplantation.	85
<b>(5.4)</b>	Living related conjunctival limbal allograft.	86
(5.5)	Patient with a history of a severe chemical injury.	86
(5.6)	Keratolimbal allograft. A, Preparation of tissue from cadaveric donor.	93
(5.7)	Keratolimbal allograft. A, Preoperative appearance of patient with severe alkali injury.	96
(5.8)	Keratolimbal allograft. A, Preoperative appearance of a patient with a total limbal deficiency secondary to a chemical injury. Note the presence of a tube shunt to control the intraocular pressure prior to the KLAL.	97
(5.9)	Schematic diagram of combined conjunctival limbal and keratolimbal allograft technique.	100
(5.10)	Combined conjunctival limbal and keratolimbal allograft. A,B, Stevens–Johnson syndrome patient with total ocular surface failure and ankyloblepharon.	101
(5.11)	Cadaveric donor – multiple grafts.	105
(5.12)	Status post penetrating keratoplasty, Ex vivo stem cell expansion. Visual Acuity 20/20	117
(6.1)	Acute limbal allograft rejection. The patient had a KLAL 2 months before. Note swollen and inflamed limbal tissue with neovascularization. Also note the	44.
	presence of an epithelial rejection line inferiorly.	115

Figure		Page
(6.2)	Late stem cell failure. Note hazy epithelium and late staining with fluorescein in most of the cornea. A wedge of normal epithelium persists	117
<b>(7.1)</b>	Approach to corneal grafting in patients with ocular surface disease.	136
(7.2)	(A) KPro front piece, (B) front piece on adhesive tape, (C) donor cornea button slid over KPro front piece, (D) KPro PMMA back plate, (E) addition of back plate, and (F) KPro unit with titanium locking	
	ring	142

### LIST OF ABBREVIATIONS

AMT Amniotic membrane transplantation

APECED Autoimmune Polyglandular Endocrinopathy -

Candidiasis - Ectodermal Dysplasia

BFGF Basic fibroblast growth factor

Bid Twice a day

BP Blood pressure

CAMs Cell adhesion molecules

CAU Conjunctival autologous

CBC Complete blood count

c-CLAL Cadaveric conjunctival limbal allograft

CD8+ Cytotoxic T cell

CIN Conjunctival intraepithelial neoplasia

CK Cytokeratin

CLAU Conjunctival-limbal autologous

CP Cicatricial pemphigoid

CXR Chest –x- ray

DMEM Dulbecco's Modified Eagle Medium

EC Endothelial cells

EGF Epidermal growth factor

FGF Fibroblast growth factors

HBV Hepatitis B virus

HCV Hepatitis c virus

HIV Human immunodeficiency virus

HLA Human leukocyte antigen

HML-1 Human mucosal lymphocyte antigen

IL-1 Interleukin-1

IOP Intraocular pressure

K Keratin

KLAL Keratolimbal allogeneic

LFT Liver function tests

lnr-CLAL Living nonrelated conjunctival limbal allograft

lr-CLAL Living-related conjunctival-limbal allogeneic

LSCD Limbal stem cell deficiency

LSCT Limbal stem cell transplantation

Mg Magnesium.

MMC Mitomycin C

MMPs Matrix metalloproteinases

NV New vascularization

OB Ocular burns

OCP Ocular cicatricial pemphigoid

PAF Platelet activating factor

PEDF Pigment epithelium-derived factor

PMC Post mitotic cells

PSA Prostate-Specific Antigen

Qd Once a day

Qid 4 times a day

RCT Randomized controlled trial

Sc Stem cell

SJS Stevens - Johnson syndrome

SSSS Staphylococcal scalded skin syndrome

TAC Transient amplifying cells

TDC Terminally differentiated cells

TIMPs Tissue inhibitors of matrix metalloproteinases

TNF Tumor necrosis factor

TPA Tissue plasminogen activator

UPA Urokinase plasminogen activator

VA Visual acuity

VEGF Vascular endothelial growth factor

(R)-HETrE (R)-Hydroxyeicosatrienoic acid

(TAC) Transient amplifying cell

'3T3' "3-day transfer, inoculum 3 x 105 cells."

5FU 5 fluorouracil

#### Chapter (1):

#### **Anatomy and Physiology of Limbal Stem cells**

#### **Limbus and Stem Cell Anatomy and Function**

The limbus is the anatomical transition of sclera and conjunctival epithelium into cornea and is believed to be the location of the epithelial stem cells of the cornea. At the limbus, the stratified columnar conjunctival epithelium moves to the stratified squamous epithelium of the cornea, and the vascular substantia propria of the conjunctival epithelium ends in a rich vascular limbal plexus. The vascular plexus is believed to be important in providing nutrients and oxygen to the mitotically active limbal stem cells. Additionally, the limbus may function to restrict conjunctival cells from the corneal epithelium. The stem cell population responsible for repopulation of the corneal epithelium remains poorly described. [1]

Early studies documented a centripetal movement of corneal epithelial cells from the limbus to the central cornea, suggesting that proliferating precursor cells were present at the limbus. The concept of limbal based stem cells was further supported by the observation that it was impossible to create permanent corneal epithelial defects in laboratory animals