Title: **Medically Reversible Limbal Stem Cell Disease: Clinical Features and Management Strategies**

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Disclosures/Disclaimers:

The work by SB and ARD is supported in part by unrestricted grants from "Research to Prevent Blindness" to Departments of Ophthalmology at Northwestern University Feinberg School of Medicine and University of Illinois at Chicago, respectively. ARD is the recipient of a career development award from the National Eye Institute, NIH and from Research to Prevent Blindness.

None of the authors have any financial or proprietary interest in this manuscript.

Presented at the Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, Seattle, Washington USA, May 5-9, 2013

KEY WORDS: limbal stem cell dysfunction; focal epitheliopathy; limbal niche

ABSTRACT

Purpose: To describe the clinical features and management strategies in patients whose limbal stem cell (LSC) disease reversed with medical therapy.

Design: Retrospective case series.

Subjects: 22 eyes of 15 patients seen at 3 tertiary referral centers between 2007 and 2011 with greater than 3 months follow-up.

Methods: Medical records of patients with medically reversible LSC disease were reviewed. Demographic data, etiologies, location and duration of disease and medical inventions were analyzed.

Main Outcome Measures: Primary outcomes assessed included resolution of signs of LSC disease and improvement in visual acuity.

Results: Etiologies of the LSC disease included contact lens wear only (13 eyes), contact lens wear in the setting of ocular rosacea (3 eyes), benzalkonium chloride toxicity (2 eyes) and idiopathic (4 eyes). Ophthalmologic findings included loss of limbal architecture, a whorl-like epitheliopathy or an opaque epithelium arising from the limbus with late fluorescein staining. The superior limbus was the most common site of involvement (95%).The corneal epithelial phenotype returned to normal with only conservative measures including lubrication and discontinuing contact lens wear in 4 patients (4 eyes) while in 11 patients (18 eyes) additional interventions were required after at least 3 months of conservative therapy. Medical interventions included topical corticosteroids, topical cyclosporine, topical vitamin A, oral doxycycline, and/or punctal occlusion. All eyes achieved a stable ocular surface over a mean follow-up of 15 months (range, 4-60 months). Visual acuity improved from a mean of 20/42 to 20/26 ($P < 0.0184$).

Conclusions: Disturbances to the LSC function and/or niche may be potentially reversible by medical therapy. These cases, which represent a subset of patients with LSC deficiency, may be considered to have LSC niche dysfunction.

PRECIS

We demonstrate the reversibility of limbal stem cell disease through medical treatment and withdrawal of toxic and traumatic insults. This reversibility suggests the limbal disease may result from dysfunction of the limbal stem niche.

INTRODUCTION

An intact corneal epithelium plays an essential role in corneal clarity and function. The corneal epithelium is continuously renewed by a population of epithelial limbal stem cells (LSC) which are located in the basal layer of the limbus (1-3). Conditions such as traumatic, immunologic and genetic diseases can destroy these cells and lead to LSC deficiency (4-5). Typical findings in LSC deficiency include whorl-like epitheliopathy, progressive ingrowth of opaque epithelium and superficial neovascularization. These findings represent various degrees of corneal conjunctivalization (6-7). Patients with LSC deficiency can further develop recurrent or non-healing epithelial defects, secondary stromal scarring or melting, and ultimately significant pain and loss of vision.

There has been an increasing awareness of the importance of the limbal microenvironment, or niche, in LSC function and deficiency (4-5, 8-9). The limbal niche plays an essential role in maintaining the function of the LSCs and consists of both cellular (e.g. limbal keratocytes) as well as non-cellular (e.g. extracellular matrix) components (9-11). Major insults to the ocular surface, such as chemical injuries or severe auto-immune reactions, typically destroy the LSCs as well as their niche. However, there is evidence that in certain pathologic conditions the the function of the LSCs may be compromised because of presumed disturbances to the limbal niche (9, 12). There are a number of reports in the literature describing cases with “LSC deficiency” where the disease was reversible with medical therapy (6-7, 13-14). It is likely that such cases may in part represent dysfunction of the niche rather than or in addition to true deficiency of the LSCs. In this case series, we present 22 eyes whose LSC disease was reversible with medical therapy and highlight their clinical presentation and the role of treatments aimed at restoring the limbal microenvironment.

MATERIALS AND METHODS

We reviewed the medical records of all patients with LSC disease that reversed with medical management. A total of 15 patients (22 eyes) were identified. The patients were seen at University of Illinois Eye and Ear Infirmary, Northwestern Memorial Faculty Foundation, and Cincinnati Eye Institute from 2007 to 2011. The study was conducted in accordance with HIPAA regulations and was approved by the Institutional Review Board at each institution before initiating the study. For the purpose of this study, LSC disease was diagnosed based on characteristic clinical features such as the loss of limbal architecture including the palisades of Vogt, the presence of a whorl-like epitheliopathy or a translucent epithelium arising from the limbus, and late fluorescein staining of the involved epithelium in a wavy or whorl pattern (Figure 1). LSC disease was considered reversible or responsive to medical therapy if there was resolution of the above mentioned features. We documented patient age, gender, symptoms, limbal disease location, visual acuity, ocular examination findings, duration of disease, presumed etiologies, and systemic and ocular associations. In addition, we documented all medical interventions in these patients including patient instructions, oral and topical medications, and interventional procedures. Statistical significance of changes in mean values was determined using unpaired T-test.

RESULTS

Fifteen patients (22 eyes) met the previously mentioned inclusion criteria. Table 1 summarizes key findings of this case series including patient characteristics and course of treatment. The mean age was 39 years (range: 22 to 57 years). Patients presented with various symptoms such as eye irritation, contact lens intolerance, and blurred or decreased vision. The one clinical sign seen in all patients was progressive epitheliopathy with hazy, translucent epithelium extending centrally from the limbus. Epithelial staining was broadest adjacent to the involved limbus and extended centripetally into the cornea to varying degrees. The degree of epithelial staining varied from punctate changes to a more confluent sheet of staining, with most cases demonstrating a whorl-shaped

and wavy pattern of staining. All patients had evidence of mild to moderate tear film dysfunction and/or reduced tear break-up time. Given the differences in examination protocols between institutions, Schirmer testing was not consistently done in all patients to determine aqueous tear deficiency.

The extent of limbal involvement was estimated clinically and varied from 60 to 360 degrees (Table 2). The superior quadrant of the limbus was the most common site of involvement and was seen in 21 of 22 eyes. Isolated superior limbal involvement was seen in 7 eyes (32%), isolated inferior involvement in 1 eye (5%), while 10 eyes (45%) showed a combination of superior and other quadrant (nasal, temporal) involvement. Sub-total involvement was noted in 4 eyes (18%) seen as near 360 degrees of limbal pathology with only scattered areas of healthy limbus.

Presumed etiologies for the LSC disease included contact lens wear only (13 eyes, 59%), contact lens wear in the setting of ocular rosacea (3 eyes, 14%), and surface toxicity due to chronic benzalkonium chloride (BAK) exposure from glaucoma medications (2 eyes, 9%). In the remaining 4 eyes (18%), no other etiologies (besides associated dry eyes) were identified and ocular and systemic histories were not contributory in these eyes.

Four eyes of 4 patients had resolution of LSC disease with only conservative management. Conservative management included discontinuation of contact lens wear, aggressive lubrication with preservative free artificial tears and lid hygiene / warm compresses when indicated. Amongst these eyes, 2 eyes had a history of contact lens wear, 1 eye had contact lens wear in the setting of rosacea, and 1 eye had moderate to severe dry eyes. These patients demonstrated a mean of 4.4 clock hours (range: 1.5 to 12) of limbal involvement. In 18 eyes of 11 patients the epithelial disease persisted after a minimum of 3 months of conservative management and therefore additional medical treatments were instituted. This group demonstrated greater limbal involvement with a mean of 6.1 clock hours (range: 2 to 12) of LSC disease. Thirteen eyes had a history of

soft contact lens wear; 2 of which were in the setting of ocular rosacea. 2 eyes had chronic BAK exposure and 3 eyes had idiopathic LSC disease (in the setting of dry eyes). One of 18 eyes had resolution of LSC disease with the use of nightly topical vitamin A ointment. In the other 17 eyes, the initial treatment consisted of anti-inflammatory therapy in the form of short-term pulse topical corticosteroids either non-preserved in the form of methlyprednisolone 1% in 8 eyes (36%); or preserved in the form of loteprednol etabonate 0.5% or 0.2% (Lotemax; Alrex; Bausch & Lomb, Inc., Rochester, NY, USA) in 8 eyes (36%); or prednisolone acetate ophthalmic suspension 1% (Pred Forte, Allergan, Irvine, CA) in 1 eye (5%). Corticosteroids were used at varying frequencies, ranging from every two hours to three times a day. In all 17 eyes treated medically with steroids, a significant clinical response was noted with evidence of regressing conjunctival epithelial haze upon review a month following start of the steroid drop. In 14 eyes, steroids were tapered off by 2 to 3 months as cyclosporine 0.05% (Restasis, Allergan, Irvine, CA) was started twice daily. Three eyes of two patients could not be tapered off of steroids and continued to require long term topical steroids to prevent recurrence. One eye was ultimately fitted with a PROSE scleral lens (Boston Foundation for Sight, Needham, MA) and was able to discontinue the steroids after 9 months, while 2 eyes of another patient continues to require every other day topical methylprednisolone to prevent recurrence of the LSC disease.

After a period of anti-inflammatory therapy, punctal occlusion was performed in eyes with more significant aqueous tear deficiency. Inferior punctal plugs (Oasis, Glenview, CA) were placed in 3 eyes (14%) and complete cautery occlusion was performed in 3 eyes (14%). Two patients with rosacea were treated with warm compresses and lid scrubs twice daily plus oral doxycycline 50-100 mg twice daily. As stated above, one eye resolved solely with topical vitamin A. Vitamin A ointment was also used adjunctively in 3 eyes that concurrently were being treated with steroids.

All 22 eyes receiving either conservative or medical intervention achieved a stable ocular surface during a mean follow-up period of 15 months (range: 4-60 months) with resolution of the clinical features of LSC disease on ophthalmic examination. As a result of improvement in the ocular surface and corneal clarity, visual acuity improved in 17 eyes. In the other 5 eyes, with good starting visual acuity, the vision was subjectively improved, but remained stable by formal measurement (Figure 2). Overall the mean corrected visual acuity improved from 0.3203 in log MAR scale (mean: 20/42; range: 20/20 to 20/400) at initial presentation to 0.1127 (mean: 20/26; range: 20/15 to 20/200) ($P < 0.0184$) at final presentation. Also of note, one eye had beginning visual acuity of 20/400 due to corneal scarring and improved to 20/200. Figures 3 and 4 illustrate the response to medical treatment with improvement of corneal surface in 2 patients.

DISCUSSION

In this study we have presented a series of patients where the LSC disease resolved with either conservative measures or with medical therapy alone. Though the term “limbal stem cell deficiency” is commonly used to describe such cases, given the reversible nature of the disease, we believe that the pathophysiology may in part involve dysfunction of the LSCs niche. In our experience, these cases occur most commonly in patients with tear film insufficiency in the setting of chronic traumatic or toxic insults to the limbus, in particular, contact lens use or exposure to BAK. Previous studies describing a whorl-like or advancing wave-like epitheliopathy represent patients with similar disease process given that the epitheliopathy was likewise responsive to medical therapy in most cases (6-7, 13-14). Regardless of the pathogenesis, based on the clinical findings and the already existing nomenclature, it may be more acceptable to still classify such patients as having partial LSC deficiency.

An interesting clinical feature of these cases is the mixed phenotype of the cells growing onto the cornea. In particular, in some of the early cases before having a

continuous sheet of late staining epithelium, there appears to be single clones of late staining cells that follow a whorl-like path. This has led us to form a hypothesis that pathologically the early cases may actually represent a form of metaplasia. This is in part based on the experimental studies by Stepp et. al. who have shown that trauma from large corneal wounds can lead to proliferation and differentiation of clusters of limbal cells into “corneal goblet cells” (12). Thus, in the setting of trauma or inflammation, the function of the LSCs or their niche may be disturbed giving rise to goblet cells that migrate onto the cornea. This would explain the often clonal appearance of the late staining epithelium on the cornea. This hypothesis remains to be tested in humans.

Our medical management is primarily aimed at restoring the limbal micro-environment. This involves a stepwise approach based on two fundamental strategies: 1) stopping traumatic/toxic insults to the limbus and 2) optimizing the ocular surface environment by improving the tear film, controlling inflammation and promoting differentiation of healthy epithelium. Our series included 4 eyes of 4 patients whose LSC function returned to normal with conservative management and 18 eyes of 11 patients who had failed to improve after a minimum of 3 months of conservative therapy and contact lens wear discontinuation. As mentioned earlier, nearly all of our patients demonstrated a compromised tear film. Therefore, in addition to stopping traumatic or toxic insults, optimizing the tear film with preservative free lubricants and aggressive treatment of the lid margin disease is one of the primary interventions.

An interesting and consistent observation in this study was the demonstration of a clinically significant response to steroids, suggesting that chronic subclinical inflammation plays a significant role in the pathogenesis of such cases of LSC disease. Topical cyclosporine was likewise used effectively as maintenance treatment once inflammation had been controlled. We hypothesize that inflammation disturbs the normal milieu of the limbal niche and leads to dysfunction and/or aberrant differentiation of the LSCs (12, 15).

Other non-surgical treatment strategies to improve ocular surface health may be useful in such cases of LSC dysfunction/deficiency. Modalities such as topical retinoids are well known for promoting differentiation of mucosal epithelium and have been used in the treatment of squamous metaplasia (16-18). While we did not use autologous topical serum drops, they may provide another alternative for promoting epithelial health. The PROSE scleral lens may be useful in the treatment of LSC disease because it provides a micro-environment for promoting a healthy limbal niche and prevents blink related trauma from lids and soft contact lenses (19).

If left untreated, chronic and persistent damage to the limbal niche may lead to permanent loss of the niche, and hence LSC deficiency, requiring surgical intervention including superficial keratectomy and amniotic membrane and/or limbal stem cell transplantation (6, 14, 20-22). The goal of surgical intervention is to restore the limbal micro-environment. As noted in the series by Jeng et al, corneal conjunctivalization recurred in one patient despite superficial keratectomy, highlighting the fact that even with surgical intervention, restoration of a normal limbal niche is imperative for preventing long term recurrence (6). Based on our experience, surgical intervention without restoration of the limbal niche will have limited success and is best pursued after achieving maximal medical improvement.

There are several limitations to this study. While the description of the presenting signs and clinical symptoms was consistent at all 3 institutions, the use of adjunctive diagnostic tests of ocular surface health such as Schirmer testing, vital dye staining with Lissamine green/Rose Bengal and qualitative/quantitative analysis of tear film was not uniformly done at all centers. Additionally, given the retrospective nature of this study, impression cytology was not performed. There were also variations in the treatment modalities used at each institution as described above.

We recommend the following treatment strategy for the management of LSC dysfunction (Table 3). As a first step, all toxic or traumatic stimuli should be discontinued including contact lens use and all potentially toxic eye drops. At the same time, the tear film should be optimized using preservative-free artificial tears and aggressive treatment of associated lid disease. Patients that have failed a few months of conservative medical management should be treated with topical corticosteroids (preferably non-preserved if available) and re-assessed after a few weeks to determine the clinical response. Topical cyclosporine can be used to maintain ongoing anti-inflammatory activity as steroids are being tapered. After inflammation is controlled, punctal plugs or cautery may be considered in patients with more significant aqueous deficiency. Adjunctive measures such as topical vitamin A and autologous serum drops may be considered to further improve the tear film and epithelial health while scleral lenses may be used for recalcitrant cases. With future research into the role played by the limbal niche in maintaining the LSCs more specific and effective therapies for LSC deficiency should become available clinically.

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FIGURE AND TABLE LEGENDS

Figure 1: The left slit-lamp photograph of a 39 year old male (patient #9 in table 1) with LSC disease demonstrating a demarcation line between healthy and unhealthy epithelium (arrow). Fluorescein staining was used to further highlight these differences (right image).

Figure 2: Visual acuity before and after treatment for LSC disease.

Figure 3: A 57 year-old female (patient #12 in table 1) with history of dry eyes and glaucoma for 10 years presented with red and irritated eyes with progressive decrease in visual acuity. Exam revealed an opaque epithelial growth superiorly with late fluorescein staining (left image). With continued treatment, the LSC disease resolved (right image) with significant improvement in symptoms and visual acuity from 20/50 to 20/25.

Figure 4: A 40 year-old male (patient #14 in table 1) with a 23-year history of soft contact lens wear presented with LSC disease. Examination revealed an irregular opaque corneal epithelium extending from superior limbus (left image). Late fluorescein staining was present (middle image). Following medical treatment, the conjunctival type epithelium completely regressed (right image) with improvement of visual acuity to 20/25.

Table 1. Summary of 22 eyes of 15 patients with medically reversible LSC disease.

Table 2. Distribution of limbal involvement in 22 eyes with medically reversible LSC disease.