Annular amniotic membrane and autologous limbal transplantation after chemical burn

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ABSTRACT: In limbal stem cell deficiency (LSCD), limbic cells are unable to maintain corneal epithelium integrity. Patients with LSCD may suffer recurrent ulceration, chronic inflammation and loss of visual acuity due to the replacement of normal corneal epithelium by conjunctival epithelium. A 60-year-old male with LSCD after a chemical burn with cement (alkali) in the left eye 15 years ago complained of progressive visual loss. The left eye showed a dull cornea with loss of transparency due to conjunctivalization of the entire cornea. LSCD was diagnosed and limbal autograft plus amniotic membrane transplantation was proposed. During the procedure, the corneal stroma was transparent, so the central six millimeters were left free of amniotic membrane. Amniotic membrane graft can cause loss of corneal transparency that can last for several months. If the central corneal stroma is transparent and regular, the transient interim opacity can be avoided by annular amniotic membrane transplantation, thus speeding up recovery of visual acuity.

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CASE REPORT

The epithelial cells of the ocular surface are replaced by stem cell proliferation. Corneal stem cells, which are classically located in the palisades of Vogt, are distributed in the basal epithelium. Conjunctival stem cells are uniformly distributed throughout the bulbar and fornix conjunctiva. Limbal stem cells are the principal source for regeneration of the corneal epithelial cells. The intact limbus acts as a barrier against corneal vascularization from the conjunctiva and invasion by conjunctival cells from the bulbar conjunctiva. In limbal stem cell deficiency (LSCD), the limbal stem cells cannot maintain the integrity of the corneal epithelium, and the conjunctival epithelium becomes the only source of corneal re-epithelialization, leading to conjunctivalization of the corneal epithelium.

Patients with this disease may suffer recurrent ulceration, re-epithelialization delay, surface neovascularization, chronic inflammation and loss of visual acuity, due to the substitution of normal corneal epithelium by conjunctival epithelium. Impression cytology is the gold standard diagnostic test for LSCD. Diagnosis is confirmed by the presence of goblet cells on the corneal surface (despite the low sensitivity of this test, because the absence of these goblet cells does not rule out LSCD). LSCD may be caused by inflammatory, congenital, idiopathic or iatrogenic diseases, or by injury. They can be classified according to the cause of the deficiency.

CASE PRESENTATION

A 60-year-old male was referred to our clinic for recurrent pain and redness in the left eye, associated with progressive loss of visual acuity. Relevant clinical history included a chemical burn by cement (alkali) in the left eye (OS) 15 years ago. Visual acuity was 1.0 in the right eye (OD) and counting fingers in the OS. Biomicroscopy of the OD was normal, while in the OS, the cornea was dull and had lost transparency due to conjunctivalization of the entire cornea. Fluorescein staining of OS cornea was abnormal, showing a conjunctival pattern (Figure 1). Epithelial neovascularization was predominantly superior. We also found some shortening of the lower conjunctival
Figure 1. A) Transparent cornea, right eye. B) Loss of corneal transparency, irregular, thickened epithelium and predominantly superior neovascularization in left eye. C) Normal corneal epithelium without fluorescein uptake in right eye. D) Abnormal fluorescein uptake due to increased epithelial permeability in left eye.

fornix due to fibrosis (Figure 2). Digital intraocular pressure (IOP) was somewhat reduced in the OS, which also showed some loss of corneal sensitivity (we used a qualitative test, with a cotton-tipped applicator). Diagnosis was total limbal stem cell deficiency due to a chemical burn. Since the ocular surface of the fellow eye was healthy, a limbal autograft from that eye was proposed to the patient.

The surgical procedure was carried out under retrobulbar anesthesia. A 360° conjunctival peritomy was performed and the abnormal corneal epithelium was removed from the OS (Figure 3A). The donor areas were marked and amniotic membrane was transplanted in the damaged eye, in the form of a graft, covering the cornea and perilimbal sclera. The amniotic membrane was fixed with Tissucol® (Baxter S.L., Ribarroja del Turia, Spain) (Figure 3B). The two limbal autografts from the healthy fellow eye were sutured into the left eye at 12 o’clock and 6 o’clock (60°) with interrupted suture of 10/0 nylon over the amniotic membrane. The donor areas were then re-covered with amniotic membrane to encourage re-epithelialization (Figure 3C). Considering the good appearance of the central cornea (regular and transparent), the central 6 millimeters were not covered with amniotic membrane. The complete thickness of the amniotic membrane was removed (Figure 3D). A bandage contact lens (Purevision®, Bausch&Lomb S. A., Alcobendas, Spain) was placed on the OS and treatment was initiated with topical ciprofloxacin 3 mg/ml and topical dexamethasone sodium phosphate 1 mg/ml, both every 6 hours, and hyaluronic acid 0.15% and threalose 3% eye drops.

At the first postoperative follow-up visit, slight paracentral de-epithelialization was observed in the OD with mild epithelial defect in the donor sites. A bandage contact lens was placed in the OD to reduce the pain in the early postoperative period. The appearance of both the amniotic membrane graft and the limbal autograft in the left eye was good. To promote epithelialization, 20% autologous serum eye drops, obtained and produced by the hospital
Figure 2. A) Predominantly upper neovascularization affecting anterior stroma. B) Discrete shortening of lower fornix conjunctiva due to fibrosis.

Figure 3. A) Peritomy 360° and epitheliectomy. B) Marking of donor sites and amniotic membrane graft area. C) Obtaining of limbal graft and membrane patch from donor sites. D) Fixing limbal autografts and central opening of amniotic membrane. (Original illustration by Dr Manuel Romera www.ilustracionmedica.es).

Laboratory, were added for both eyes. Three days after the procedure, de-epithelialization of the OD had resolved. One week after the procedure, the cornea in the OS had become more regular, with some subepithelial opacity, although there was more transparency in the central zone in comparison with the peripheral cornea, due to the 6 mm in the center that were left free of amniotic membrane. One week later, the donor limbus was almost fully epithelialized. Some small dehiscence was observed in the nasal area of the limbal autograft in the 12 o’clock position (Figure 4).

Two weeks after the procedure, there was irregular staining in the temporal cornea and nasal epithelial defect. Neovascularization was also observed in the upper peripheral cornea (Figure 5), so the topical steroid dose was increased (dexamethasone sodium phosphate 1 mg/ml every two hours). At three weeks, the central visual axis remained clear despite the appearance of superficial neovessels (Figure 6). One month after the procedure, visual acuity was 0.1 in the OS. Persistent superficial neovascularization was observed, but the appearance of the epithelium was normal. The visual axis was free of abnormal conjunctiva, with mild subepithelial opacity. Two and a half months after the procedure, uncorrected visual acuity was 1.0 in the OD and 0.3 in the OS. Mild limited pannus was observed in the donor sites of the OD. Peripheral neovascularization in the superior nasal
quadrant, peripheral and self-limiting conjunctivalization at 4-5 o’clock, and mild epithelial irregularity were observed in the OS (Figure 7).

An OCT was performed on the OS, showing a regular corneal stroma slightly thinner than the OD (Figure 8).

At six months, the OS had achieved a corrected visual acuity of 0.7. The characteristic symptoms of LSCD had disappeared. Peripheral conjunctivalization at 4-5 o’clock persisted, but this was self-limiting and did not affect the visual axis. Central corneal epithelium was regular and transparent (Figure 9). The patient is currently being followed up with topical lubricants and regular check-ups in our clinic.

Figure 4. One week after surgery. A) More regular cornea with some subepithelial opacity. B) Dehiscence of limbal graft, upper nasal area. C) Well-positioned lower limbal graft.

Figure 5. Two weeks after surgery. A) Irregular staining of temporal cornea. B) Nasal de-epithelialization. C) Superior pannus.
Figure 6. Three weeks after surgery. A) Active superficial neovascularization in superior area of amniotic membrane. B) Fluorescein-negative epithelialized cornea.

Figure 7. Two and a half months after surgery. A) Mild epithelial irregularity. B) Peripheral neovascularization in superior nasal quadrant. Self-limiting peripheral conjunctivalization at 4-5 o’clock position.

Figure 8. Corneal OCT both eyes.
DISCUSSION

In chemical burns, in addition to the direct damage caused by the chemical agents, clinical sequelae include ischemia and an intense inflammatory reaction, which increases damage to the limbal stem cells. Treatment of LSCD varies depending on its extent. Mild LSCD is treated with lubricants, and if necessary, de-epithelialization. Partial stem cell deficiency requires amniotic membrane transplantation, which will act as a basal membrane to help the residual limbal stem cells repopulate the ocular surface with corneal-type epithelial cells. In cases of total LSCD, a limbal graft is needed alongside the amniotic membrane transplantation to aid the growth of normal epithelial cells, since amniotic membrane transplantation alone is insufficient for restoring correct corneal re-epithelialization. In the case of bilateral disease, a limbal allograft can be obtained from a healthy relative of the patient or cadaver tissue with high HLA compatibility. Since the grafted tissue is in contact with vascularized tissues, chronic immunosuppression is required to reduce any problems derived from immunological rejection, and the long-term results are poor. In cases with unilateral involvement, autologous transplantation is preferred, thus avoiding the risk of graft rejection. The beneficial effect of combining amniotic membrane transplantation with a limbal graft lies in restoring an intact basal membrane that is usually damaged in LSCD. The amniotic basal membrane is very similar to the corneal basal membrane, and also provides many growth factors. It acts as a substrate, encouraging epithelial cell migration and adhesion, aiding the proliferation of progenitor cells in the corneal epithelium and promoting their differentiation. The stromal portion of the amniotic membrane provides additional benefits due to its anti-inflammatory and anti-angiogenic effects, and to the release of several growth factors and protease inhibitors. The control of postoperative inflammation is fundamental for obtaining satisfactory results.

If the chemical burn has been sufficiently severe as to affect the corneal stroma, causing thinning, irregularity or opacification of the cornea, a keratoplasty will also be necessary once the initial inflammatory period has passed. An amniotic membrane graft can lead to a loss of corneal transparency that could last several months. In our case, after extraction of the fibrovascular membrane, the patient had good transparency in the visual axis of the cornea, so we decided to leave the central 6 millimeters free of amniotic membrane. If the central corneal stroma is transparent and regular, annular amniotic membrane transplantation avoids the transient opacity, speeding up the recovery of visual acuity (uncorrected visual acuity was 0.3 in the OS at two months). However, our patient had epithelial irregularity two and a half months after the procedure that might not have appeared if the central amniotic membrane had not been removed. It is important to inform the patient of the risks involved in a limbal autograft from a healthy eye, as the procedure may cause LSCD in the donor eye. In summary, in cases in which subsequent keratoplasty is not required, annular amniotic membrane transplantation could allow faster recovery of visual acuity.
REFERENCES