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Gentian violet (GV) ink associated reaction in a case of preloaded Descemet membrane endothelial keratoplasty: Case report

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ABSTRACT

Purpose: This report describes a case of Descemet membrane endothelial keratoplasty (DMEK) graft failure after non-clearing bullae over the area of the orientation mark on the graft.
Methods: Case report.
Results: The summary of the clinical presentation and workup is described, followed by a brief overview of the DMEK procedure and the gentian violet (GV) ink used to ensure the correct orientation of the DMEK graft.
Conclusions: GV has a good safety profile; however, there are rare cases of adverse events. Therefore, alternative approaches should be explored, such as the use of intraoperative optical coherence tomography, reviewing a video recording of the insertion step, adjusting the insertion technique, or using asymmetrical trephine marking on graft edges.

1. Introduction

Descemet membrane endothelial keratoplasty (DMEK) has become the favored technique for treating eyes with corneal endothelial dysfunction. DMEK allows selective monolayer transplantation of the endothelium, including the DM. This technique confers some advantages, including reduced endothelial cell loss, better visual outcomes, and low immunological rejection rates, ranging between 0% and 7%.[1,2] Hence, DMEK potentially confers favorable functional and anatomical outcomes.[1,2]

The main contributor to primary graft failures has been the upside-down orientation of tissue.[2] Using vital dyes to mark the graft improves the surgeons' ability to identify the correct graft orientation. Various vital stains are used for marking, and their safety has been established at low concentrations.[3]

In endothelial keratoplasty, gentian Violet (GV) is one of the most commonly used vital stains for marking the donor Descemet membrane (DM)/stromal surface. The effect of 0.01 % and 0.001 % GV staining on the cornea is reported to be safe, with no toxic effects.[3] However, significant GV cytotoxicity was observed at 0.10 % or higher concentrations.[4]

The GV ink is composed of these two components: the gentian violet pigment itself and the alcohol carrier for the ink. Before placing the mark on the tissue, the ink is painted onto the metal S stamp surface. When the tissue is marked, the excess alcohol will diffuse through the Descemet membrane and destroy the endothelium. Therefore, it is paramount to allow alcohol to evaporate from the S stamp surface before proceeding with marking the tissue.[5]

Despite the manufacturer's 'nontoxic and nonirritating label, GV ink might affect corneal endothelium layer vitality. We report a case of corneal bullae that was limited to the S orientation mark in a case that underwent DMEK using a precut, pre-stamped, and preloaded graft.

2. Case report

A 70-year-old male was referred to King Khalid Eye Specialist Hospital, Riyadh, Saudi Arabia, to manage bilateral cataracts and Fuchs endothelial corneal dystrophy. His uncorrected visual acuity (UCVA) was 20/100 in both eyes. There was no history of ocular surgery or other ocular diseases. The patient had bronchial asthma that was controlled with a salbutamol inhaler as necessary.

The patient underwent uneventful sequential cataract surgery followed by DMEK in the right eye. The graft was imported, and the tissue was preloaded with an S stamp marked and certified by The Eye Bank Association of America (EBAA).

Subsequently, an uneventful DMEK was performed on the left eye. The graft was also imported, and the tissue was preloaded in a modified Jones tube with a marked S stamp and certified by EBAA. The S stamp

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was dark in color with blurry and broad-edged borders. The graft endothelial cell density was 2882 mm^2, with a diameter of 9.5 mm, and the death to implant time was seven days. Intraoperatively, the epithelium was debrided as the view was poor, and Sulfur Hexafluoride (SF6) tamponade was maintained for 15 minutes, and then a 9 mm SF6 bubble was left in situ.

Postoperatively, the patient was advised to maintain a supine position as much as possible for the first 48 hours. The eye was treated with regular postoperative medical therapy, including topical prednisolone acetate 1 % and antibiotic drops. On the first postoperative day, slit-lamp examinations revealed an attached lenticule, well-positioned, with the correct orientation as evident by the S mark and a clear center.

The follow-up course was unremarkable and epithelium healed on third post-operative day; however, on the fourth day postoperatively, an epithelial bulla appeared corresponding to the S mark area on the left eye which was located paracentrally and extending to cover the visual axis. A few days later, the graft was stable and attached despite the presence of bullae. Anterior segment optical coherence tomography (AS-OCT; MS-39; Costruzione Strumenti Oftalmici, Firenze, Italy) was performed, and the patient was discharged home with a prescription for prednisolone acetate eye drops every 4 h, ofloxacin, 5 % sodium chloride, and sodium hyaluronate eye drops (Fig. 1A).

Six weeks after the DMEK surgery, the patient presented with uncorrected visual acuity (UCVA) of 20/300 and pinhole acuity of 20/160-1 in the left eye. A slit lamp examination showed an attached lenticule, the S mark in the correct orientation, the suture was in place, and no corneal epithelial defect. However, the bullae were still present and limited to the S marking (Fig. 1C and D). Therefore, the patient was advised to continue the medical regimen with a topical prednisolone of 1 % every 6 h daily.

Three months postoperatively, the vision worsened to 20/400 UCVA. Examination revealed a stable DMEK graft except for persistent bullae with mild edema limited to the area of the S mark. Since the rest of the cornea was clear, debridement of the bullae was performed using a 30G needle at the slit lamp. The patient was started on a more frequent dosage of topical prednisolone (Q3H, Q4H, then QID to be tapered weekly until the next visit) and 5 % sodium chloride drops.

Four months postoperatively, a slit lamp examination of the left eye indicated the persistence of the bullae with significant stromal edema around the area of the S mark. While the peripheral cornea was clear, the patient was provisionally diagnosed with DMEK failure-related S mark toxicity since the surgery was atraumatic. Subsequently, the patient underwent uncomplicated Descemet's stripping automated endothelial keratoplasty (DSAEK) with good visual recovery and postoperative sequelae (Fig. 1E).

3. Discussion

The success of DMEK depends on donor graft preparation, surgical procedure, and the absence of postoperative complications. A failed corneal graft can result from either primary or secondary graft failure. Primary graft failure is characterized by the presence of a persistent diffusely edematous corneal graft that fails to clear two months after the surgery. This could be due to severe iatrogenic corneal endothelial cell loss during graft preparation, implantation, manipulation, an "upside-down" transplant, or graft detachment. Alternately, the deterioration of vision due to the loss of corneal transparency in the previously functional corneal graft is defined as secondary graft failure. This is frequently caused by late endothelial graft failure, immunological rejection, and glaucoma.[6,7] Other causes of secondary graft failure include infection, trauma, and epithelial ingrowth.[6,7]

GV has been used in medicine for almost 100 years in many applications. It is a water-soluble triphenyl-methane dye that preferentially stains living cells. In addition to ophthalmic applications, GV is also used as a skin marker in orthopedic, neurologic, cardiovascular, and plastic surgery.[4,8] The potential cytotoxicity of the dye was investigated in several studies in which the dye was used in rabbit eyes and rat eyes at concentrations of 0.01 % and 0.001 %, and there was no evidence of

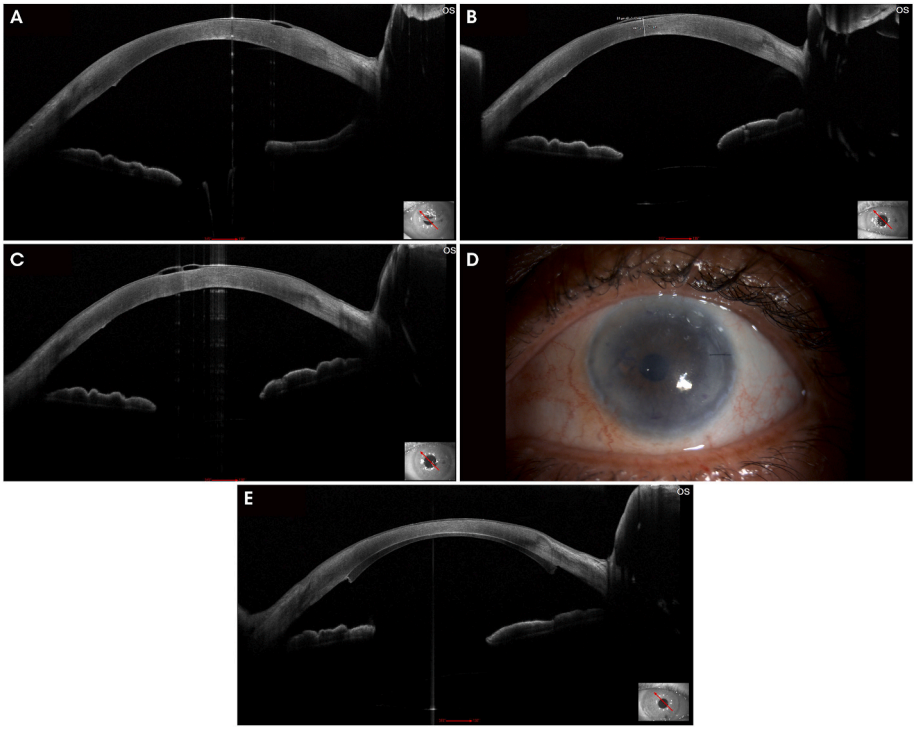

Fig. 1. A. Anterior segment optical coherence tomography (AS-OCT) image in the first week post-Descemet membrane endothelial keratoplasty (DMEK) surgery. B. AS-OCT image three weeks after DMEK surgery showing enlarged bullae. C. AS-OCT image six weeks after DMEK surgery showing persistent bullae over to the S mark area and attached DMEK graft. D. A corresponding Slit lamp photo six weeks after the surgery shows a correct graft orientation. E. AS-OCT image after Descemet's stripping automated endothelial keratoplasty (DSAEK) showing attached lenticule and smooth epithelial surface.

histopathological corneal toxicity.[9,10] On a cellular level, GV was found not to be significantly affected by the process in which the calcium concentration increases or by the extrusion mechanism of corneal endothelial cells.[4]

Cytotoxicity to corneal endothelial cells and all the corneal layers was investigated in rabbits, where structural changes were evaluated by light microscopy and transmission electron microscopy.[11,12] The studies reported that concentrations of 0.10 % and 0.50 % were likely to induce corneal damage, while lower concentrations were generally safe, respectively.[11,12] Ide et al. assessed the endothelial damage on DSAEK donor tissue using a GV marking pen.[8] Their in vitro model showed that the pattern of endothelial damage was limited to the application area.[8]

In our case, we had persistent bullae and corneal edema limited to the S mark site for months after the surgery, which was unresponsive to conservative management and debridement with the needle. The bullae did not resolve despite the more invasive approach, suggesting a different etiology other than mechanical causes. Our patient had good outcomes with a DMEK graft in the right eye. Additionally, an experienced surgeon performed all the procedures for this patient. No tags or irregularities were seen on the posterior surface of the cornea, indicating no mechanical causes could be attributed to the formation of the bullae and corneal edema in the left eye. The left eye was successfully managed with DSAEK. When evaluating the donor graft, it is essential to determine that there is no history of diabetes for the donor, which is a risk factor linked to early failure post-DSAEK.[13]

Cases of GV-associated toxicity after keratoplasty are rarely reported in the literature. Vincent et al. reported two cases that underwent DSAEK with donor grafts marked with GV.[14] In the first postoperative week post-DSAEK, both cases had marked corneal edema in the area of the GV markings and bullae.[14] However, unlike our case, the edema gradually resolved over weeks, and the vision improved to 20/40.[14] In a recently published paper, two cases of persistent localized DMEK detachments secondary to GV ink have been reported. Authors have attributed the cause to the higher concentration of Isopropyl alcohol solvent in the marker used for these cases.[15] In such cases, we speculate the reason behind these incidents that Isopropyl alcohol has not been allowed to evaporate off from the metal stamp surface, which resulted in the diffusion of alcohol through the Descemet membrane and caused endothelial cell loss.

In conclusion, stromal side marking is a valuable method to confirm the orientation of the DMEK graft. However, the adverse reaction to GV ink in keratoplasty has only been clinically recorded in limited cases. Considering this issue, the dry ink method technique can be considered on donor grafts to avoid unnecessary corneal endothelial damage. Alternative approaches to confirm the correct orientation of the DMEK graft should be explored such as intraoperative optical coherence tomography, adjusting the insertion technique, or use of asymmetrical trephine marking on graft edges can be considered.[5,16–18]

4. Patient consent

This report does not contain any personal information that could lead to the identification of the patient. Therefore, consent to publish the case report was not obtained. The case report was waived by the IRB at King Khaled Eye Specialist Hospital.

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CRediT authorship contribution statement

Rawan Abdulaziz Alosaimy: Conceptualization, Data curation, Methodology, Writing – original draft. **Halah Bin Helayel:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **Muhammad Ali Ahad:** Conceptualization, Data curation, Methodology, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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