Assessment of endothelial cell density after deep anterior lamellar keratoplasty

Ahmed H. Ebied[a], Hossam-Eldein M.A. Khalil[b], Hazem E. Haroun[b], Islam F.A. Elbeih[c], Ahmed T.S. Saif[d]

[a]Department of Ophthalmology, Faculty of Medicine, Cairo University, Giza, [b]Department of Ophthalmology, Faculty of Medicine, Beni-Suef University, Beni Suef, [c]Department of Cornea, National Eye Institute, Cairo, [d]Department of Ophthalmology, Faculty of Medicine, Fayoum University, Fayoum, Egypt

Correspondence to Ahmed T.S. Saif, MD, 5 Sherif Street, Babel Louk Square, Cairo 11111, Egypt. Tel: +20 100 196 0266; e-mail: ats@fayoum.edu.eg

Received: 19 January 2024
Revised: 24 February 2024
Accepted: 19 April 2024
Published: 24 August 2024

Delta Journal of Ophthalmology 2024, 25:135–141

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Background
The fundamental aim of successful Keratoplasty is to obtain a clear corneal graft and to maintain its survival. Previous studies have demonstrated the importance of endothelial cell density (ECD) in maintaining graft transparency and survival after keratoplasty.
Objective
This study aimed to assess the changes in corneal ECD following deep anterior lamellar keratoplasty (DALK) and to evaluate its visual and surgical outcomes.
Patients and methods
This prospective observational study was conducted at the National Eye Institute Rod-Elfarag, Cairo, Egypt, between July 2020 and February 2022. It included 50 eyes of 50 patients, with pathologies affecting the epithelium and/or stroma for whom DALK was performed. Patients with comorbidities affecting the endothelium were excluded from the study. Preoperative, intraoperative, and postoperative data, including ECD, were collected. Postoperative assessments were conducted at 1, 3, and 6 months.
Results
The mean age of the patients was 24.80±2.81 years (range=21–30 years), with 60% of the patients being males. The preoperative diagnoses included keratoconus, granular dystrophy, macular dystrophy, and corneal scarring. The ECD significantly decreased from a baseline value of 2726.6±188.53 cells/mm^2 to 2591±175.79 cells/mm^2 at 1 month, 2461.3±155.43 cells/mm^2 at 3 months, and to 2349.9±104.58 cells/mm^2 at 6 months postoperatively ($P<0.001$). The best corrected visual acuity improved significantly from a preoperative value of 0.06±0.02 to 0.61±0.15 at 6 months, postoperatively ($P<0.001$). The postoperative complications included a double anterior chamber (in 2 eyes) and wrinkling of Descemet's membrane (in 2 eyes).
Conclusion
DALK led to a significant reduction in ECD over the first 6 months postoperatively, with improvement in best corrected visual acuity. The study highlights the importance of considering ECD changes and visual outcomes in DALK patients. Preservation of the patient's endothelium may contribute to better long-term outcomes, with careful consideration of the potential complications.

Keywords:
corneal transplantation, deep anterior lamellar keratoplasty, endothelial cell density, surgical complications, visual acuity

DJO 25:135–141
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1110-9173

Introduction

The fundamental aim of successful keratoplasty is to obtain a clear corneal graft and to maintain its survival. Previous studies have demonstrated the importance of endothelial cell density (ECD) in maintaining graft transparency and survival after keratoplasty [1].

The average yearly rate of endothelial cell loss within the initial 3–5 years following penetrating keratoplasty (PKP) (7.8%/year) exceeds the natural endothelial cell loss rate (0.52%/year) [2,3]. A major factor for late graft failure is the cumulative endothelial cell loss, reaching up to 50% within 10 years after PKP [4].

To obtain long-term protection of corneal graft transparency, deep anterior lamellar keratoplasty (DALK) has emerged as the preferred procedure in recent years. In this approach, the corneal endothelium of the recipient is kept intact and the anterior layers of the donor cornea, without endothelium and Descemet's membrane (DM), are transplanted which eliminates the possibility of endothelial rejection.

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Today, sophisticated DALK surgical techniques have enabled sufficient stromal dissection and the preparation of a smooth recipient bed without interface problems. With DALK, the loss of endothelial cells appears to be similar to the physiological loss, and impairment of corneal graft transparency due to tissue mismatch can be prevented [5]. However, postoperative endothelial cell loss was still observed in DALK, which could be related to surgical injury and postoperative inflammation. In general, the reported long-term endothelial cell loss is lower in DALK than in PKP [6].

DALK is currently the first-choice operative procedure for patients with corneal diseases that do not involve the endothelial layer, including keratoconus, stromal scars, and lattice dystrophy [7,8]. Patients with these corneal diseases may require transplantation during the first 3 decades of life. Long-term graft survival is expected in young patients. Removal of the damaged corneal stroma can be achieved by manual dissection with a surgical blade and scissors, microkeratome-assisted lamellar cutting, or femtosecond laser-assisted cutting. Detachment of the DM from the corneal stroma can also be achieved using air injection (the 'big-bubble' technique), hydro-delamination through a sclero-corneal flap, or sodium hyaluronate injection. While a successful big-bubble procedure allows the removal of all recipient stroma, a layer of the recipient posterior stroma usually remains with other techniques [9,10].

This study aimed to assess the ECD changes after DALK and to evaluate its surgical and visual outcomes.

Patients and methods

This is an observational prospective study that was conducted at the National Eye Institute Rod-Elfarag, Cairo, Egypt between July 2020 and February 2022. The Ethical Committee of the Faculty of Medicine, Beni-Suef University approved the study in 7/6/2020 (FMBSUREC/07062020/Elbeih, FWA #: FWA00015574). The study adhered to the principles of the Declaration of Helsinki and all patients signed a written informed consent to participate in the study and for publication of data before enrollment in the study.

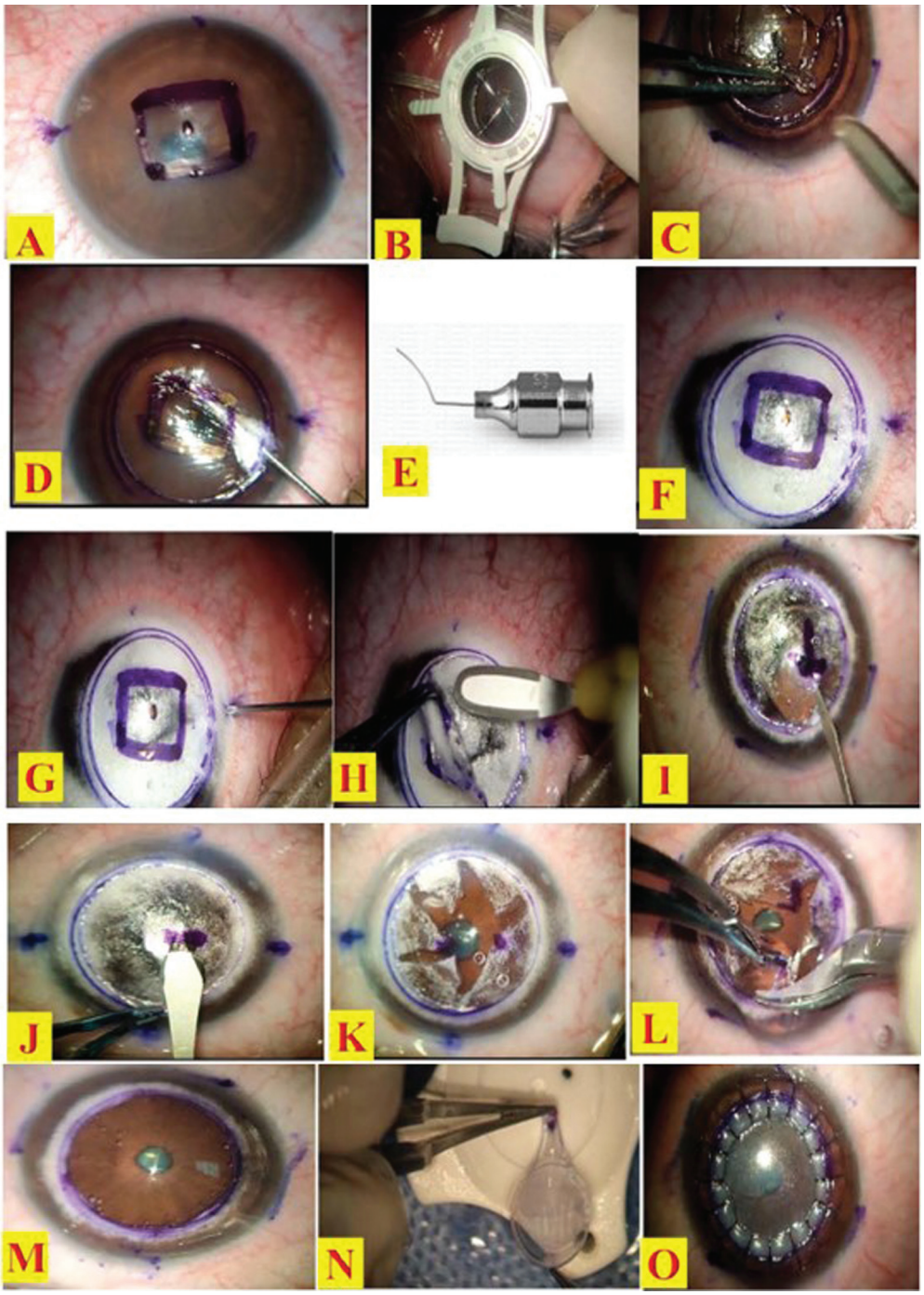
The study included patients aged 20–50 years of either sex with any pathology affecting the epithelium and/or stroma for whom DALK was performed. Only phakic eyes were included in the analysis to ensure consistency in the study population. Patients with other anatomical comorbidities, any pathology such as glaucoma or uveitis, and ocular surface disorders that may affect the endothelium, and patients with previous history of herpes which may cause endotheliitis or kerato-uveitis were excluded from the study.

A thorough ophthalmic evaluation was conducted including visual acuity measurement, anterior segment evaluation using slit lamp biomicroscopy to assess the anterior segment including the cornea and lens. For posterior segment evaluation, an indirect ophthalmoscope was utilized if a clear view was attainable. On the other hand, if visibility was compromised due to factors like opacities, ultrasound imaging was employed. Further investigations (according to the regulations by the Ministry of Health) included electroretinogram (ERG) (HVM-MonPackONE (METROVISION, PERENCHIES, France) and visual evoked potential (VEP) (HVM-MonPackONE, METROVISION, PERENCHIES, France) to evaluate retinal and optic nerve function, respectively. Anterior segment optical coherence tomography (AS-OCT) (RS-3000 Advance 2, NIDEK CO., LTD, Hiroishi-cho, Gamagori, Aichi 443-0038, JAPAN) provided detailed imaging of the anterior segment structures. Additionally, corneal ECD was assessed preoperatively using a Topcon specular microscope (Topcon SP-2000P Specular Microscopy, TOPCON corporation, Tokyo, Japan), with exceptions made for cases with dense corneal opacities or dystrophies where specular microscopy was deferred until the first day postoperatively.

Surgical technique

The anatomical center of the cornea was marked (Fig. 1a). Marking the anatomical center of the cornea is a very important step in keratoplasty to avoid graft decentration with complications such as a high incidence of graft rejection and high postoperative astigmatism. Marking of the corneal center was done by using a caliber that was adjusted to make a mark 4.5 mm from the superior, inferior, nasal, and temporal limbus, respectively. The center of the resulting square is the anatomical center of the cornea. This was followed by partial thickness trephination of the host cornea up to 60–80% depth using Hessburg Barron suction trephine (Tecfen suction trephine, Tecfen Medical, Santa Barbara, California, USA) (Fig. 1b and c). A blunt tip air injection cannula with a 5 ml filled syringe was inserted into the mid-peripheral stroma and was pushed forward 3–4 mm toward the center of the cornea, parallel to the corneal plane (Fig. 1d and e). Air was injected with increased

Figure 1



Steps of deep anterior lamellar keratoplasty. A) Marking the anatomical center of the cornea. B) Hessburg Barron suction trephine. C) Partial thickness trephination of the cornea. D) insertion of air cannula. E) air cannula. F) big bubble appearance. G) Paracentesis. H) The anterior and middle stroma were removed with a crescent knife. I) Big bubble roof opening. J) An incision was made by the keratome to the posterior stroma to reach the Descemet's membrane (DM). K) posterior stromal lamellae segmentation. L) segment removal. M) Bare Descemet's membrane. N) Stripping of the donor Descemet's membrane. O) Interrupted sutures.

pressure to dissect the DM from the stroma by forming a big bubble (Fig. 1e). Paracentesis was performed to decrease the intraocular pressure (Fig. 1g). The needle or blade used should be vertical while performing paracentesis to avoid injury to the DM. The anterior and middle stroma were removed with a crescent knife (Fig. 1h). An incision was made by the keratome to the posterior stroma to reach the DM (Fig. 1i and j). The remnants of the posterior stromal lamellae were removed with blunt tip corneal scissors (Fig. 1k, l, and m).

The donor cornea was cut from the endothelial side with a 0.25–0.5 mm larger Barron donor punch

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(Fig. 1n). The endothelium was stained with trypan blue and was completely stripped from the button. The donor cornea was placed on the recipient bed and fixed with 16 bites of interrupted sutures (Fig. 1o).

The postoperative regimen included topical postoperative antibiotics, steroids, and lubricants.

A comprehensive ophthalmological evaluation was conducted for all patients at 1, 3, and 6 months, postoperatively. The examination included uncorrected and best-corrected visual acuity (UCVA and BCVA), slit lamp biomicroscopy for detailed examination of the anterior segment, and measurement of spherical equivalent and astigmatism in diopters (D) to gauge the refractive status. The corneal ECD was evaluated utilizing the Topcon SP. 2000P specular microscope. Indirect ophthalmoscopy was performed to assess the posterior segment, while AS-OCT provided detailed imaging of the cornea and anterior chamber (AC) structures. This comprehensive approach ensured thorough monitoring and assessment of postsurgical outcomes and patient's ocular health.

Statistical analysis

The data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) software version 20.0. (SPSS, IBM Corp, Armonk, NY, USA). The qualitative data were described using numbers and percent. The Shapiro–Wilk test was used to verify the normality of distribution. The quantitative data were described using range (minimum and maximum), mean, SD, median, and interquartile range (IQR). Paired *t* test was used for normally distributed quantitative variables to compare between two periods. The analysis of the variance test with repeated measures was used for normally distributed quantitative variables to compare between more than two periods or stages, and a Post Hoc test (Bonferroni adjusted) was used for pairwise comparisons. The significance of the obtained results was determined at the 5% level.

Results

The study included 50 eyes of 50 patients. The mean age of the patients was 24.80±2.81 years (range=21–30 years) with 30 (60%) patients being males and 20 (40%) being females. The preoperative diagnosis was keratoconus in 20 (40%) cases, granular dystrophy in 10 (20%) cases, macular dystrophy in 10 (20%) cases, and corneal scarring in 10 (20%) cases.

The mean baseline ECD was 2726.6±188.53 cells/mm^2. It decreased significantly to 2349.9±104.58 cells/mm^2 after 6 months ($P<0.001$, Table 1). The specific data for patient number 6 are illustrated in Fig. 2.

The mean preoperative BCVA was 0.06±0.02 (range=0.03–0.08). It improved significantly to 0.76 ±0.14 (range=0.50–1.00) at 3-month and 6-month postoperatively ($P<0.001$, Table 2).

The postoperative complications were reported in 3 (6%) cases. Double AC occurred in two cases, one of them resolved spontaneously after 2 weeks (Fig. 3a) and the other required rebubbling (Fig. 3b). Wrinkling of the central part of DM was reported in two cases, one of them was already diagnosed with double AC and underwent rebubbling (Fig. 3b and c) and the other had a spontaneously attached DM (Fig. 3d).

Discussion

In present study, there was a higher proportion of males (60%) compared with females (40%), indicating a potential sex disparity in the incidence of corneal conditions necessitating keratoplasty. This agrees with previous studies by Van Dooren *et al.* [11] and Hashish *et al.* [12], who reported variations in sex distribution among patients undergoing keratoplasty.

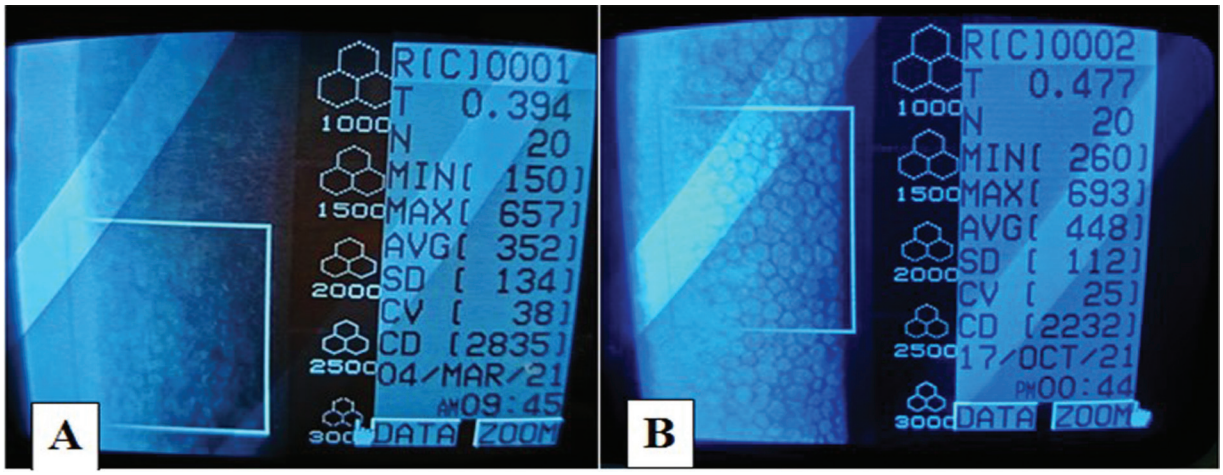
The mean age of the patients was 24.80±2.81 years, which is a relatively young population undergoing keratoplasty. Older age was reported by Van Dooren *et al.* [11] and Hashish *et al.* [12], reflecting potential differences in patient demographics across studies.

Table 1 Endothelial cell density changes at the different study periods (n=50)

ECD (cells/mm^2)	Baseline	1 m	3 m	6 m	F	*P*
Min–max	2345–2988	2234–2876	2134–2678	2122–2478	371.313	<0.001*
Mean±SD	2726.6±188.53	2591±175.79	2461.3±155.43	2349.9±104.58		
Median	2778	2610.5	2456	2347		
IQR	2567.0–2789	2456–2678	2346–2564	2311–2433		
P_1		<0.001*	<0.001*	<0.001*		
% Reduction		4.96±1.27	9.68±1.65	13.64±3.10		

*Statistically significant. ECD, endothelial cell density; F, F test (ANOVA) with repeated measures, significance between periods were performed using a Post Hoc test (adjusted Bonferroni); IQR, Interquartile range; p, *P* value for comparing between the studied periods; *P*1, *P* value for comparing between baseline and each postoperative period.

Figure 2



Specular microscopy data of a keratoconus case. a) Preoperative. b) postoperative.

Table 2 Best corrected visual acuity changes at the different follow-up periods (n=50)

BCVA	Baseline	1 m	3 m	6 m	F	*P*
Min–max	0.03–0.08	0.30–0.80	0.50–1.00	0.50–1.00	1049.812	<0.001*
Mean±SD	0.06±0.02	0.61±0.15	0.76±0.14	0.76±0.14		
Median	0.06	0.65	0.80	0.80		
IQR	0.04–0.06	0.60–0.70	0.70–0.80	0.70–0.80		
P_1		<0.001*	<0.001*	<0.001*		
% Change		1072.50±368.09	1361.67±354.44	1361.67±354.44		

*Statistically significant. BCVA, best corrected visual acuity; F, F test (ANOVA) with repeated measures, significance between periods were performed using a Post Hoc test (adjusted Bonferroni); IQR, interquartile range; p, *P* value for comparing between the studied periods; *P*1, *P* value for comparing between baseline and each postoperative period.

The primary indications for keratoplasty, in the current study, were keratoconus, granular dystrophy, macular dystrophy, and corneal scarring. This distribution agrees with previous studies [12,13], highlighting the diverse etiologies necessitating corneal transplantation.

The ECD is a crucial determinant for long-term graft survival following keratoplasty. In the present study, there was a significant reduction in the ECD over time, which is consistent with Van Dooren *et al.* [11], Acar *et al.* [14], and Mousa *et al.* [15]. The observed decline underscores the importance of monitoring endothelial health postoperatively to optimize graft outcomes.

Comparing outcomes between PKP and DALK, the current study supports superior endothelial cell survival and visual rehabilitation in DALK. This aligns with previous studies [11,14,16], highlighting the potential advantages of preserving the recipient endothelium in DALK.

In the present study, the BCVA improved significantly postoperatively, which agrees with other investigators [12,17,18]. Notably, DALK was associated with better postoperative visual acuity compared with PKP [19], emphasizing its efficacy in enhancing visual outcomes.

The postoperative complications varied between PKP and DALK, with DALK generally exhibiting fewer complications. This is consistent with previous reports [12,15,17,18], suggesting a favorable safety profile for DALK compared with PKP.

The limitations of the current study include the small sample size and the short follow-up duration. Future studies with larger cohorts and longer follow-up are warranted to further elucidate the comparative effectiveness and safety of DALK compared with PKP.

Conclusion

There was a significant reduction in ECD over the first 6 months following the DALK procedure. The comparative advantage of DALK in preserving recipient corneal endothelium and promoting better cell survival underscores its significance as a favorable option for corneal transplantation, particularly when

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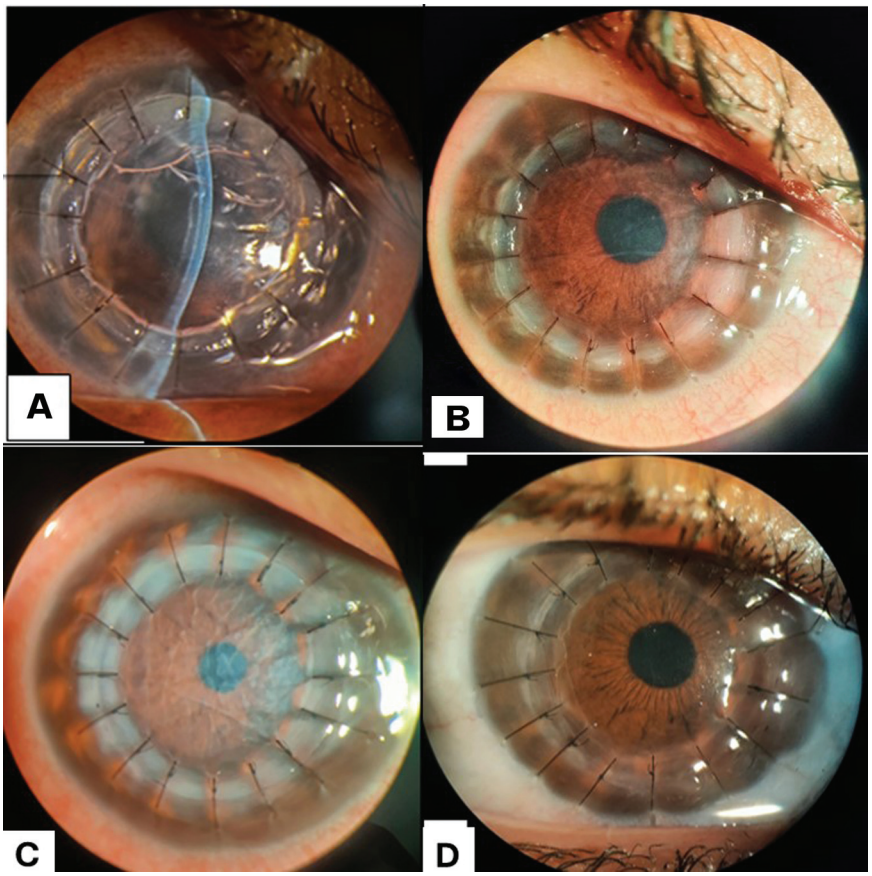
Figure 3



Postoperative complications. a) Double anterior chamber. b) Central wrinkles in the Descemet's membrane (DM). c) Postoperative graft edema. d) Spontaneously attached Descemet's membrane.

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considering long-term graft outcomes and patient prognosis.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Mishra SK, Joshi A, Ginu PM, Sati A, Kumar SV. Corneal transplantation: A walk to vision. Med J Armed Forces India 2023; 79:645–650.

2 Ghareeb AE, Figueiredo MS, Pradhan SP, Curnow E, Armitage WJ, Figueiredo FC. Long-term graft survival and decline in endothelial cell density following penetrating keratoplasty with organ-cultured corneas. Ophthalmol Ther 2022; 11:1131–1146.

3 Ragab M, SAIF MY, Gouda A. Assessment of Endothelial cell Density in Pterygium: A Cross-sectional Study. NILES J Geriatric Gerontology 2020; 3 (Geriatric Ophthalmology):34–39.

4 Patel SV, Lass JH, Benetz BA, Szczotka-Flynn LB, Cohen NJ, Ayala AR, *et al.* Postoperative endothelial cell density is associated with late endothelial graft failure after descemet stripping automated endothelial keratoplasty. Ophthalmol 2019; 126:1076–1083.

5 Melles GRJ, Remeijer L, Geerards AJM, Beekhuis WH. A quick surgical technique for deep lamellar keratoplasty using visco-dissection. Cornea 2000; 19:427–432.

6 Feizi S, Javadi MA, Karimian F, Bayat K, Bineshfar N, Esfandiari H. Penetrating keratoplasty versus deep anterior lamellar keratoplasty for advanced stage of keratoconus. Am J Ophthalmol 2023; 248:107–115.

7 Kawashima M, Kawakita T, Den S, Shimmura S, Tsubota K, Shimazaki J. Comparison of deep lamellar keratoplasty and penetrating keratoplasty for lattice and macular corneal dystrophies. Am J Ophthalmol 2006; 142:304–309.

8 Krumeich JH, Knulle A, Krumeich BM. Deep anterior lamellar (DALK) vs. penetrating keratoplasty (PKP): a clinical and statistical analysis [in German]. Klin Monbl Augenheilkd 2008; 225:637–648.

9 Anwar M, Teichmann KD. Big-bubble technique to bare Descemet's membrane in anterior lamellar keratoplasty. J Cataract Refract Surg 2002; 28:398–403.

10 Shiuey EJ, Zhang Q, Rapuano CJ, Ayres BD, Hammersmith KM, Nagra PK, *et al.* Development of a nomogram to predict graft survival after penetrating keratoplasty. Am J Ophthalmol 2021; 226:32–41.

11 Van Dooren BTH, Mulder PGH, Nieuwendaal CP, Beekhuis WH, Melles GRJ. Endothelial cell density after deep anterior lamellar keratoplasty (Melles technique). Am J Ophthalmol 2004; 137:397–400.

12 Hashish AM, Awad EA, Sabry D, El-Awady HE, El-Metwally MN. Evaluation of deep anterior lamellar keratoplasty for anterior corneal stromal pathology. J Egypt Ophthalmol Soc 2021; 114: 21.

13 Nanavaty MA, Vijjan KS, Yvon C. Deep anterior lamellar keratoplasty: A surgeon's guide. J Curr Ophthalmol 2018; 30:297–310.

14 Acar BT, Vural ET, Acar S. Changes in endothelial cell density following penetrating keratoplasty and deep anterior lamellar keratoplasty. Inter J Ophthalmol 2011; 4:644.

15 Mousa R, Aboulenin K, Elgendy N, Elsayed M. Corneal endothelial cell loss following Deep Anterior Lamellar Keratoplasty. NILES J Geriatric Gerontology 2024; 7:226–236.

16 Sugita J, Kondo J. Deep lamellar keratoplasty with complete removal of pathological stroma for vision improvement. Br J Ophthalmol 1997; 81:184–188.

17 Schaub F, Enders P, Adler W, Bachmann BO, Cursiefen C, Heindl LM. Impact of donor graft quality on deep anterior lamellar Keratoplasty (DALK). BMC Ophthalmol 2017; 17:204–212.

18 Yüksel B, Kandemir B, Uzunel UD, Çelik O, Ceylan S, Küsbeci T. Comparison of visual and topographic outcomes of deep-anterior lamellar keratoplasty and penetrating keratoplasty in keratoconus. Int J Ophthalmol 2017; 10:385–390.

19 Tan DT, Anshu A, Parthasarathy A, Htoon HM. Visual acuity outcomes after deep anterior lamellar keratoplasty: a case-control study. Br J Ophthalmol 2010; 94:1295–1299.

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