Original Article

# Outcome of therapeutic deep anterior lamellar keratoplasty in advanced *Acanthamoeba* keratitis

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**Purpose:** To report and analyze the outcomes of therapeutic deep anterior lamellar keratoplasty (DALK) in patients with advanced *Acanthamoeba* keratitis (AK). **Methods:** Medical records of microbiologically confirmed AK, underwent DALK from 2004 to 2017, were reviewed and the data related to early and late outcome including complications were retrieved. Outcome of cases with largest diameter of infiltrate ≥8 mm at the time of surgery (advanced keratitis) were analyzed and compared with those with less severe keratitis (infiltrate size less than 8 mm). **Results:** Out of 23 patients of AK in whom DALK was performed, ten (43.4%) patients had advanced keratitis. Mean age of these patients was 38.7 ± 8.6 years (range, 25 to 56). Median visual acuity at presentation was 2.78 (IQR, 1.79–3.0) that improved to 1.79 (IQR, 0.70–2.78) postoperatively. Early complications included recurrence of AK in 2 (20%), Descemet's membrane detachment in 5 (50%), and persistent epithelial defect in 3 (30%) cases. Overall, 6 (60%) grafts failed, whereas 4 (40%) patients had clear graft at their last follow-up. Median follow-up of these cases was 5 months (IQR, 1.4–11.4). One graft developed stromal rejection, which resolved with increased dose of corticosteroids. In comparison, DALK performed for less severe keratitis ($N$ = 13) had 1 (7.6%) recurrence and 2 (15.8%) grafts failure (OR, 8.25). The probability of one-year graft survival and eradication of infection was 32% and 74.1%, respectively, in advanced cases compared to 91.6% and 83.9% in less severe cases. **Conclusion:** Outcome of DALK in advanced *Acanthamoeba* keratitis is less favorable compared to those carried out for less severe keratitis cases.

**Access this article online**

**Website:** www.ijo.in

**DOI:** 10.4103/ijo.IJO_307_19

**Quick Response Code:**

**Key words:** *Acanthamoeba* keratitis, deep anterior lamellar keratoplasty, Microbial keratitis

Acanthamoeba is relatively uncommon cause of corneal infection accounting for less than 5% of all microbiology positive cases.[1-6] However, the cases are associated with significant morbidity on account of several challenges associated with its management. These include: Delayed presentation and diagnosis, nonavailability of effective and approved therapy, and need for relatively longer duration of treatment. Medical treatment using Biguanides[7] either singly or in combination[8-10] with hexamidines/Azoles is reported to be successful in eradicating infection in 60–90%[1,7] cases. Cases who presented late are quite difficult to manage by medical therapy alone. They are mostly caused by trauma rather than caused by contact lens.[2]

Therefore, surgery plays an important role in the management of this disease. While therapeutic penetrating keratoplasty (TPK)[11-18] is the classical procedure performed for the eradication of infection, more recent literature suggests successful eradication of infection even with deep anterior lamellar keratoplasty (DALK).[19-21] The very first report of successful DALK for AK cases was published in 2007 by[20] Parthasarathy *et al*. Later in 2009, the same group published comparison of full-thickness keratoplasty with lamellar procedure and found superior graft survival, lower risk of complications including recurrence of infection, endophthalmitis, and glaucoma. Yet another group (Sarnicola *et al*.) recommended early surgery for superior graft survival. In most of these reports, the average size of infiltrate at the time of surgery was 6 mm.[20,21] There is no report on the outcomes of DALK in patients with advanced keratitis (ADK).

This knowledge is important because the literature review of fungal keratitis clearly suggests higher risk of recurrence of infection after DALK if performed in patients with ADK (larger infiltrate and involvement of posterior stroma).

The objective of this study was to evaluate the outcome (both short and long term) of DALK performed for advanced acanthamoeba keratitis cases and compare it with those performed for less severe disease.

## Methods

The study was conducted at an advanced tertiary eye care center. It was approved by the institutional review board (LEC 09-13-078) and followed the tenets of declaration of Helsinki. Medical records of all microbiology proven

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Received: 12-Feb-2019 Revision: 28-Aug-2019
Accepted: 12-Sep-2019 Published: 14-Feb-2020



**Cite this article as:** Bagga B, Garg P, Joesph J, Mohamed A, Kalra P. Outcome of therapeutic deep anterior lamellar keratoplasty in advanced *Acanthamoeba* keratitis. Indian J Ophthalmol 2020;68:442-6.

*Acanthamoeba* keratitis cases managed at the center between July 2004 and December 2017 were reviewed to identify cases where surgery notes documented the successful completion of DALK for the treatment of active keratitis. In all cases of microbiologically proven *Acanthmamoeba* keratitis, institute protocol was to treat with combination of topical PHMB 0.02% and Chlorhexidine 0.02%. These topical medications were not commercially available and needed to be prepared from stock solution (20%) in microbiology lab by diluting with CMC eye drops. In the event of worsening of infection even after intensive medications, it was managed by surgical management in terms of DALK or PK depending on the depth of involvement. Worsening of infection was defined as increase in the size of infiltrate and progressive thinning of corneal stroma. In all the cases, category 2 corneal tissues with compact stroma and without epithelial sloughing were used. Prior to use, all corneal tissues were stored in MK medium with maximum storage of 4 days. We followed the standard protocol for use in DALK as laid by Eye bank association of India. As in all the cases, DM was peeled off prior to the transplantation, endothelial cell count was given least preference. Postoperatively anti-*Acanthamoeba* medications were continued in all cases for a minimum 2 weeks. Topical prednisolone acetate 1% was started in all cases in a week time provided there is no clinical suspicion of residual infection. Following data were gathered from the review of medical records of included cases: Demographic characteristics, clinical features at the time of presentation and at the time of surgery, microbiology results, and the details of prior and post diagnosis treatment. Depth and extent of infiltrate was measured by the use of slit-lamp examination. For very advanced cases where cornea was either perforated or there was extensive corneal involvement till Descemet's membrane, PK was advised or else DALK was planned in other cases. Surgery sheets were reviewed to gather information on trephine size for recipient and donor preparation, the technique of DALK, and intraoperative details. All patients were followed up in the institute for variable duration and received anti-*Acanthamoeba* treatment with PHMB and Chlorhexidine along with corticosteroids in the immediate postoperative period. At each follow-up visit, the patients underwent detailed clinical examination and observations related to visual acuity graft status including evidence of infiltration, edema, and Descemet membrane status. In addition, other findings in anterior and posterior segment as well as intraocular pressure were noted in medical records. Patients with postoperative complications were managed appropriately. For the purpose of this study, we classified all patients with infiltrate size of ≥ 8 mm in the widest meridian at the time of surgery as ADK and compared the outcomes of these cases with those with less severe keratitis (LSK). This classification in advanced and LSK was based on the classification of severity scale of microbial keratitis. In the cases with *Acanthamoeba* keratitis as well, size of the infiltrate is considered as one of the risk factors of bad prognosis.[1,22]

The statistical analysis was performed using the software Origin v7.0 (OriginLab Corporation, Northampton, MA, USA). The continuous data were checked for normality using Shapiro-Wilk test. Mean and standard deviation described the normally distributed data, whereas median and inter-quartile range (IQR) described the data that are not normally distributed. The categorical data were described in proportions. While comparing the two outcome groups, Student *t*-test was used for comparisons between parametric data and Mann–Whitney test for nonparametric ones. Fisher's exact test was used to compare the categorical data. A *P* value of <0.05 was considered statistically significant.

## Results

We identified total 23 patients of *Acanthamoeba* keratitis [Fig. 1], in whom DALK was performed for the control of infection. Out of which, 10 (43.4%) of the cases were categorized as ADK. Mean age of the patients was 38.7 ± 8.6 years (range 25 to 56 years). Males and females were distributed equally (five each) in this group. Median size of infiltrate at the time of presentation was 8 mm both vertically and horizontally. Deeper layer of stroma was involved in 6 (60%) out of 10 cases. Median duration of presentation from the onset of infection was 13.5 days (range, 2–180 days). Three (30%) cases had vision equivalent to perception of light with projection of rays, while median visual acuity for remaining cases was 2.78 (IQR, 1.79–3.0). The median duration of treatment with anti-*Acanthamoeba* drugs prior to DALK was 12 days (IQR, 7 to 60 days). Median follow-up of these cases was 5 months (IQR, 1.4–11.4 months). Anti-*Acanthamoeba* drugs (same as before) were continued for a median duration of 23.5 days (IQR, 18 to 30 days) postoperatively. Topical corticosteroids (Prednisolone acetate 1%) were also started in the immediate postoperative period with the median interval of 4 days (IQR, 1–9 days) from surgery. Postoperatively, the best-corrected visual acuity was only light perception in one case and no light perception in one case. In all other cases, median Log MAR visual acuity was 1.79 (IQR, 0.70–2.78).

Early (noticed either immediate or <1 week postoperative) complications included the recurrence of infection, Descemet's membrane detachment (DMD), and surface-related complications such as filamentary keratopathy and nonhealing epithelial defect. The recurrence of AK [Fig. 2] was noticed in 2 (20%) cases. Therapeutic PK was done for one eye, while for other case evisceration was performed. DMD was noticed in 5 (50%) cases. Air descemetopexy was performed for all of them, which lead to reattachment of DM with clearing of the graft in 2 cases while in other 3 grafts, edema persisted despite attached DM. Three cases developed persistent epithelial defect, which were managed with lubricants and tarsorrhaphy.

On long-term follow-up, 1 out of 4 clear grafts developed stromal rejection [Fig. 3] that resolved completely with the increased dose of topical steroids.

Overall, out of 10 cases, 6 (60%) grafts failed and 4 (40%) were clear at the last follow-up. The probability of graft survival and recurrence-free graft survival in these cases at the end of one year were 32% and 74.1%, respectively.

On comparing outcomes [Table 1] of these cases with advanced (ADK) (*N* = 10) with that of LSK (*N* = 13), we found that in LSK, only 2 (15.8%) grafts failed (odds ratio, 8.25). The probability of graft survival and recurrence-free graft survival in at the end of one year were 91.6% and 83.9%, respectively. Clinical details of cases with failed graft (8 in 23) and recurrence of infection (3 in 23) are elaborated in Tables 2 and 3, respectively.

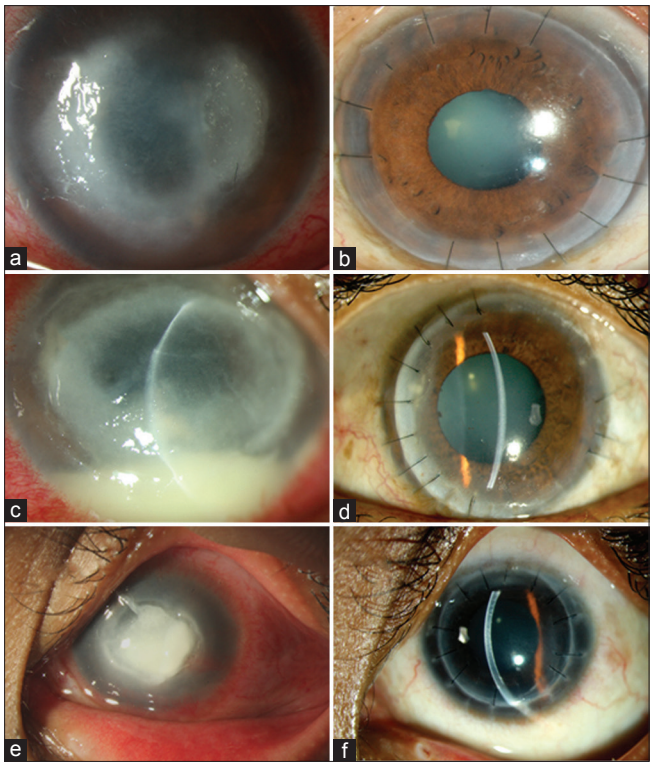


**Figure 1:** Successful outcome of TDALK done to treat *Acanthamoeba* keratitis with severity of varied intensity. (a and b) shows moderate severity, while (c and d) shows very advanced infection with scleral involvement, and (e and f) shows central infection partially responding with dense and deep vascularization

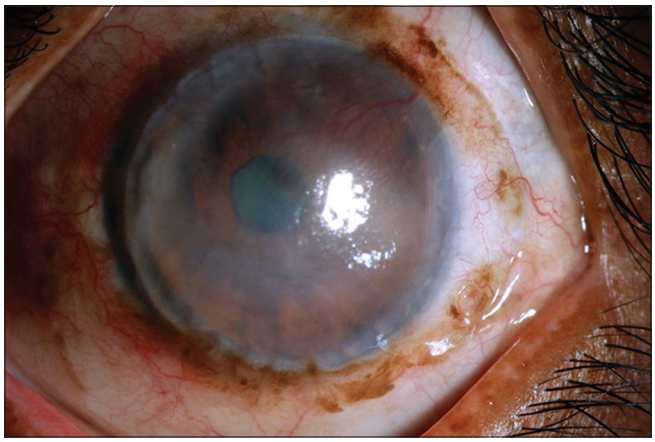

**Figure 3:** Stromal rejection of the graft characterized by the occurrence of stromal edema with deep vessels in the interface

## Discussion

Surgery plays an important role in the management of microbial keratitis. The most common procedure performed comprises the excision of diseased tissue and replacing with healthy corneal tissue. The primary goal of such a surgical procedure is the eradication of infection. Restoration of vision is secondary objective. Traditionally, the procedure is comprised of full-thickness excision of cornea and replacing it appropriate sized full-thickness healthy corneal tissue (penetrating keratoplasty). However, the procedure is associated with many complications, especially because the procedure is carried out

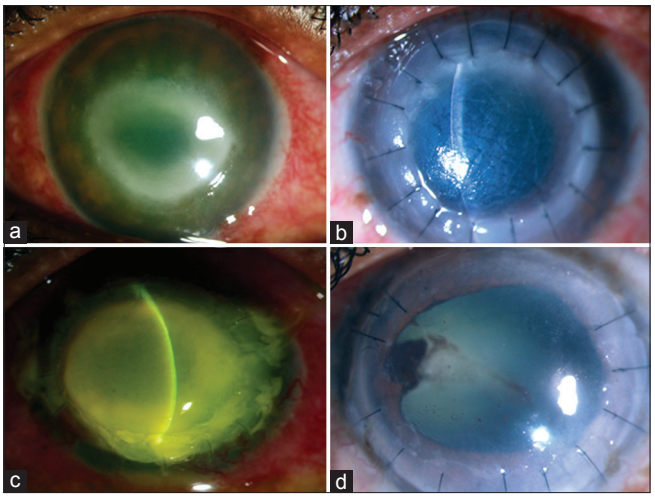


**Figure 2:** Case with AK. (a) Recurrence of *Acanthamoeba* infection after DALK. Persistent stromal edema (b) with cellularity along with interface haze characterizes the recurrence of infection, treated medically and worsened (c) and treated with TPK with good outcome (d)

in inflamed eyes. Deep anterior lamellar keratoplasty is a closed globe procedure and has been shown to be effective in eyes with active infection. Several reports have highlighted superiority of the procedure over full-thickness penetrating keratoplasty. While most of these reports described its role in early cases, i.e., cases with 6-mm infiltrate, its role in patients with ADK is not clear. The procedure in ADK has potential of a) higher risk of recurrence of infection not only from peripheral cornea but also from the underlying stroma Descemet membrane complex and b) complications of DALK such as DM detachment. Therefore, we decided to undertake this study. Although the primary goal of the surgery, i.e. eradication of the infection, was achieved in 8 of the 10 cases, overall outcome in terms of clear graft was achieved in only 4 out of 10 cases. These outcomes are much inferior to outcomes observed in less severe cases requiring relatively smaller size of the graft with 11 of 13 patients achieving clear graft in that cohort. Eyes in a group of ADK had more posterior involvement. In our series, 6 eyes out of 10 (60%) eyes with ADK, and 3 (23%) of 13 eyes with LSK were having posterior stromal involvement. The poor outcomes were primarily due to higher risk of complications in the immediate postoperative period in the form of incomplete eradication of the infection, DMD, and epithelial problems. We continued postoperative treatment with anti-*Acanthamoeba* medications for 23.5 days (IQR, 18–30 days) to avoid recurrences based on our experience although some authors suggested longer duration of treatment. Duration of treatment prior to DALK in both the groups were different due to severity of keratitis at presentation. For mild-to-moderate cases, median duration of preoperative medications was 2 (0.8–3) months, while it was 0.4 (0.25–2) months in advanced cases to control infection and save the eye. As most of the cases with AK resolve medically, we also tried in most of the cases to control the infection with medical therapy.

Higher rate of recurrence of infection observed in this series could be due to difficulty with big bubble in patients requiring larger graft resulting in failure to achieve bare Descemet's membrane and consequently incomplete removal of stroma. The information about whether the big bubble was achieved was missing in many records; therefore, the assumption is

**Table 1: Difference between advanced and mild-to-moderate forms of keratitis based on the size of infiltrate**

| Groups | Advanced Keratitis (size >8 mm) | Mild-to-moderate Keratitis (<8 mm) | *P* |
|---|---|---|---|
| No of cases | 10 | 13 | |
| Age (years), mean±SD | 38.7±8.6 | 31.7±12.2 | 0.14 |
| Duration of symptoms (days), median (IQR) | 13.5 (7 to 30) | 15 (10 to 30) | 0.42 |
| Size of infiltrate (median in mm) | 8×8 | 4.5×5.3 | 0.0001 |
| Depth of infiltrate | DS-6, MS-1, AS-3 | DS-3, MS-4, AS-6 | 0.10 |
| Use of topical steroids prior to DALK | 1 (10%) | 3 (23.1%) | 0.60 |
| Duration of treatment (months), median (IQR) | 0.4 (0.25 to 2) | 2 (0.8 to 3) | 0.10 |
| Follow-up period (years), median (IQR) | 0.4 (0.1 to 1) | 1.2 (0.4 to 2.9) | 0.10 |
| Size of trephine (mm), median (IQR) | 9.5 (8.5 to 10) | 8 (8 to 8.25) | 0.02 |
| Big bubble: Manual | 2:4 | 4:5 | 1.00 |
| DMD | 5 (50%) | 3 (23.1%) | 0.22 |
| Recurrence | 2 (20%) | 1 (7.7%) | 0.56 |
| Failure | 6 (60%) | 2 (15.4%) | 0.04* |
| Rejection | 1 (10%) | 3 (23.1%) | 0.60 |
| Histopathology positive for Cysts | 10 (100%) | 7 (63.6%) | 0.09 |

(*Odds ratio=8.25, 95% confidence interval 1.15-59.01), DS: Deep Stromal, MS: Mid Stromal, AS: Anterior stromal

**Table 2: Eight cases of failed DALK with their causes of failure and their outcome**

| Case | Cause of failure | Management | Outcome | Visual acuity |
|---|---|---|---|---|
| 1 | Persistent graft edema then developed PED | Penetrating keratoplasty (PK) with cataract surgery with IOL after 5 months | Good | 20/60 |
| 2 | DMD, persistent graft edema | Descemetopexy, cataract surgery | Poor | PL, PR rec |
| 3 | Graft infiltrate, DMD | Evisceration | Poor | PL negative |
| 4 | Recurrence of infection | Therapeutic PK | Poor | PL, PR inaccurate |
| 5 | Recurrence of infection | Therapeutic PK | Poor | HM, PL + |
| 6 | Graft infiltrate with extensive necrosis (bacterial infection) | Tissue adhesive | Poor | CFCF |
| 7 | DMD, with secondary infection | Descemtopexy with interface fluid drainage | Poor | CFCF |
| 8 | Recurrence of infection | Therapeutic PK | Good | 20/200 |

**Table 3: Demographical, clinical, microbiological, histopathological features and outcome of *Acanthamoeba* recurrences on the graft**

| Case no. | Preoperative clinical and demographic features | | Duration after DALK | Location of recurrence and clinical features | Microbiology | Histopathology | Management and Outcome |
|---|---|---|---|---|---|---|---|
| 1 | Age/sex-<br>Duration before diagnosis<br>Ulcer size-<br>Duration of treatment<br>Technique- (DALK) | 25-year-old female, 3 days, 7.6×7.7 mm, 75 days Manual technique for DALK | 62 days | Superior GHJ and severe persistent graft edema with pigments on endothelium | Smears-negative<br>Culture -*Acanthamoeba* | Cysts and trophozoites Present | Therapeutic PK Persistent graft edema and later graft infiltrate (Poor) |
| 2 | Age/sex-<br>Duration before diagnosis<br>Ulcer size-<br>Duration of treatment<br>Technique- (DALK) | 56-year-old male, 30 days, 8×8 mm, 48 days Manual technique | 30 days | Severe graft edema with endoexudates wit pigments | Smears- cysts<br>Culture-*Acanthamoeba* grown | Cysts were present | Therapeutic PK no recurrence Failed graft (poor) |
| 3 | Age/sex-<br>Duration before diagnosis<br>Ulcer size-<br>Duration of treatment<br>Technique- (DALK) | 47-year-old male, 15 days, 4×5 mm, 20 days BB for DALK | 27 days | Graft edema with superior arcuate infiltrate with pigments on endothelium | Smears-*Acanthamoeba*<br>Culture-*Acanthamoeba* grown | Cysts are seen | Therapeutic PK done Good no recurrence |

not supported by data. There were total 5 corneal surgeons who operated and had the experience of performing DALK in noninfectious cases.

The second complication observed in this series was DMD. This higher risk of DMD could be attributed to intraoperative microperforation while dissecting stroma in an inflamed eye. Further, despite successful descemetopexy, clear graft could be achieved in only two cases. The higher risk of graft failure despite successful reattachment suggests poor endothelial functions in these patients with advanced keratitis. Probably, the extension of inflammation into deep stroma results in adverse impact on endothelial function, which gets further compromised with DMD and the additional procedure of DMD. The occurrence of recurrence of infection or intraocular spread was not seen and also was not different in eyes with or without DMD in either of the group.

Like any other large graft, the procedure of DALK for advanced keratitis results in higher risk of epithelial complications that manifested in this series in the form of filamentary keratopathy and persistent epithelial defect. Illingworth and Cook in 1998 proposed partial limbal stem cell dysfunction in patients of AK secondary to persistent limbitis. Therefore, one must adopt additional measures to promote and protect epithelial healing.

Once cornea recovers from initial surgery, the long-term outcomes are as good as for those performed for small infiltrate. One patient in this series developed allograft rejection, which was related to discontinuation of corticosteroids by the patient. Even for this case, clear graft was obtained on reinstitution of corticosteroid therapy. Overall, in our series 4 out of 23 cases developed stromal rejection on long-term follow-up but all of them recovered completely with the increased dose of steroids. The incidence of allograft rejection in the series was similar to the incidence[23,24] reported for noninfectious indications, i.e., keratoconus and stromal dystrophy.

## Conclusion

The experience with this case series clearly suggests that the DALK procedure performed for advanced keratitis cases (>8 mm infiltrate size) is associated with higher risk of complications. Most of the complications are directly or indirectly related to technical challenges associated with the procedure; therefore, a physician more familiar with full-thickness keratoplasty might find it appropriate to perform penetrating keratoplasty for ADK cases. Therefore, a comparison of DALK and penetrating keratoplasty for such cases will be valuable in providing evidence-based decision on procedure of choice for such cases.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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