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RESEARCH ARTICLE Indian Journal of Animal Research

Application and Evaluation of Full-thickness Keratoplasty for the Management of Irreparable Corneal Affections in Dogs

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10.18805/IJAR.B-5474

ABSTRACT

Background: Corneal transplant or full-thickness keratoplasty (FTKP) is considered as last line of treatment for severely damaged or opaque corneas where other treatment protocol could not be effective. There is lack of previous literature based on Indian context for corneal transplant in dogs hence present study was planned, cases of irreparable corneas were selected, operated and technique along with outcomes was evaluated.

Methods: Total six clinical cases (n=6) of irreparable corneal diseases in different breeds of dogs were selected after complete necessary preoperative examinations including ophthalmological tests and ocular ultrasound. Healthy corneas were harvested along with sclera rim from dogs euthanized for untreatable conditions like trauma and systemic diseases. After collection, corneal tissue was stored in an air-tight container within 'MK-medium.All the cases were operated successfully under general anaesthesia. Both fresh and frozen grafts were utilized for cornea transplant.

Result: The mean value of donor graft size was 9.58±0.49 mm whereas recipient's lesions size was 6.87±1.22 mm. The mean value of operative time was recorded as 73.33±14.25 minutes. Sutures used to affix donor graft with recipient's bed ranged from 6 to 12 in numbers. After cornea transplant, only 2-eyes remained visual (due to surrounding transparent window) with 100% graft rejection in this study. Efficacy of this technique was determined by dividing the number of cases with visual outcomes to the total number of the eyes operated. Severe graft reaction, graft rejection, graft opacity, graft melanosis and development of post-surgical cataract were reported as postoperative complications.

Key words: Dog, General Anaesthesia, Irreparable corneal diseases, Penetrating keratoplasty.

INTRODUCTION

Infected corneas of dogs can be treated medically or surgically depending on severity of corneal diseases (Gelatt and Samuelson, 1982). Usually better response is achieved through medical management at early stage with confirmatory diagnosis of corneal diseases but delayed or severely traumatised irreparable corneas demands surgical interventions to achieve visual or tectonic eyes (Hollingsworth, 2003). Moreover unsuccessful surgical management in corneal diseases can leads to panophthamitis followed by eye-globe enucleation (Maggs, 2018). There are many surgical techniques like pedicle grafting from bulbar conjunctiva, keratotomy, superficial keratectomy, use of cryogens and keratoplasties were described in previous literatures (Hakanson, 1987; Azoulay, 2014; Dorbandt *et al.,* 2015; Lacerda *et al.,* 2016; Rajasekaran, 2021; Qureshi *et al.,* 2022) for verity of surgical affections of canine corneas with different success rates. For irreparable corneal affections, cornea transplant can be a good option and even after graft rejection, this procedure can save reaming surrounding cornea transparent and thereby eye remains visual (Hansen and Guandalini, 1999). However in diseases where complete cornea gets affected then tectonic outcomes are usually expected and many pet-owners prefers it over eye enucleation. The Term "Keratoplasty" consists surgical procedure which involved replacement of diseases corneal tissue with healthy cornea (Qureshi, 2020). Results of full-thickness keratoplasty (or penetrating keratoplaty) in animals were well documented in earlier studies (McEntyre, 1968; Mueller and Formston, 1969; Coster, 1981; Brooks *et al.,* 2008; Lacerda*et al.,* 2016) but promising results are still lacking. Corneal tissue after transplant shows comparatively less immune reaction and hence possesses higher success rates over other organ transplant in human ophthalmology (Coster, 1981; Volker Diebe*et al.,* 1982; Wilson, 1990; Boisjoly *et al.,* 1993; Panda *et al.,* 2007). Therefore six clinical cases of dog affected with irreparable unilateral corneal affections such as bullous keratopathy, corneal sequestrum, full thickness

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How to cite this article: Sharma, R.K., Mahajan, S.K., Devi, N.U., and Mohindroo, J. (2025). Application and Evaluation of Full-thickness Keratoplasty for The Management of Irreparable Corneal Affections in Dogs. Indian Journal of Animal Research. 1-8. doi: 10.18805/IJAR.B-5474.

Submitted: 14-08-2024 **Accepted:** 27-03-2025 **Online:** 19-06-2025

corneal defect and corneal edema were selected, operated and evaluated for penetrating keratoplasty.

MATERIALS AND METHODS

Total 6-clinical cases {bullous keratopathy (n=1), chemical burn (n=1), corneal sequestrum (n=1), full-thickness corneal defect (n=1) and in corneal oedema (n=2)} affected with irreparable severely damaged corneas were presented to Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana. Detailed preoperativecase presentation findings are included in Table 1. All these cases were selected for full-thickness keratoplasty after comprehensive blood work and detailed ocular examinations. Fresh and frozen corneas were utilized for transplant in accordance to availability at the time of case presentation. Donor corneas along with 1-2 cm sclera rim were harvested from dogs died or euthanized due to systemic/traumatic diseases other than eye diseases. Immediate after harvesting, donor corneal tissue was placed in a commercially available ophthalmic antibiotic solution ("McCarey-Kaufmanmedium"[1]) and stored. For utilization of tissue as fresh graft (collected 48-hours prior to surgery), corneas were preserved refrigerated at 4°C. While for utilization of frozen grafts, the containers were frozen at -22°C and used as per need within 1 month of period. All the selected dogs were prescribed and advised to administer topical medicines to prevent panophthalmitis. Systemic antibiotic was also administered for 3-days prior to surgery. Eye medications which included topical lacrimomimetic (0.5%) carboxy methyl cellulose sodium[2], topical antibiotic eye drops (0.3%) gatifloxacin[3], topical immunosuppressant (0.1%) cyclosporine[4] from the day of reporting till the scheduled day of surgery. Where necessary, systemic antibiotics, non-steroidal anti-inflammatory medications and other supplements were given based on the findings of the pre-operative evaluation.To ensure ocular asepsis on the day of surgery, gatifloxacin eye drops were applied to the affected ocular surface at intervals of 15 to 20 minutes, beginning one hour before surgery and continuing until the procedure started. General anaesthesia was used for surgical interventions in present study. A combination of Butorphanol[5] @ 0.2 mg/kg body weight, Atropine[6] @ 0.02-0.04 mg/kg body weight and Acepromazine maleate[7] @ 0.02-0.05 mg/kg body weight through intramuscular route were used in all dogs for premedication. For induction Propofol®[8] @ 4 mg/kg body weight was administered slowly by intravenous route. For Maintenance Isoflurane[9] kept at 1-2% with 100% oxygen was utilized. The patient's head was elevated and positioned to the surgeon's preference by placing padding material beneath the patient's head. Hair was clipped (not shaved) from the surrounding area (periorbital area and eyelids) and a 5% povidone iodine solution was applied. Before the procedure started, the dorsal and ventral conjunctival fornixes were also flushed with povidone iodine solution (1:50 ratio). To cover the eye during surgery, sterile surgical drapes or commercially available eye drapes with an operating window in the middle were used. The eyelids were held in place with a Lieberman speculum and the surgeon operated while seated in front of the patient. Both fresh (n=3) and frozen (n=3) homologous corneas collected from donor dogs were applied to replace the recipient corneas affected with irreparable corneal affections (opaque/weakened/infected) that do not permit vision. All efforts were made specially to eliminate ocular bacterial infections, if any. For surgery eyes were prepared similarly mentioned in earlier techniques of present study. Two different sized sterilized corneal trephines were used for trephining of donor and receipt corneas, respectively. The corneal lesions to be removed were positioned centrally within the minimum sized corneal trephine. In recipient eye, holding the corneal trephine perpendicular to the corneal lesions, it was twisted both clockwise and counter-clock wise until approximately 80% of the corneal stroma was incised. A stab was made using 2.8 mm keratotome in circumscribed incision made through corneal trephine and ultimately it allowed entry into the anterior chamber. Diseased cornea was removed with the help of keratoplasty scissors. Anterior chamber was collapsed immediate after incisions where larger corneal defects were presented and thereby larger recipient's corneal tissue were removed. Donor cornea was first placed over Teflon block with endothelial side up (identified through attached scleral-rim) and donor corneal button was harvested with 0.5 mm larger sized corneal trephines. Corneal button was grabbed carefully (to prevent endothelial damage) using Lim's forceps and placed over the recipient's bed where diseased corneal was already trephined. Patson spatula (double ended) was used to secure the corneal button in position on recipient's bed during application of cardinal sutures. The corneal graft was sutured in each quadrant with four 8-0 simple interrupted Vicryl sutures. Suture needle was carefully penetrated upto 2/3rd of stroma in both donor and recipient's corneal tissue thereby

[1] MK Medium, Eye bank, Hyderabad

[2]Refresh Tears®, Piramal Enterprises Ltd, Madhya Pradesh, India

[3]Gatiquin, Aditi Pharmaceuticals Pvt. Ltd, Solapur, India

[4]Ophthocare, Corise Healthcare Pvt. Ltd, Maharashtra, India

[5]Butodol®, Neon Laboratories Ltd, Mumbai, India

[6]Tropine*, Neon Laboratories Ltd, Mumbai, India

[7]Ilium-Acepril-10®, Troy Laboratories Pvt. Ltd, Australia

[8]Neorof®, Neon Laboratories Ltd, Mumbai, India

[9]Forane®, Aesica Queenborrough Ltd, UK

penetration of endothelium was prevented. Once the donor button was stabilized with cardinal sutures, additional sutures were placed in simple interrupted manner. Partial temporary tarsorraphy was done and removed after 2-weeks.All animals were administered with systemic antibiotics (Inj. Cefotaxime @ 25 mg/kg body weight, b.i.d.for 5 days) and NSAIDs (Inj, Meloxicam @ 0.2 mg/kg body weight, o.d. for 3 days) intramuscularly to prevent further infections and reduce post-operative inflammation.Topical lacrimomimetic eye drops (Carboxy methyl cellulose sodium) was instilled 4-times a day for 1 month post-operatively. Topical antibiotic eye drops (Gatifloxacin) was instilled on every 2 hour basis during the 1st post-operative week which was tapered to every 4 hour basis, 8 hour basis and 12 hour basis in the successive post-operative weeks on the basis of fluorescein dye test (FDT). Topical cycloplegic eye drops (1%Tropicamide) instillation was started for 1st-week after surgery (one-drop in a day) to relieve uveal-spasm. Topical steroid independent of FDT were started from 2nd week of surgery till recovery (to stop initiation of graft reaction) and topicalimmunosuppressant drug (0.1% Cyclosporine, 2-drops t.i.d.) was started from the day of surgery till eye become comfortable. Owners were advised to place dogs in dark room forfirst month post-operatively.Corneal clarity was graded from 0 (crystal clear), 1 (clear), 2 (hazy), 3 (very hazy) and 4 (opaque) whereas corneal pigmentation score was calculated according to Charbiwala (2019). This extent of corneal pigmentation was assessedusing particular grading system in accordance to Charbiwala, (2019). Total area of deposition of brown to blackish pigment either melanin or interms of melanocytes over the ocular surface (mainly cornea) was referred as degree/extent of corneal pigmentation. In all the casesof ocular surface pigmentation, corneo-scleral limbus was used as periphery (rim) andthen ocular surface within circle was divided into total 12 sectors'. It is the graded as ½ (Single line of pigment in a sector), 1 (Single sector affected with pigmentation), 1+1 (Pigment extending upto the resting pupil edge), 2+1 (Visual axis is affected {pigment extending beyond the resting pupil edge}) and 1 point perclock hour (Pigment extending just beyond the limbus).

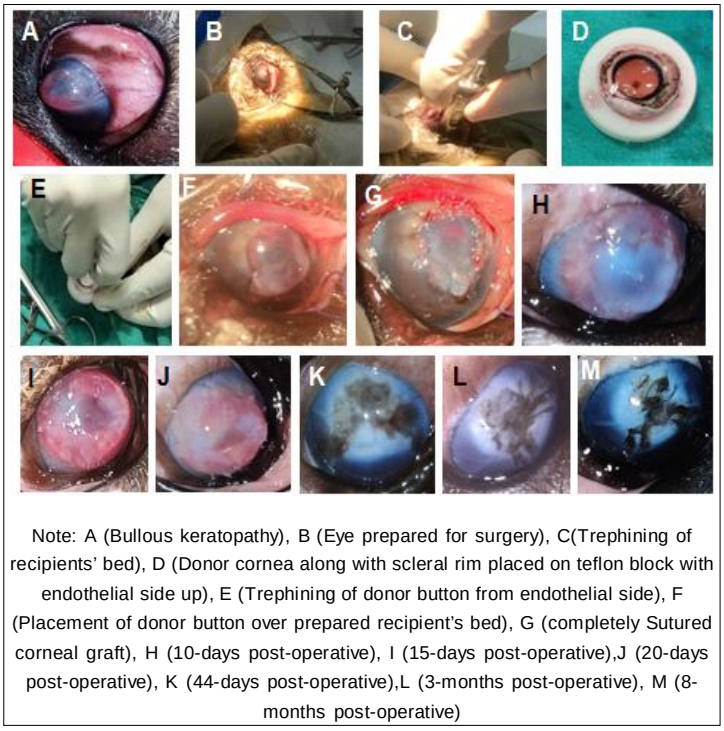
RESULTS AND DISCUSSION

Indications of corneal transplant for irreparable corneal affections in small animals were already mentioned in earlier literature (Townsend *et al.,* 2008; Gelatt *et al.,* 2013; Maggs, 2018). In present study, fresh (n=3, C1 to C3) and frozen grafts (n=3, C4 to C6) were applied in equal distribution and independent of type of corneal affection. Size of affected cornea was also calculated in four-cases (mean was 6.87±1.22 mm, median was 6.2 mm, ranges from 4.9 to 10.2 mm) whereas in two cases (C2 and C4) it was not possible because of complete cornea involvement. The mean surgical time was recorded as 73.33±14.25 minutes. All the grafts were stabilised with the help of absorbable sutures (size- 8-0, polyglactin 910). In research of Lacerda *et al.* (2016) on penetrating keratoplasty, similar to present study, polyglactin 910 (9-0 in their study, but 8-0

Table 1: Detailed preoperative/case presentation findings.

Case no.	Corneal affection	Pattern of blood vessels	Nature of corneal oedema around lesion		Corneal clarity score and menace reflex	Fluorescien dye test	Shirmer tear test (mm/min)	Time duration between day of case presentation to day of surgery
C1	Bullous keratopathy	Tree	Moderate	3	Present	(-)	27	9-days
C2	Chemical corneal burn	Hedge	Severe	4	Absent	(+)	28	24-days
C3	Corneal sequestrum	Not observed	Mild	3	Absent	(-)	13	2-days
C4	Corneal oedema	Tree	Severe	4	Absent	(-)	24	3-days
C5	Corneal oedema	Tree	Severe	4	Absent	(-)	16	1-day
C6	Perforated cornea	Mixed (Hedge + Tree)	Moderate	4	Absent	(-)	Not done	1-day

in present study) absorbable suture material was applied to stabilize the graft but they followed cardinal suture pattern (12-6-9-3 O'clock positions). Townsend *et al.*(2008) transplanted cornea for treatment of feline sequestrum and used non-absorbable polyamide (9-0) sutures. McEntyre (1968) applied 7-0 silk sutures in canine cornea transplant. They placed simple interrupted pattern in few millimetre gap (1-2 mm). In present study, the mean value of number of sutures used during FTKP to affix the donor cornea with recipients' bed was recorded as 8.83±1.01, with median of 9 ranges from 6 to 12 (in numbers). In a study (McEntyre, 1968) on experimental keratoplasty on dogs, range of total number of interrupted sutures was 8 to 16 along with continuous suture application. In his study, wound leakage and out-riding of donor edges were reported even after use of total 16 simple interrupted sutures. However such intra-operative complications might remained undiagnosed in present study hence not documented. Mueller and Formston (1969) explained that a more satisfactory method of preparing donor full-thickness graft was to trephine the transplant tissue from endothelial side of donor corneal button (corneal trephine should be firmly placed over the



Note: A (Bullous keratopathy), B (Eye prepared for surgery), C(Trephining of recipients' bed), D (Donor cornea along with scleral rim placed on teflon block with endothelial side up), E (Trephining of donor button from endothelial side), F (Placement of donor button over prepared recipient's bed), G (completely Sutured corneal graft), H (10-days post-operative), I (15-days post-operative),J (20-days post-operative), K (44-days post-operative),L (3-months post-operative), M (8-months post-operative)

Fig 1: Surgical procedure and long term follow-up of penetrating keratoplasty in a dog affected with Bullous keratopathy.

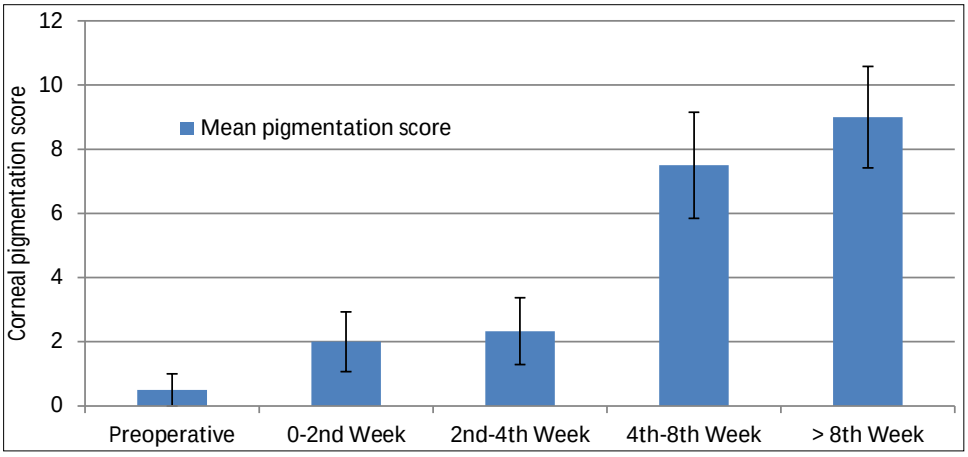


Fig 2: Mean ± S.E. of corneal pigmentation at different time intervals.

Table 2: Follow-up and postoperative outcomes.

Case no.	Corneal clarity score, Menace reflex, corneal pigment score (postop.)			Graft status and Postoperative complications				
				at 7th -day	15-days	1-months	2-months	>2 months
C1	3	0	11	Graft reaction started and covered the whole periphery of graft, both graft and surrounding cornea oedematous moderately (Menace-ve)	Blood vessels deeply invaded both donor and recipients' corneal tissue, severe graft reaction, severe corneal oedema (menace-ve), blepharospasm+photophobia	Graft reaction still persisted and cornea was severely oedematous (menace -ve),application, blepharospasm + photophobia + epiphora	Graft reaction reduced completely after aggressive topical corticosteroid graft melanosis started around periphery of completely opaque grafts (menace+ve)	At 9th months of follow-up cataract was diagnosed in USG but sluggish menace still observed, graft failure, completely opaque graft
C2	4	A	5	Severe graft reaction is observed at dorsal periphery of transplanted looks moderately oedematous while slightly transparent ventral portion of donor corneal tissue was noticed	Graft reaction covered complete transplanted cornea, epiphora, donor tissue, rest of cornea (menace-ve)	Graft became almost opaque, graft rejected, no menace photophobia, blepharospasm	Completely rejection of transplanted cornea, vitreous changes in USG-examination, melanosis observed at ventro-nasal portion of ocular surface	Conjuctivilization of complete trans planted cornea, no menace, owner advised encucleation but denied due to sentimental issues
C3	2	D	D	Graft reaction started at periphery, moderated corneal oedema surrounding transplanted graft, remaining healthy corneal window looked transparent (Menace +ve)	Severe graft reaction, whole graft was invaded by blood vessels, graft become reddish and opaque, corneal oedema still persisted (menace +ve)	Complete rejection of transplanted graft, anterior synechia observed clearly and iris adhered with opaque graft but for rest of iridial tissue PLR was positive, graft melanosis started from dorso-nasal region (Menace+ve)	Dead	Dead
C4	4	A	A	Oedematous cornea + epiphora+ Menace (-ve)	Graft reaction started covering periphery of transplanted tissue (menace -ve), severe oedematous cornea	FDT (+ve) and ulcer seen over the transplanted cornea, moderate cornea still persisted (menace -ve)	Dead	Dead

Table 2: Continue...

Table 2: Continue...

C5	4	A	12	Severe graft reaction, corneal oedema, cornea transplanted graft, bulged and protruded outside (menace-ve)	Graft reaction covered whole attached graft, photophobia, blepharospasms (menace-ve)	Corneal melanosis started from ventro-nasal region, corneal opacity, menace (-ve)	Severe corneal melanosis, completely opaque graft, graft rejected (menace-ve)	Completely opaque severe corneal melanosis, dog became blind
C6	3	0	8	Graft reaction seen moderately, surronding corneal tissue covered with tree-pattern of blood vessels, menace (-ve)	Severe graft reaction, graft became completely reddish, graft rejected, belpharospasm, photophobia (menace was sluggish)	Complete graft rejection, surrounding cornea became transparent with use of aggressive topical steroid theraphy (Menace was sluggish and turned +ve)	Owner was not reported for follow-up but contacted telephonically and noticed that menace turned positive due to surrounding healthy transparent cornea with opaque graft	Follow-up not available

Sluggish (0), Present (P), Absent (A), Dead (D).

endothelium and then disk is punched out) which was similarly followed in present study. Procedure of cornea collection was also similarly followed in accordance to their study and they described that cornea with a rim of sclera should be dissected from intact globe; the ciliary body adherent to the scleral rim should be removed together with iris and lens. Many hurdles and complications like intra-operative iatrogenic damage to lens and vitreous (C5), faulty entry into anterior chamber (C1, C2 and C5), intra-operative iris protrusion (C5), difficulty to identify the exact epithelial and endothelial surface of donor corneal button after harvesting and thus the button was not utilized ahead (C4), oedematous/ swellon donor corneal button (C4 to C6) and difficulty in placement of suture knot due to oedematous margins of recipients' cornea and donor corneal button (C4 to C6) were recorded as Intra-operative complications. All these complications were similarly reported in research of Qureshi (2020) on canine keratoplasties. The mean value of donor graft size (in diameter) utilized in FTKP was recorded as 9.58 ± 0.49 mm. Same value is recorded as size of trephines for donor button retrieval. The mean value of recipient's bed size was 9.08 ± 0.49 mm. In research of Lacerda *et al.* (2016), graft size ranged from 3 to 15 mm (median 7.5 mm). Donor graft must be larger than the recipients' bed which ranged from 0.1 mm to 0.5 mm in previous literature (McEntyre, 1968; Mueller and Formston, 1969; Gelatt *et al.,* 2013; Lacerda *et al.,* 2016; Qureshi 2020) because graft-shrinkage during post-operative period. McEntyre (1968) utilized 7.0 mm corneal trephine for a depth of 0.4 mm for incision in recipient's cornea. He mentioned that to minimize damage and save maximum of endothelial cells, recipient's button was excised with a bevelled incision.

Previous researchers (McEntyre, 1968; Mueller and Formston, 1969; Coster, 1981; Gelatt *et al.,* 2013; Lacerda *et al.,* 2016) stated that irreversible oedema, graft reaction (invasion of blood vessels towards donor tissue) and graft-opacity/fibrosis were considered as gross clinical signs of graft rejections after FTKP. The main reason behind all these clinical signs was considered as endothelial destruction which is usually not easy to identify. Similar clinical signs were also reported in present study in post-operative periods. Association of graft failure with numbers of invaded vessels in postoperative period in per cornea quadrant (away from limbus at least 2mm) was considered as risk factors of graft rejection in earlier studies ((McEntyre 1968). Similarly in present study grafts were invaded by vessels within a week of surgery.

Total 4-dogs of present study were survived whereas 2-dogs died in later period due to other systemic causes. The mean time of disappearance of corneal sutures or mean value of time taken to dissolve absorbable sutures was recorded as 37.6±3.26 days. The surgical Procedure and long term follow-up of penetrating keratoplasty in dog affected with Bullous keratopathy (C1) is shown in Fig 1.

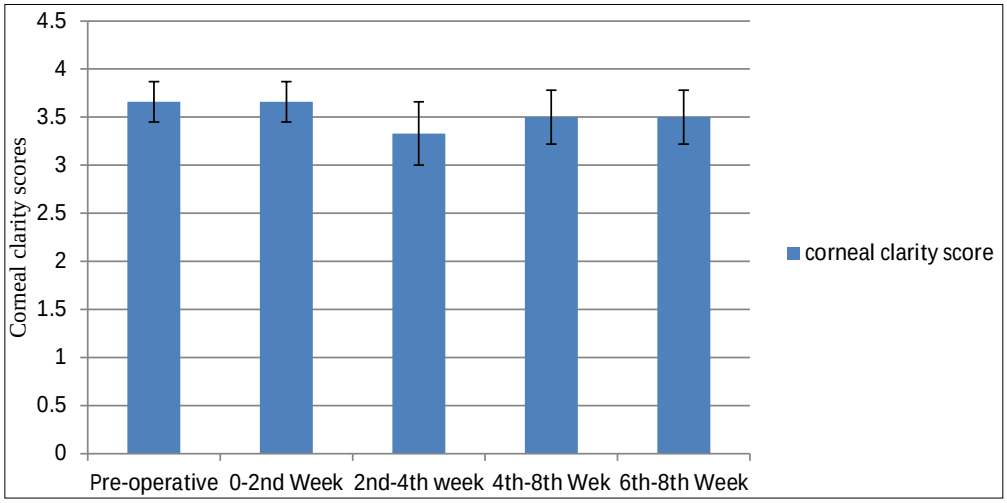


Fig 3: Mean ±S.E. of corneal clarity score at different interval.

Postoperatively, overall corneal clarity score 2, 3 and 4 was reported in total 1-case (C3; upto 1-month, afterwards dog died), 2-cases (C1 and C6) and 3-cases (C2, C4 and C6) respectively (Fig 3). Up to 1-months total 3-cases (C1, C2 and C6) showed positive menace reflex. 2-cases died and excluded from follow-up statistics (Table 2). In total 2-cases (C1 and C6) were reported with sluggish menace reflexes. Therefore, postoperatively visual outcome was 50% (2/4) and tectonic outcome was 100% (4/4) in present study. Post-operatively, mean value of corneal pigmentation score (for 4-cases, C1, C2, C5 and C6) was 9.00±1.58 (Fig 2). Pre-operatively visual outcome was recorded as 33.33% (2/6) which was remained same in post-operative period up to 6-months of follow-up. Moreover topical steroid was advised for long term application to reduce the scar/opacity but prolonged outcome was not available.There was only one report (Lacerda *et al.,* 2016) in which rate of graft-failure/ rejection has been mentioned as 56% which was almost similar to medical practice (Wilson and Kaufman, 1990; Panda *et al.,* 2007) but many reports of medical science possess comparatively less rejection rates than veterinary practice (McEntyre, 1968; Mueller and Formston, 1969; Gelatt *et al.,* 2013; Lacerda *et al.,* 2016; Qureshi, 2020).

CONCLUSION

Good tectonic outcomes were achieved with use of full-thickness keratoplasty to manage irreparable corneas in dogs. Out of 6-eyes, 2-eyes at last stages of diseases were saved and existing visual capacity was preserved. Severe graft reaction, corneal opacity, graft-melanosis and graft rejections were noticed and recorded as complications of this study.

ACKNOWLEDGEMENT

The financial support was provided by ICAR under DIMSCA-project and GADVASU, Ludhiana for completion of present research.

Conflict of interest

No conflict of interest.

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