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Management of deep corneal ulcer in a dog using autologous platelet rich fibrin (PRF) membrane#

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Abstract

A six-year-old male spitz dog was presented with deep corneal ulcer in left eye and cataractous changes in right eye. Menace response, cotton ball test and pupillary light reflex were found negative for both eyes. Fluorescein dye test was positive for left eye and no improvement was noticed on medical management. Hence, surgical intervention with platelet rich fibrin (PRF) membrane grafting was resorted to for the protection of the ulcer bed. Autologous platelet rich fibrin membrane was grafted over the corneal ulcer bed under general anaesthesia, followed by third eyelid membranoplasty. Topical antibiotic and anti-inflammatory drops were advised for five days post-operatively and the dog had an uneventful recovery.

Keywords: *Autologous graft, corneal ulcer, platelet-rich fibrin membrane*

Corneal ulcers are the most frequent ocular emergency in dogs (Antonia *et al*., 2014). An ulcer involves the loss of corneal stroma besides the loss of epithelium (Jose *et al*., 2013). The majority of these lesions were brought about by trauma, including foreign bodies, inadequate tear film, dysfunctional eyelids, aberrant eyelashes, bacterial as well as viral infections. Exposure keratopathy and xerophthalmia can lead to corneal lesions in dogs having buphthalmic eyes (Venugopal, 2013; Syam *et al*., 2011).

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Analgesics, anti-inflammatory medications and antibiotic therapy are used in the conventional method of treating corneal ulcers. To hasten the healing of both soft and hard tissues, platelet-rich fibrin membrane (PRFM) can be employed as a scaffold for corneal ulcers. It comprises of autologous platelets and leucocytes in a complex fibrin matrix. Being a biomaterial for interposition and healing, it is found to hasten mucosal healing and wound closure because it releases fibrin and growth factors (Choukroun *et al*., 2006). There is dearth of literature in medical and veterinary ophthalmology about the use of regenerative therapy using PRFM for corneal ulcer healing and hence this case is placed on record.

A six year old male spitz dog weighing 10 kg was presented to University Veterinary Hospital, Kokkalai with the history of cloudiness of the left eye (OS - Oculus Sinister) since six days (Fig. 7) and cataract noticed in the right eye (OD – Oculus Dexter). The animal was presented with high stepping gait suggestive of visual deficit. The dog had normal feeding habits and on ophthalmic examination, cloudiness of the left cornea was noticed with oedema and pannus formation.

The patient underwent a thorough ophthalmic examination, which included tests like the Schirmer tear test (OD: 16mm/min, OS: 18mm/min), fundus examination, menace response test (OU) and pupillary light reflex test (OU) were negative. A positive fluorescein dye test in the left eye confirmed the presence of corneal ulcer. A complete blood count (CBC), peripheral blood smear and buffy coat smear examination were performed and found to be normal. The affected eye was thoroughly cleansed and debris and necrotic material were flushed out of corneal ulcer with normal saline. It was advised to apply D-panthenol eye gel twice daily and to instill bromfenac eye drops two drops twice daily for seven days, along with tear substitute eye drops containing carboxy methylcellulose (0.5%) two drops four times a day for the same period of time. Since the animal did not show any satisfactory clinical improvement and the lesion became a deep corneal ulcer, surgical correction making use of regenerative therapy using PRFM was resorted to.

Prior to surgery, 10mL of fresh blood was drawn from the saphenous vein into two 10mL centrifugation tubes without the addition of anticoagulants. The tubes were immediately centrifuged at 542.4 G (3200 rpm for 10min). Following centrifugation, red blood cells accumulated at the bottom of the tube, platelet-rich fibrin gel at the middle and platelet-poor plasma (PPP) was accumulated at the top of the tube. Using a pair of tongs, the fibrin gel in the middle of the sample was gently removed from the tube and the platelet rich fibrin membrane (Fig. 2) was prepared by pressing the fibrin gel (Fig. 1).

The surgical procedure was performed under general anaesthesia. Preanaesthetic medication was given using, inj. atropine sulphate at the dose rate of 0.045 mg/kg body weight (B.W) intramuscularly, inj. tramadol hydrochloride at the dose rate of 2.0 mg/kg B.W. intramuscularly and inj. xylazine hydrochloride at the dose rate of 1.0 mg/kg B.W., administered intramuscularly. General anaesthesia was induced with inj. ketamine hydrochloride at the dose rate of 2.5 mg/kg B.W. intravenously and inj. midazolam at the dose rate of 0.05 mg/kg B.W. administered intravenously. Anaesthesia was maintained using isoflurane at 2% in 100% oxygen, following endotracheal intubation. The affected eye was rinsed with normal saline solution, followed by 1 % povidone iodine collyrium. Ulcer bed was debrided with a sterile cotton swab (Fig.3) and PRFM was placed to cover whole cornea. It was sutured to bulbar conjunctiva using 5-0 polyglactin 910 suture material with simple interrupted sutures (Fig.4). Third eyelid membranoplasty was done using polyglactin 910 size 2-0 in horizontal mattress suture pattern (Fig.5). The third eyelid membranoplasty sutures were removed on the seventh post operative day.

Seven days after surgery, entire area of the ulcer was covered by epithelialisation, granulation tissue and corneal neovascularisation. Fluorescein dye test was found negative one week after the treatment. There was no sign of secondary infection or inflammation. In the region of the corneal ulcer, the PRF membrane triggered the formation of granulation tissue and following topical application of anti-inflammatory and antibiotic

Fig. 1. Separation of PRF

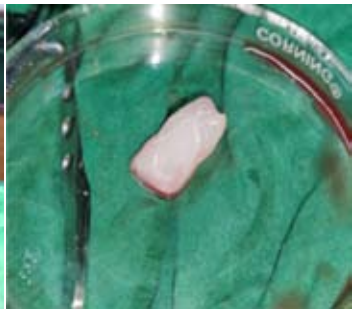

Fig. 2. PRF clot

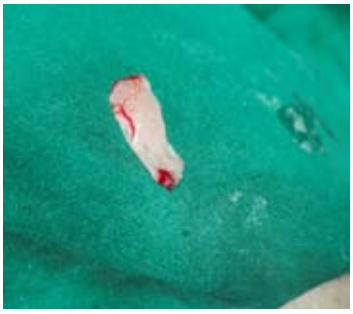

Fig. 3. PRFmembrane

Fig. 4

Fig. 5

Fig. 6

Surgical procedure: **Fig. 4**. Debridement of ulcer edges. **Fig. 5**. PRF membrane completely covering the cornea and suturing PRF membrane over conjunctiva. **Fig. 6**. Third eyelid membranoplasty

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Deep corneal ulcer treated with PRF membrane: **Fig. 7.** Day 0 **Fig. 8.** Day 7 **Fig. 9.** Day 14 **Fig. 10.** Day 21 **Fig. 11.** Day 28

eye drops, the granulation tissue completely disappeared over a period of three weeks. At the end of four weeks, it was evident that the corneal neovascularisation had regressed by 80 per cent with the anterior chamber of the eye distinct under cornea.

Deep corneal ulcers are characterised by stromal collagen loss. In order to control the inflammation and infection to promote stromal repair and to restore cosmetic appearance and function, various medical and surgical treatment protocols were tried. Several techniques have been adopted in small animal practice for the treatment of corneal ulcers, including biomaterial grafts (Jose *et al*., 2013). In the current case, autologous PRF membrane graft was used in the surgical treatment of corneal ulcers, yielding extremely good anatomical and visual outcome.

The PRF membrane can be implanted as a simple surgical intervention because it is a quick, affordable and repeatable approach. This procedure involved the use of a second generation of the autologous platelet concentrate as designed by Choukron *et al*. (2006) for uses in jaw-facial surgery. The therapeutic impact was produced by thrombocytes, leukocyte cytokines, stem cells and a number of growth factors found in the fibrin matrix, including platelet-derived growth factor, cytokines, vascular endothelial growth factor, transforming growth factor and thrombospondin-1 (Kang *et al*., 2011). In corneal ulcer treatments, nictitating membrane was placed temporarily over the graft materials to mechanically shield the surgical site from eyelid movements and to keep the membrane from drying out and to help it stick to the surrounding tissue. In the present case, it was found that the nictitans membrane application produced positive outcomes, safeguarding the membrane and stopped it from drying out. According to Anoop *et al*. (2015), following corneal stromal wound healing, there was transformation of keratocytes to fibroblast. Collagen, which was laid in a disorganised manner, resulted in scar formation. In the present case, epithelialisation was achieved in a period of 10 days. The transplant totally disintegrated and integrated with the surrounding corneal tissue after seven days (Demir *et al*., 2022). Granulation tissue present at the corneal ulcer bed and border region started melting gradually after two weeks and the cornea became translucent, with resolution of oedema and pannus. Healing of the cornea and restoration of vision following treatment confirmed the effectiveness of autologous PRF membrane grafts in the management of deep corneal ulcers (Can *et al*., 2016).

Summary

Based on the case study presented here, it was inferred that autologous PRF membrane-based regeneration therapy for corneal ulcers is a successful technique for the management of deep stromal corneal ulcers. The graft offered satisfactory ocular support as well as positive functional and aesthetic outcomes. Platelet Rich Fibrin (PRF) membrane is a good technique to promote wound healing for the management of corneal ulcer in dogs.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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