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Short Communication

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.4.831-834

Canine autologous platelet rich fibrin membrane transplantation for corneal reconstruction after the excision of dermoid cyst in two pups

Laiju M. Philip[1*], B.M. Nijin Jos[1], Ajin S. Kumar[1], Alan V. Stephen[1], Reji Varghese[1], K.D. John Martin[1] and Syam K. Venugopal[1]

[1]Dept. of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala- 680 651, Kerala Veterinary and Animal Sciences University Kerala, India

Citation: Philip, L.M*, Nijin Jos, B.M., Kumar, A.S., Stephen, A.V., Varghese, R., John Martin, K.D. and Venugopal, S.K. 2024. Canine autologous platelet rich fibrin membrane transplantation for corneal reconstruction after the excision of dermoid cyst in two pups. *J. Vet. Anim. Sci.* **55** (4):831-834

Received: 05.05.2024 Accepted: 22.07.2024 Published: 31.12.2024

Abstract

Two Rottweiler puppies were presented to the University Veterinary Hospital, Kokkalai, KVASU, with the history of epiphora from the right eye for a period of three weeks. On ophthalmic examination, a dermoid cyst could be observed at the lateral border of the right cornea in both the dogs. Dermoid cysts were excised under general anaesthesia by superficial keratectomy followed by conjunctivectomy. Autologous platelet rich fibrin membrane (PRFM) was transplanted over the wound bed by suturing onto the bulbar conjunctiva followed by third eyelid membranoplasty. After the transplant, the corneal epithelisation process took place over two weeks. The cornea returned to normal transparency after five weeks. At eight weeks following the transplant, the dog had little neovascularisation and slight corneal scarring. In conclusion, canine autologous PRFM transplantation can aid corneal healing following the removal of dermoid cysts in dogs.

Keywords: *Autologous platelet rich fibrin membrane, corneal reconstruction, dermoid cyst*

Dermoid is a choriostoma, which is histologically normal tissue that forms at an unnatural place during embryonic development (Syam *et al*., 2001; Balland *et. al*., 2015). The clinical signs of ocular dermoid include epiphora, corneal pigmentation, corneal ulceration, blepharospasm and keratitis (Martin, 2005; Lee *et. al*., 2005). According to Martin (2005), breeds including the Dachshund, Basset hound, Dalmatian, Doberman pinscher, German Shepherd dog, Golden Retriever *etc*. had a hereditary predisposition for ocular dermoid. Erdikmen *et al*. (2013) reported that surgical excision was a successful treatment option. Depending on the size and depth of the defect following excision of dermoid, corneal tissue adhesives, limbal stem cell transplants, third eyelid and conjunctival flaps, amniotic membrane application, therapeutic bandage contact lenses and autologous sliding lamellar keratoplasty are preferred in surgical treatment (Kaya and Pekel, 2015). The ability of biomaterials to produce faster healing was examined and tested and platelet derived concentrates have remained distinct among these. Platelet rich fibrin membrane (PRFM), a second-generation platelet concentrate was found to be appropriate for the reconstruction and healing of the corneal surface owing to its properties of mechanical and chemotactic support (Choukroun *et al*., 2006; Dohan *et. al*., 2006; Oncu and Alaaddinoglu, 2015).

**Corresponding author: laiju@kvasu.ac.in, Ph. 9447996512*

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The current cases were undertaken to assess the healing of the cornea after surgical excision of dermoid in two dogs followed by PRFM grafting since its effect on corneal wound healing has not yet been established.

Two female Rottweiler puppies, aged six and seven months respectively, were presented to University Veterinary Hospital, Kokkalai, Thrissur, Kerala with the history of discharge from both eyes along with presence of hair in the left eye. Ophthalmic examination of the puppies revealed slightly thick and elevated tissue at the temporal limbus and bulbar conjunctiva with hairs arising from it with chronic ocular discharge (Fig. 1, 2). Hyperaemia of conjunctiva was observed because of continuous irritation of conjunctiva with the tuft of hairs and the condition was diagnosed as unilateral dermoid cyst extending from the sclera to the cornea across the limbus.

Fig. 1. Dermoid on left cornea in Dog 1

Fig. 2. Dermoid in left eye in Dog 2

A complete blood count (CBC), peripheral blood smear and buffy coat smear examination were performed. Animals were administered with topical antibiotic and anti-inflammatory drops for seven days and surgical correction was resorted to.

Canine autologous PRFM preparation and preservation were different from those previously described for human PRFM application in which the whole blood was centrifuged at 3000 rpm for 10 minutes for human descemetocele cases. Before surgery, 10 ml of the autologous blood was drawn into a sterile centrifuge tube without addition of any anticoagulants and it was centrifuged at 3000 rpm for 12 minutes. Above the sedimented red cells, a clump of fibrin matrix rich in platelets was observed, with platelet poor plasma above it (Fig. 3). With the use of forceps, the platelet rich fibrin matrix was carefully lifted from the tube and the red blood cells gathered at the bottom were cut off with sterile scissors (Fig. 4). After that, it was manually compressed between sterile gauze pads to make it into a membrane, which would later be sutured to the corneal defect following dermoid removal (Fig. 5). The length and width of the PRF membrane graft were modified to match with those of the corneal lesions, ensuring that the graft was only marginally larger than the defect.

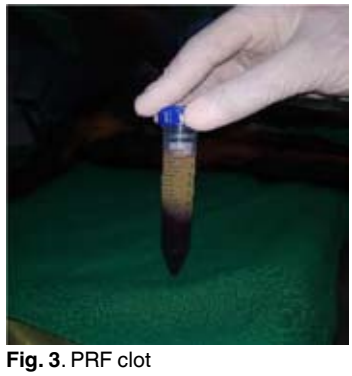

Fig. 3. PRF clot

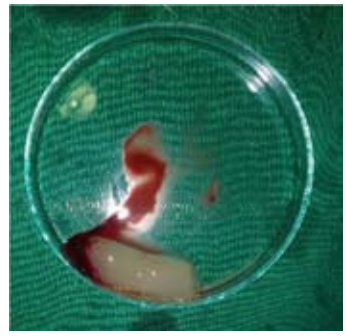

Fig. 4. PRF clot after separation

Fig. 5. PRF membrane after centrifugation

Both the puppies were premedicated with inj. atropine sulphate at the dose rate of 0.045 mg/kg, inj. tramadol hydrochloride at the dose rate of 2.0 mg/kg and inj. xylazine hydrochloride at the dose rate of 1.0 mg/kg body weight intramuscularly. General anaesthesia was induced with inj. ketamine hydrochloride at the dose rate of 2.5 mg/kg and inj. midazolam at the dose rate of 0.05 mg/kg body weight intravenously. Anaesthesia was maintained using isoflurane at two per cent in 100 per cent oxygen.

Eyes were rinsed with normal saline solution, followed by one per cent povidone iodine collyrium. The globe was fixed with stay sutures using 3-0 polyglactin 910 suture material and the affected animals were subjected to superficial keratectomy and conjunctivectomy. The conjunctival dermoid was incised around its periphery, using keratome and was excised. This was followed by grafting of the PRFM over the defect (Fig. 6) and was sutured to bulbar conjunctiva using 5-0 polyglactin 910 suture material (Fig. 7). Third eyelid membranoplasty was done using 5-0 polyglactin 910 in horizontal mattress suture pattern (Fig. 8, 13). An Elizabethan collar was advised for four weeks, to avoid self-mutilation. Third eyelid membranoplasty sutures were removed after seven days. Postoperatively, antibiotic and anti-inflammatory eye drops along with d-panthenol ophthalmic gel were advised in both the cases for seven days post grafting.

After the removal of third eyelid sutures on the seventh day postoperative, examination of the eyes indicated that the PRFM graft was completely absorbed (Fig. 9, 14). The cornea appeared opaque. Antibiotic and anti-inflammatory eye drops were prescribed from seventh day onwards after the removal of membranoplasty sutures. The keratectomy incision fully epithelialised by fourteenth

day. At the end of four weeks, it was observed that the corneal neovascularisation had regressed by 80 per cent with the anterior chamber of the eye distinct under cornea. Every week after treatment, corneal transparency and resolution of pannus were evaluated (Fig. 9, 10, 11). A Combination of steroid and antibiotic eye drops was prescribed from the third week onwards for one week for reducing the vascularisation of cornea. After the fourth week of surgery, the corneal transparency returned to normal (Fig. 11). The vision and menace response in both dogs were normal. The fluorescein dye test was used to evaluate the integrity and healing of the cornea which was found negative one week after the treatment.

Fig. 6. Placement of graft

Fig. 7. Suturing of graft

Fig. 8. Third eyelid membranoplasty

Dermoid is a congenital disorder that frequently goes undetected until later in life, unless they are big enough to see, or when secondary issues are manifested. All the elements of healthy skin, including the epidermis, dermis, sebaceous glandular tissue, fat tissue, hair follicles and blood vessel, are present in a dermoid (Crispin, 2002; Maggs, 2008). In dogs, the occurrence of ocular dermoid was most frequently found near the temporal limbus (Maggs, 2008). Similar findings were observed in the present case scenario. Mann (1930) classified ocular dermoids into three types *viz.,* type 1 (limbal or epibulbar), type 2 (corneal), and type 3 (encompassing the entire front region). Type 1 and type 2 dermoids were observed in Dog 1 and Dog 2, respectively. The median age of dogs presented with ocular dermoid was six months and the condition was found to be unilateral in both the cases and a similar finding was observed by Erdikmen *et al*. (2013). The corneal defect that occured from dermoid excision required the same kind of care as other corneal injuries. Although none of the dogs in the present study required a canthotomy, it could occasionally be necessary to completely expose the conjunctival dermoids. Unfortunately, it might be challenging to estimate the stromal invasion of a dermoid before surgery.

Fig. 9. Healing of corneal transplant on postoperative day 7

Fig. 10. A large reduction in granulation with a mild corneal transparency noticed on day 21

Fig. 11. Minimal scar tissue with an apparently clear cornea on day 45

The most common indications for membrane grafts were ulcers that were large, deep or perforated, encompassing 50% or more of the corneal thickness, and refractory to conventional therapies. The procedure could be used to heal corneal ulcer or corneal defects after superficial keratectomy. The platelet-rich fibrin (PRF) membrane, a new second-generation fibrin concentrate rich in autologous platelets as described by Choukroun *et al*. (2006), was used in the current investigation. Grafting of PRFM had been used successfully in humans. Hence, the same techniques had been chosen to apply in the surgical treatment of canine ocular dermoid because of the anti-inflammatory properties of the membrane and the healing with minimal scarring. The fibrin matrix was converted into a membrane graft as recommended by Alio *et al.* (2013). The membrane transplant served to conceal the corneal defect. In this instance, the PRFM graft was placed as an adjuvant follow-up therapy for canine corneal dermoid after keratectomy. The epithelialisation of the cornea was completed over a period of 14 days and the cornea became more transparent over a period of time. The corneal healing received complete structural support from the membrane transplant. The graft material fused with the cornea within a week of treatment and healing progressed further in the following four weeks. The corneal opacity was moderate. Fast epithelialisation is essential for repairing the ocular surface and PRFM graft served the purpose. Application of topical antibiotic-corticosteroid

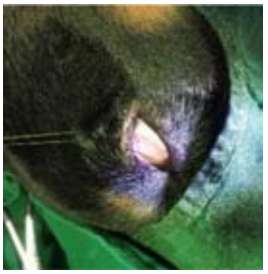

Fig. 12. PRFM grafting in dog 2

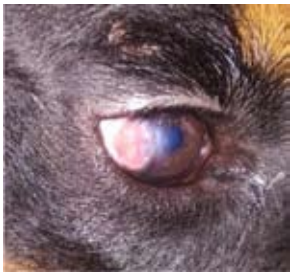

Fig. 13. Third eyelid flap in dog 2

Fig. 14. Mild granulation tissue formation with an apparently clear cornea on Day 7

formulations lessened postoperative corneal scarring and enhanced the attainment of ultimate clarity of the cornea once corneal epithelialisation is complete, as indicated by the lack of fluorescein dye retention (Lee *et al*., 2005).

Summary

Early corneal healing with minimal scarring recorded in these two cases of corneal dermoid treated with keratectomy and canine autologous platelet-rich fibrin membrane (PRFM) transplant is suggestive that PRFM is a safe and effective treatment option for corneal reconstruction in dogs.

Acknowledgements

The authors acknowledge the University Veterinary Hospital, Kokkalai and Mannuthy and College of Veterinary and Animal Sciences, Mannuthy for the facilities provided.

Conflict of interest

The authors declare that they have no conflict of interest.

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