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Corneal ulcer management using human amniotic membrane in dogs: A report of 6 cases

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Abstract
The present study was conducted on 6 clinical cases of dogs irrespective of age, sex and breed which were having corneal ulcer to evaluate the efficacy of surgical procedure. Dogs presented with corneal ulcer were confirmed by clinical examination, direct ophthalmoscopy, fluorescein dye test and then subjected to human amniotic membrane transplantation. All the dogs were subjected to topical moxifloxacin eye drops and flurbiprofen eyedrops prior and post-surgery at the rate of two drops four times daily for a period of 2 weeks and 5 days respectively. Healing of the ulcer were studied based on vision function tests, schirmer tear test, gross appearance of the cornea, corneal clarity before and 7th, 14th and 28th post-operative days. Negative fluorescein staining was seen on 7th post-operative day in most of cases suggests that the corneal epithelialization was rapid and was complete by 7th post AMT and corneal transparency was regained by 28th postoperative day in most of the cases.

Keywords: Cornea, corneal ulcer, amniotic membrane

1. Introduction
The cornea forms the anterior portion of the eyeball that refracts light onto the retina. Corneal ulceration, often known as ulcerative keratitis is one of the most common ocular problems with a variety of aetiologies in companion animals, and occurs when there is a breach of the epithelium and exposure of the stroma. Significant treatment is important to restore and preserve vision and minimize pain. The principles of corneal wound ulcer treatment include removal of the primary cause, reduction of inflammation, control of infection, enhancement of corneal healing and minimization of corneal scarring (Ledbetter and Gilger, 2013; Maggs *et al.*, 2012) [24, 42]. When the corneal epithelium breaks down, exposing the underlying corneal stroma, a corneal ulcer develops. Based on the depth corneal ulcers were classified as superficial, deep, descemetocele and based on ease of healing classified as complicated, uncomplicated, refractory, progressive. Superficial ulcerations involve the corneal epithelium and basement. Membrane with minimal or no stromal involvement. Deep ulcerations extend to one half the stromal depth or greater, and descemetoceles extend to the level of Descement's membrane (Moore, 2003) [27].
Lacrimation, blepharospasm, photophobia, conjunctival hyperaemia, corneal oedema, and perhaps miosis and aqueous flare were the symptoms of this condition. These clinical indications, as well as the corneal stroma's retention of topically applied fluorescein dye, were used to diagnose a corneal ulcer. In most cases, uncomplicated superficial ulcers heal quickly with little scarring. However, complicated deep ulcers, such as those caused by microbial infection, might induce vision loss due to corneal scarring or, in the case of corneal perforation, anterior synechia formation (Gellat *et al*., 2013) [11].
Different surgical methods have been used for the correction of deep corneal ulcers includes tissue adhesives (Watte *et al*., 2004; Bromberg, 2005) [38, 4], conjunctival grafts, corneo-conjunctival transposition (CCT) (Andrew *et al*., 2001; Graham *et al*., 2016; Gogova *et al*., 2020) [2, 15, 13], fresh or frozen corneal transplants (Laguna *et al*., 2015; Lacerda *et al*., 2017) [23, 22] and the use of biomaterials: porcine small intestinal submucosa (Vanore *et al*., 2007) [36], pericardium (Dulaurent *et al*., 2014) [8], renal capsule (Andrade *et al*., 1999) [1], urinary bladder (Chow and Westermeyer, 2016) [5], and amniotic membrane (Costa *et al*., 2019) [6]
Amniotic membrane consists of a combination of tissue and cells, which when used as a biological dressing, helps wound healing by providing a foundation for soft tissue regrowth. Growth factors and cytokines with anti-inflammatory, anti-bacterial, anti-immunogenic, and and anti-fibrotic qualities were delivered by biologically active cells in the epithelial and stromal layers of the amnion (Perepelkin *et al.*, 2016) [32].

The present study was under taken to evaluate the efficacy of amniotic membrane in management of corneal ulcers in dogs.

2. Material and Methods

The study was conducted on six clinical cases of dogs with corneal ulcer irrespective of breed, age, and gender presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru.

Dogs presented with corneal ulcer was confirmed by clinical examination, direct ophthalmoscopy, fluorescein dye test and will be subjected to human amniotic membrane transplantation. All the dogs were subjected to topical moxifloxacin eye drops and flurbiprofen eyedrops prior to surgery at the rate of two drops four times daily to reduce existing subclinical inflammation and pre-operatively restricted solid food for about 12 hours and 6 hours for the water was considered.

All the dogs were premedicated with atropine sulphate @ 0.04 mg/kg body weight subcutaneously and xylazine @ 1 mg/kg body weight intramuscularly. After 15 - 20 minutes, general anaesthesia was induced and maintained with thiopentone sodium @ 12.5 mg/kg body weight intravenously and administration of atropine sulphate resulted in reduction of salivary secretion whereas xylazine paresthesia administration provided good sedation and muscle relaxation. Induction of anaesthesia was smooth and uneventful. No intraoperative anaesthetic complications were noticed in any of the cases.

All the dogs were subjected to topical moxifloxacin eye drops prior to surgery at the rate of two drops four times daily and pre-operative fasting will be considered. The eyelashes of the affected eye were clipped close to the palpebral border. Accumulated ocular discharge were removed using sterile cotton swab and the area around the eye was cleansed with povidone iodine solution, the eye was aseptically prepared by irrigating it with diluted povidone 1:50 solution. Globe was fixed with eye speculum and the Freeze-Dried Amniotic Membrane (FD-AM) was hydrated with saline and then applied to the cornea in such a way that stromal side of the membrane faces towards the cornea. The FD-AM was sutured with polyglactin 910 no. 6-0 to the bulbar conjunctiva using interrupted sutures at 6 places. After surgery the eyelid speculum was removed carefully and the eyeball was repositioned, operated eye was irrigated with normal saline and temporary tarsorrhaphy was done using trulon no. 2-0. All the dogs were administered moxifloxacin eyedrops @ 2 drops 4 times a day for 2 weeks and flurbiprofen eyedrops @ 2 drops 4 times a day for 5 days. Systemic antibiotic such as cephalexin was used @ 25 mg/kg twice daily for 7 days. Advised to follow post-operative medications and to apply Elizabethan collar, to protect the eye from self-inflicted trauma. Post- operatively on 7th, 14th, and 28th day, all the cases were evaluated by using vision function tests, haemato-biochemical parameters, schirmer tear test, fluorescein dye test and gross appearance of the cornea.

3. Results

In the present study all the ulcers were centrally located and bacterial keratitis was the detected as the most frequent causative factor followed by nasal trichiasis and trauma. Clinical parameters like heart rate, respiratory rate and rectal temperature did not show any significant variation. Haematological parameters like Haemoglobin, PCV, TLC, TEC, PLT and DLC remained within normal levels and no significant changes were observed. Biochemical parameters like Creatinine and ALT remained within normal levels and non-significant changes were observed.

3.1 Clinical assessment of corneal healing: The efficacy of AMT was monitored based on improvement in clinical signs such as epiphora, ocular discharge, fluorescein dye test, vision function tests, corneal vascularization, and opacity before the surgery and on 7th, 14th, 28th post- operative days. The time to complete corneal epithelialization was confirmed by negative stain retention. The reduction in lacrimation is tested by STT.

3.1.1 Schirmer's tear test: In the present study the Mean ± SE values of STT before surgery and on 7th, 14th, 28th post-operative days were 24.66±0.49, 20.0± 0.51, 18.0 ± 0.73, & 16.83±0.54 mm/min. The recorded values were within the normal range however the readings were higher initially and postoperatively reduced significantly. Similar findings were seen by Korittum *et al.* (2019) [20], this could be due to the excessive lacrimation due to pain and ocular irritation (Williams and Burg, 2017) [41].

3.1.2 Fluorescein dye test: The fluorescein staining was seen in all the cases on the day of presentation this could be due to the fluorescein sodium stain was taken up by exposed corneal stroma and defines the margins of the corneal ulcer green (Singh *et al.*, 2004) [34]. The exposed corneal stroma takes up the fluorescein dye due to its hydrophilic nature, while the corneal epithelium does not stain due to its lipophilic nature (Moore, 2001 and Morreale, 2003) [27, 28]. Out of 6 cases, fluorescein dye test was found to be negative in 5 cases on 7th day, whereas positive in one case and which showed negative by 14th day. This could be due to the epithelialisation of the cornea due to the grafted human amniotic membrane. The time to complete corneal epithelialization, confirmed by a negative fluorescein retention at the corneal lesion as given by Vongasakul *et al.* (2009) [17] and Korittum *et al.* (2019) [20].

3.1.3 Vision function tests: Palpebral reflex was found normal (+++) in all the cases throughout the study period both before and after AMT (100 percent). However, the significance of the palpebral reflex is debatable, as Ofri (2008) [31] believes that it can be present in animals that are apparently blind.

Menace reflex and cotton ball test were absent (-) in five out of six animals and one Animal showed sluggish to absent (+) finding on the day of presentation and became normal (+++) by the end of the study period after AMT. A positive menace response is a blink reflex elicited by the animal to the sudden threat movement near the eye (Martin, 2001) [25]. The function of the optic nerve (CN-II, sensory) and the facial nerve is represented by the menace test (CN-VII, motor). A clear optic medium, a functioning retina, and intact optic and facial nerves were required for a positive menace response (Moore, 2001) [28]. It's a subcortical reflex that occurs when the visual system is suddenly stimulated (such as foreign body moving toward eye). The threat reflex causes the palpebral fissure to close and the head to turn away from the noxious stimulus. The findings of this study were consistent with those of Felchle and Urbanz (2001) [9], who used the menace reflex to confirm visual function and outcome and cotton ball test termed positive if the patient focus on an object that is moved or dropped in front of them and it follows the course of the object (Beranek and Vit, 2007) [3].

Pupillary light reflex was absent (-) in four out of six cases, normal to sluggish (++) response to this test was seen in two animals on the day of presentation and all the animals were showing positive normal response (+++) to this reflex by the end of the study period. The PLR test effectively assesses the function of the retina, optic nerve, and iris sphincter muscle (Felchle and Urbanz, 2001) [9] however in contrast to this Mitchell (2011) [26] claimed that the PLR was not a function of vision because the blind animals had normal PLR in the case of cataracts or occipital brain lesions, but animals with adequate vision had no PLR in the situation of iris atrophy. In the present study extensive corneal opacity due to corneal oedema, deep and large sized epithelial defect prevents the light to pass through pupil and thereby prevent the pupil constriction. Therefore, majority of animals showed absent menace and pupillary light reflex (Cullen and Grahn, 2005) [7]. The improvement in the responses for visual function tests on the subsequent days in the study period can be attributed to the reduction in oedema and improvement in clarity of the cornea resulted due to the anti-inflammatory effects of the decellularized human amniotic membrane graft (Tseng and Li, 1999; Wichayacoop *et al.*, 2005 and Korittum *et al.*, 2019) [35, 40, 20].

3.1.4 Post-operative appearance of grafted ulcer

The grafted ulcer appeared granulating in appearance in all the dogs on 7th day of observation. On subsequent days of observation, the ulcer appeared shallow and light pink, extent of granulation was found reduced, and surface had become smooth and continuous with adjacent cornea. These findings could be attributed to the healing effects of the grafted human amniotic membrane. Fernandes *et al*. (2005) [10] reported that the human amniotic membrane acts like a basement membrane and facilitates the migration of epithelial cells. It reinforces adhesion of basal epithelial cells, promotes epithelial differentiation, prevents epithelial apoptosis, and improves corneal sensitivity and tear film stability. Echeverry *et al*. (2018) [14] reported that the AM facilitates migration of epithelial cells and acts as a barrier to infectious agents, promotes rejuvenation and reduces pain. The time to complete epithelialization was confirmed by negative fluorescein stain retention. Re-epithelialization of the cornea receiving AM transplantation followed by tarsorrhaphy was complete on day 7 post-operatively in five out of six animals and in one animal it was on 14th post-operative day. Human AM contains number of growth factors (Koizumi *et al*., 2000) [19] that favours epithelial healing while its basement membrane facilitates the migration of epithelial cells and re-establishes adhesion between new epithelial cells and underlying basement membrane (Khodadoust *et al*., 1968) [18]. Vongsakul *et al*. (2009) [37] reported mean epithelisation time as 7.33±0.21 days with canine amniotic membrane in conjunction with third eyelid flap for created corneal ulcers. However, Kruse *et al*. (1999) [21] observed mean epithelisation time and amniotic membrane dissolution time was four weeks and twelve weeks respectively following use of multilayer cryopreserved human amniotic membrane. Success rate of technique was evaluated in terms of restoration of vision and resumption of normal corneal transparency. Vision was restored in all 6 eyes and normal corneal transparency was resumed in four out of six eyes and two eyes showed a focal spot of haziness on 28th post-operative day. Kalpravidh *et al*. (2009) [17] observed normal restoration of corneal transparency within 35th post-transplantation day with canine amniotic membrane transplantation.

3.1.4.1 Vascularization of Cornea

In the present study all the dogs showed mild to extensive superficial corneal vascularization on 0th day of observation. This is could be due to the nonspecific response to the corneal injury (Gilger *et al.*, 2014). The superficial vascularization progressively resolved on subsequent days and was not observed on 28th day of observation. The corneal vascularization observed on initial post-operative days related to the healing processes stimulated by filling the deep corneal lesions with the membrane (Rozin *et al*., 2020) [33].

The reduction in the superficial vascularization could be attributed to the anti-angiogenic properties of the decellularized human amniotic membrane which mitigates ocular surface neovascularization (Nakamura *et al*., 2004) [30]. This was in accordance with Hao *et al.* (2000) [16], who reported that the IL-10 and IL-Ira blocked angiogenesis by increasing the secretion of TIMP-1 and decreasing the action of matrix metalloproteinase -2 and -9.

3.1.4.2 Corneal clarity

In the present study, all the animals were showing moderate to severe degree of corneal opacity on the day of presentation and which reduced on subsequent days. On 28th post-operative day, four animals have regained complete transparency and two animals had a focal spot of haziness. The improvement in the corneal clarity could be attributed to the fact that the basement membrane of AM is similar to that of corneal, conjunctival collagen composition and antifibrotic effects of the decellularized human amniotic membrane. Human amniotic membrane suppresses the transforming beta growth factor signalling which was responsible for fibrosis (Tseng and Li, 1999) [35] and also due to the anti-inflammatory and epitheliotropic effects of the decellularized human amniotic membrane (Tseng and Li, 1999; Wichayacoop *et al*., 2005 and Wichayacoop *et al.*, 2009) [35, 39, 40].

Fig 1: Day 0 image of deep central corneal ulcer with intense corneal Oedema

Fig 2: Day 7th post-operative image of healing corneal ulcer with mild neo-vascularization

Fig 3: Day 14th post-operative image of healing corneal ulcer with slight corneal oedema

Fig 4: Day 28th post-operative image of healed corneal ulcer with regain of normal corneal transparency.

4. Conclusion

In conclusion, Freeze dried human amniotic membrane graft used for management of corneal ulcer has epitheliotropic, anti-inflammatory, anti-angiogenic and anti-fibroblastic properties, provided rapid epithelization without neovascularization and scarring of the cornea which is important for corneal healing and hence human amniotic membrane transplantation is proved to be an effective surgical technique for the management of corneal ulcer in dogs

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