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Treatment of corneal ulcer in brachycephalic breeds using subconjunctival infiltration of autologous platelet-rich plasma-non-invasive therapy

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Abstract

A research study was conducted to understand and evaluate the effect of subconjunctival platelet-rich plasma (sprp) on corneal ulcers in brachycephalic dogs. A total of six dogs with different morphologies of corneal ulcers more than 1mm in size were used in this study, irrespective of age and sex. As a pre-treatment evaluation, a detailed ophthalmic examination was conducted on both eyes. Diagnostic tests like ABST, Schirmer tear test, corneal impression cytology, and fluorescent dye test were conducted on affected eyes. After collecting cephalic blood samples from six dogs, platelet-rich plasma was obtained by centrifugation. All six dogs received an autologous platelet-rich plasma, which was instilled via subconjunctival injection in the affected eye on days 0, 7, 14 and 21. Clinical outcome was monitored by evaluation of epithelial defects, corneal opacity, corneal vascularisation, duration of blepharospasm, duration of ocular discharge and ulcer size measurement. As post-treatment care, artificial tears and antibiotic drops were instilled thrice daily till the corneal healing. Application of subconjunctival platelet-rich plasma was associated with early healing in superficial and stromal ulcers.

Keywords: Autologous platelet-rich plasma, brachycephalic dogs, corneal ulcer

Introduction

Ulcerative keratitis or corneal ulcer is defined as the loss of epithelium in addition to loss of corneal stroma, accompanied by a variable degree of reflex uveitis in dogs (Mandell and Holt 2005) [10]. Whitley (2000) [15] classified corneal ulcers by the depth of corneal involvement as superficial, deep stromal, and descemetocoele. Superficial corneal ulcers were further classified as uncomplicated, progressive, or refractory. Deep stromal ulcers were divided into progressive and non-progressive types. Etiological factors for ulcerative keratitis may be trauma, eyelid defects, lacrimal system disorders, fungal infections, bacteria and viruses (Roberto *et al.*, 2018) [13]. Incidence of corneal ulcers in brachycephalic dogs was twenty times more likely to be affected than non-brachycephalic dogs Packer *et al.*, 2015 [11] and more common in brachycephalic breeds like Chinese pugs (Chinchu, 2010) [3]. Predisposing factors for corneal ulcer in brachycephalic breeds were shallow orbit, lagophthalmos, more scleral exposure, short craniofacial ratios and nasal folds. Ulcerative keratitis in brachycephalic dogs was usually recurrent and located in a central or paracentral corneal position (Bouhanna *et al.*, 2008) [1]. Various treatment options were available to treat corneal ulcers, including either medical treatment (antibiotic therapy, regenerative therapy) or surgical treatment. Brachycephalic breeds are more prone to anaesthetic risk due to their anatomical features. So, minimally invasive treatment is required to treat corneal ulcer in brachycephalic breeds. Structural mechanisms of the wound healing process require several raw materials such as growth factors, vitamins, glucose and so on. The easiest and most efficacious source to obtain these raw materials is the blood. Until recently, various blood derivatives were used for this purpose, such as fetal calf serum, umbilical cord serum and autologous/allogeneic serum. Platelet-rich plasma (PRP) is a blood-derived product that provides a higher concentration of essential growth factors by concentrating platelets in a small volume of plasma and enhances epithelial wound healing in ocular surface disease in dogs (Reda *et al.*, 2020) [12]. The growth factors (GF) include platelet-derived GF (PDGF), transforming GF- β (TGF- β), vascular endothelial GF (VEGF), fibroblastic GF (FGF), insulin-like GF (IGF) and epidermal GF (EGF).

These GF lead to accelerated endothelial and epithelial regeneration, stimulate angiogenesis, collagen synthesis, soft tissue healing and homeostasis (Jee *et al.*, 2016) [8]. PRP is a well-known angiogenic, anti-inflammatory, non-toxic, non-immunogenic and anticatabolic agent (Yang *et al.*, 2018) [16]. PRP application on the corneal surface revealed an acceleration and improvement in corneal epithelial wound healing. Either drop or clot form of PRP can be used for subconjunctival application. This study aimed to investigate the effect of subconjunctival PRP injection on the corneal epithelium wound healing process in brachycephalic breed dogs.

Materials and Methods

A total of six brachycephalic dogs diagnosed with corneal ulcer of more than 1mm in size were presented at the Department of Veterinary Surgery and Radiology, Veterinary Clinical Campus, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, from October 2022 to March 2023, and were selected for the present study.

The animal particulars and history regarding time of occurrence, presence of systemic diseases, any medication undertaken and the etiological factors were recorded. Under ophthalmic examination, physical examination of both the eyes, examination of the affected cornea and ocular reflexes were recorded on day 0, 7, 14 and 21. Diagnostic tests like antibiotic sensitivity test (ABST), Schirmer I tear test, corneal impression cytology, and fluorescent dye staining were carried out on the affected eye on days 0, 7, 14 and 21.

Preparation of Autologous Platelet Rich Plasma

PRP preparation was carried out under strict sterile conditions using sterile and disposable materials. A total of 3-5ml of whole blood was collected from the cephalic vein in sterile 4.5ml tubes with an anticoagulant base of EDTA. The tubes with blood samples were centrifuged once @ 1600 rpm for 10 minutes to separate the whole blood into 3 fractions. The lower red layer with the vast majority of the red cells, the upper yellow layer representing plasma and in between a thin white layer, the buffy coat was obtained. In the plasma layer, the upper one-third was discarded, and the lower two-thirds of around 2.5 to 3 ml was taken in a sterile disposable syringe, which was used as subconjunctival infiltration since it contains a number of growth factors and proliferating factors.

Subconjunctival treatment and post-treatment care

All six brachycephalic dogs with corneal ulcer were subjected to infiltration of 1ml autologous platelet-rich plasma using a sterile and disposable insulin syringe in the palpebral conjunctiva on day 0, 7, 14 and 21. As post-treatment care, artificial drops and antibiotic drops based on the results of ABST were advised to be instilled three times a day, along with an E-collar, to prevent self-mutilation.

Method of clinical evaluation

The effectiveness of the procedure for restoring the transparency and functional capacity of the cornea was carried out by the examination of the eye and cornea, Schirmer I tear test, corneal impression cytology and fluorescein dye staining and other complications if any, after treatment on days 7, 14 and 21. Clinical examination of the affected cornea was performed. The parameters included

were Transparency-Based on Corneal opacity level scale score: 0-absence, cornea totally transparent, 1-mild, slight opacity traces, observed through oblique illumination, 2-moderate, salient opacity but not affecting viewing of the back chamber, 3-intense, impairing viewing of the back chamber and 4-stromal total opacity, preventing back chamber viewing as described by Gandolfi *et al.*, 2021 [5]. Location of ulcer (axial/paraxial/inferior nasal/inferior temporal/perilimbal). Border (well demarcated/feathery), as described by Thomas *et al.*, 2005 [14]. Nature of corneal vascularisation-Based on vascularisation scale score: 0-absence, 1-mild, 2-moderate and 3-intense, as described by Gandolfi *et al.*, 2021 [5]. Depth of the ulcer (Superficial/stromal/Descemetocoele) as described by Gelatt *et al.*, 2013 [15]. Corneal ulcer healing-Based on scale score: 0-no corneal healing, 1-partial healing and 2-complete healing as described by Gandolfi *et al.*, 2021 [5].

Results and Discussion

A total of six dogs irrespective of age, sex and etiology presented with corneal ulcer sized more than one mm were taken for the study. In the present study, majority of the cases were pug (83.33%) followed by Shih tzu (16.67%). Over-representation of pugs in this study could be attributed to their increased popularity as a pet, which is supported by Chinchu (2010) [3]. All were aged between 2-4 years. Males (66.67%) were likely to be affected more than female. Left eye with corneal ulcer were presented more than right eye. Etiological factors of all the cases were trauma (Mandell and Holt 2005) [10]. Time of occurrence of injury between 3-7 days. On physical examination of eyes: Eyelids and conjunctiva were normal on both the eyes throughout the period of observation. Clinical signs like scleral congestion, blepharospasm and watery ocular discharge was noticed on the affected eye of all six dogs on the day of presentation, which got resolved after treatment from day 7 onwards. On examination of cornea, corneal oedema with ulcer (1-3mm) were noticed in all the six animals on the day of presentation, by day 21 all six animals showed corneal healing with mild scar which indicates PRP was well tolerated by corneal tissue with no sign of toxicity and early healing of corneal wound was noticed (Gimeno *et al.* 2010) [7].

On examination of ocular reflexes: Palpebral reflex was positive on both the eyes throughout the period of observation. Menace reflex and pupillary light reflex were absent on five dogs on the day of presentation, by day 21 all five dogs showed positive reflexes. The menace response found normal when the cornea attained clarity. The normal PLR response in due course of treatment indicated an intact optic and oculomotor nerve. On observation of corneal transparency: One dog showed total opacity, four dogs showed intense opacity and one dog showed moderate opacity on the day of presentation, by day 21 five dogs showed transparent cornea with mild scar and one dog showed moderate corneal opacity which indicates that PRP treated superficial corneal ulcers attained transparency within 2-4 weeks (Farghali *et al.* 2021) [4].

Majority of the corneal ulcers were located in paraxial region (50%), followed by axial region (33.33%) and Perilimbal region (16.67%) with well demarcated border. Higher percentage of paraxial located ulcer were observed might be due to the inherent lower corneal sensitivity in brachycephalic breeds. On observation of corneal

vascularization: Three dogs showed moderate vascularization and remaining dogs showed no vascularization on the day of presentation. By day 21, five dogs showed no vascularization and one dog showed mild vascularization. Majority of the cases were presented with superficial ulcer (66.67%), followed by stromal ulcer (16.67%) and descemetocoele (16.67%). All six dogs showed complete corneal healing by day 21.

Schirmer I tear test (STT): The Mean $\pm$ SE of STT values were 22 ± 0.31 and 20 ± 0.42 , 20 ± 0.42 and 20 ± 0.37 , 19 ± 0.60 and 20 ± 0.49 , 18 ± 0.82 and 19 ± 0.48 of affected and contralateral eye on day 0, 7, 14 and 21 respectively. There is no significant difference noticed in the values of the affected and contralateral eye of corneal wound in the whole duration of the study (Charalambidou *et al.* 2019) [2].

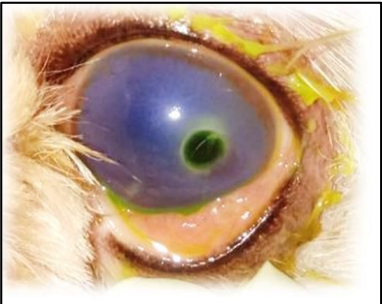
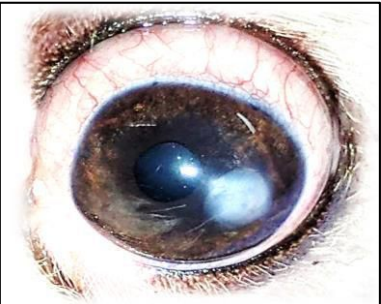
On observation of corneal impression cytology: All six dogs

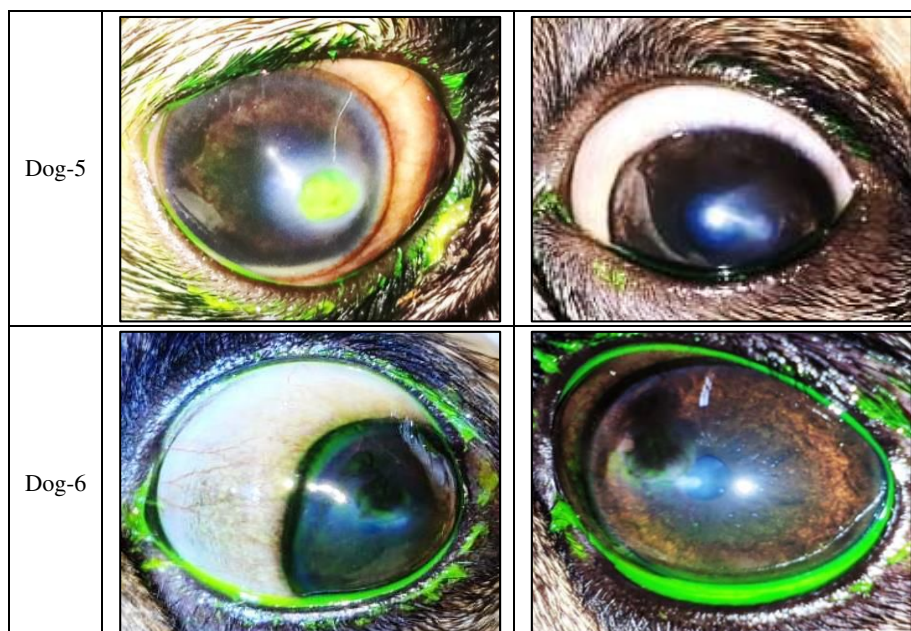
showed presence of epithelial cells and neutrophils on the day of presentation, reduction of epithelial cells with absence of neutrophils were observed from day 14 onwards which showed that PRP helped in reducing the inflammatory process due to its anti-inflammatory property.

Based on antibiotic sensitivity result, gentamicin and ciprofloxacin were most sensitive to the corneal ulcer cases of this study. All six dogs showed positive uptake of dye on the day of presentation, by day 21 all six dogs showed negative uptake of dye. Corneal ulcers were considered healed when the cornea's tested negative for fluorescein dye test (Maggs, 2008) [9].

Mean $\pm$ SE of corneal ulcer size of six dogs were 2.0 ± 0.30 . Mean $\pm$ SE of platelet in whole blood of all six dogs were 157.7 ± 20.62 , platelet in plasma was 645 ± 47.92 and platelet increase over the base line was 3.31 ± 0.27 .

Corneal affections of six dogs

Number	Day-0	Day-21
Dog-1		
Dog-2		
Dog-3		
Dog-4		



Conclusion

In the present study, freshly isolated platelet rich plasma sub conjunctival infiltration was a simple, convenient, volume limited technique with no ocular discomfort, no side effects and well tolerated by animals. Autologous PRP therapy was a deemed convenient, cost effective, non-immune provocation, minimally invasive technique and does not require general anaesthesia. The PRP concentration of 2.9 to 3.3 was ideal to enhance the healing of superficial and stromal ulcers quickly. Topical antibiotic therapy in accordance with ABST result post treatmently in all animals were found effective in preventing infection and other complications. E-collar was accommodated well by all the animals and prevented self-mutilation. No significant post treatment complications were observed during the study period. PRP application was effective in treating corneal ulcer due to its high concentration of growth factors that participate in wound healing. PRP proved to be an excellent option to quickly and effectively repair the lesion, in addition to accelerating re-epithelialization, reducing inflammation and corneal opacity, helping to re-establish eye health. PRP infiltration was one of the better alternation technique for surgical intervention of corneal ulcer cases in brachycephalic breeds.

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