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Evaluation of autologous platelet rich plasma injection into the lacrimal gland for the management of keratoconjunctivitis sicca in dogs- A randomised clinical trial#

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Abstract

The present study was conducted in six clinical cases of canine keratoconjunctivitis sicca (KCS) presented to the University Veterinary Hospitals of Kerala Veterinary and Animal Sciences University (KVASU), during the period from November 2024 to September 2025. Dogs having clinical signs suggestive of KCS were selected irrespective of age, sex, or breed and were subjected to autologous platelet-rich plasma (PRP) injection into the lacrimal gland. Clinical, ophthalmic and haematological parameters were evaluated on day 0, days 30 and 60 post operatively. Autologous PRP was prepared using a double-centrifugation method with a modified syringe technique, ensuring a platelet concentration of approximately $1\times10^6/\mu L$. The procedure was performed under general anaesthesia with strict aseptic precautions. Marked improvement in corneal clarity and ocular discharge was observed in all dogs, accompanied by a reduction in corneal neovascularisation. A significant increase in Schirmer tear test-I (STT-I) and Schirmer tear test-II (STT-II) values indicated enhanced total and basal tear production, while tear film break-up time (TBUT) showed a significant rise, reflecting improved tear film stability and ocular surface integrity. The treatment was well tolerated, with no major complications. These findings suggest that autologous PRP injection offers a minimally invasive, biologically regenerative approach for restoring lacrimal gland function in the management of canine KCS.

Keywords: *Dogs, keratoconjunctivitis sicca, platelet rich plasma, lacrimal gland injection*

Keratoconjunctivitis sicca (KCS), commonly known as dry eye disease, is a prevalent ocular disorder in dogs characterised by quantitative or qualitative tear film deficiency, leading to desiccation and inflammation of the cornea and conjunctiva (Ribeiro et al*.,* 2008; Anoop et al., 2010; Venugopal et al., 2011; Anoop et al., 2015b). The normal tear film is composed of lipid, aqueous and mucin layers which is essential for maintaining corneal health, lubrication and optical clarity (Grahn and Storey, 2004). Disruption of any component results in tear film instability, epithelial damage and secondary infections (Resmi, 2008). Clinically, affected dogs exhibit mucopurulent discharge, conjunctival hyperaemia, corneal opacity and varying degrees of visual impairment (Karthikaa et al*.,* 2020).

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Conventional therapeutic approaches involve administration of topical tear stimulants such as cyclosporine and tacrolimus, artificial tear substitutes and anti-inflammatory agents (Benitez-del-Castillo et al., 2017). However, these therapies often manage symptoms without restoring lacrimal gland function in cases of quantitative type of dry eye diseases. For chronic or refractory cases, surgical interventions like parotid duct transposition or minor labial salivary gland transplantation are employed to maintain corneal hydration. While being effective, these procedures are invasive and carry risks of postoperative complications or variable secretory adaptation (Gelatt et al., 2018).

To overcome these limitations, regenerative approaches, such as platelet-rich plasma (PRP) offer a promising, minimally invasive, biologically active solution. Due to its rich concentration of growth factors, cytokines and bioactive proteins. PRP actively promotes angiogenesis, cellular regeneration and immunomodulation (Mohammed et al*.,* 2022). In human ophthalmology, PRP injections have been shown to enhance tear production and improve corneal health in patients with severe lacrimal dysfunction (Avila et al*.,* 2019). Similarly, autologous PRP therapy in dogs has demonstrated a strong potential for restoring lacrimal gland function and improving ocular surface integrity, offering a superior and minimally invasive alternative to the surgical management of KCS (Aswani, 2022).

Materials and methods

The study was conducted in six clinical cases of canine KCS with basal tear production less than 5 mm/min during the period from November 2024 to September 2025, presented to University Veterinary Hospitals of KVASU. The dogs were selected irrespective of their age, breed and sex and were numbered from P1 to P6. All of them were subjected to detailed physical, clinical and ophthalmic examination (Table 1 and 2) and haemato-biochemical and wet film examinations were carried out pre-operatively (Sarangom et al., 2014).

Platelet rich plasma was prepared by double centrifugation method (Shin et al*.,* 2017) which was modified using syringe method developed by Laiju (2022). A minimum platelet concentration of $1\times10^6/\mu L$ was ensured. The PRP obtained was subjected to quantitative analysis by comparing the complete blood count parameters in whole blood to that of final PRP (Table 3). The Platelet enrichment Factor (PEF) was calculated by dividing the PRP platelet concentration by the whole blood platelet concentration.

Topical antibiotic and anti-inflammatory eye drops were administered preoperatively in all the dogs to prevent iatrogenic infection (Suhas, 2015). Food and water were withheld for twelve and six hours, respectively. Surgical procedure was performed under general anaesthesia, with aseptic preparation of eye and adnexa using dilute povidone iodine (1:50) collyrium (Chinju, 2010; Anoop et al*.,* 2015a; Adarsh et al., 2017). The anaesthetic protocol involved premedication with tramadol (2.0 mg/kg) and xylazine (1.0 mg/kg) both intramuscularly, followed by induction using ketamine (2.5 mg/kg) and midazolam (0.05 mg/kg) intravenously (Narayanan et al*.,* 2011; Thajunnisa et al*.,* 2020; Shareef et al*.,* 2024). Given the brief nature of the procedure, maintenance of general anaesthesia was not necessary in all cases.

For injection of PRP into the lacrimal gland in dogs, the orbital ligament was palpated in the dorsolateral region of the ocular bulb to accurately locate the site and a 25G needle was introduced through the conjunctival fornix beneath the ligament. The needle was advanced approximately 6-7 mm until the tip was positioned deeper to the orbital ligament. An amount of 0.1 mL of freshly prepared autologous PRP was then injected using a 1.0 mL syringe (Fig.1). Procedure was done as recommended by Park et al. (2013) and Bittencourt et al*.* (2016) at biweekly interval for one month. Postoperative care included Elizabethan collars, topical antibiotics, anti-inflammatory drops and artificial tears for a period of seven days.

Detailed ophthalmic examination was carried out on the day of presentation and post-surgically on days 30 and 60 which included assessment of tear production using Schirmer tear test (STT) I and II and tear film breakup time (TBUT), recording of nature of discharge, changes in corneal lustre, corneal clarity, corneal neovascularisation and conjunctival hyperaemia.

Fig. 1. Injection of PRP into the anatomical position of the lacrimal gland

Results and discussion

All the dogs included in the study were female Shih Tzu, aged between 13 and 27 months with a mean age of 19.5±2.26 months. This finding reflects the breed and sex predisposition with brachycephalic anatomy (Venugopal, 2011; Sarangom, 2012; Venugopal, 2013; Antonia et al*.,* 2014; Karthikaa et al*.,* 2020; Rajaei et al*.,* 2024) and potential androgen deficiency contributing to tear film instability and ocular surface inflammation (Worda

Table 1. Details on Schirmer tear test (STT) I and II and tear film breakup time (TBUT)

Case No:	Eye affected	Nature of discharge	STT1 (mm/min)	STT 2 (mm/min)	TBUT (s)
P1	Both	Thick mucopurulent discharge	6	1	6
P2	Left	Mucopurulent discharge	10	4	5
P3	Left	Mucopurulent discharge	8	2	5
P4	Both	Mucopurulent discharge	6	2	8
P5	Both	Thick mucopurulent discharge	7	3	6
P6	Right	Mucopurulent discharge	6	1	8

Table 2. Data on clinical ophthalmic parameters of all 6 cases

Case No:	Corneal clarity	Corneal lustre	Conjunctival hyperaemia	Corneal neovascularisation
P1	Severe haziness	Absent	Severe	Profuse
P2	Mild haziness	Absent	Mild	Mild superficial
P3	Moderate haziness	Absent	Moderate	Profuse
P4	Moderate haziness	Absent	Moderate	Profuse
P5	Severe haziness	Absent	Severe	Profuse
P6	Severe haziness	Absent	Severe	Profuse

Table 3: Quantitative assessment of PRP

	Day 0		Day 14	
PRP parameters	**Initial**	**Final**	**Initial**	**Final**
TEC ($\times10^6$/ µL)	5.81±0.28	0.46 ± 0.06	5.99±0.35	0.43 ± 0.07
Hb (g/dL)	14.73±0.95	0.2±0.06	15.23±0.75	0.28±0.07
VPRC (%)	42.7±2.52	2.82±0.32	45.83±1.84	2.28±0.49
TLC ($\times10^3$/µL)	13.11±0.91	15.18±1.25	13.83±0.84	16.37±1.07
Platelet count ($\times10^3$/µL)	317.67±21.58	1914.83±124.60	323.33±21.76	1885.33±138.02
Platelet enrichment factor		6.12±0.41		5.88±0.30

et al*.,* 2001). Consistent mucopurulent ocular discharge and signs of discomfort, indicative of inadequate tear production and impression cytology revealing secondary bacterial colonisation was observed in all the dogs. Bilateral affection was observed in three dogs, consistent with immune-mediated etiology, while three dogs showed unilateral signs suggestive of localised or neurogenic causes. Duration of clinical signs ranged from one to three months. All the dogs had a history of either topical anti-inflammatory, antibacterial, or tear replacement therapies, yet persistent symptoms underscored the recurrent nature of KCS.

Platelet rich plasma was prepared using blood collected from the cephalic vein *via* a 20G scalp vein set, minimising premature platelet activation as recommended by Dhurat and Sukesh (2014). A modified syringe based double centrifugation method (Shin et al*.,* 2017; Laiju, 2022) was employed, using a centrifugation force and duration of 200×g for five minutes and 500×g for 20 minutes at 20°C, yielding effective platelet separation (Jennes et al*.,* 2023).

Quantitative analysis of PRP (Table 3) revealed reduced erythrocyte and haemoglobin levels compared to whole blood, confirming efficient separation. Leucocyte counts were slightly higher than whole blood which might have contributed positively to ocular healing without inducing inflammation. The platelet concentration exceeded the therapeutic threshold of 1×10^6/µL (Hara and Basu, 2014) in all cases, with mean counts of 1914.83 ± 124.60 (day 0) and 1885.33 ± 138.02 (day 14). Enrichment factors averaged 6.12 ± 0.41 and 5.88 ± 0.30, aligning with Weibrich et al*.* (2004). A concentration between 1 to 1.5 million per µL was ensured after diluting the PRP with platelet poor plasma using the formula $C_1V_1=C_2V_2$ (Reji et al*.,* 2025).

Following treatment, ocular discharge patterns demonstrated progressive alterations across cases. By day 30, three dogs (P3, P4, and P6) showed improvement from mucopurulent to serous discharge, which remained unchanged by day 60. In P5, the ocular discharge became mucoid, while in P1 it persisted as mucopurulent. By day 60, discharge in P1 had shifted to mucoid, P2 exhibited scanty seromucoid discharge, and P5 progressed from mucoid to serous. These changes reflect reduced inflammation and improved tear quality, consistent with findings by Aswani (2022) and Estanho et al. (2023). Significant improvements in conjunctival hyperaemia, corneal clarity and corneal lustre were observed, indicating therapeutic efficacy.

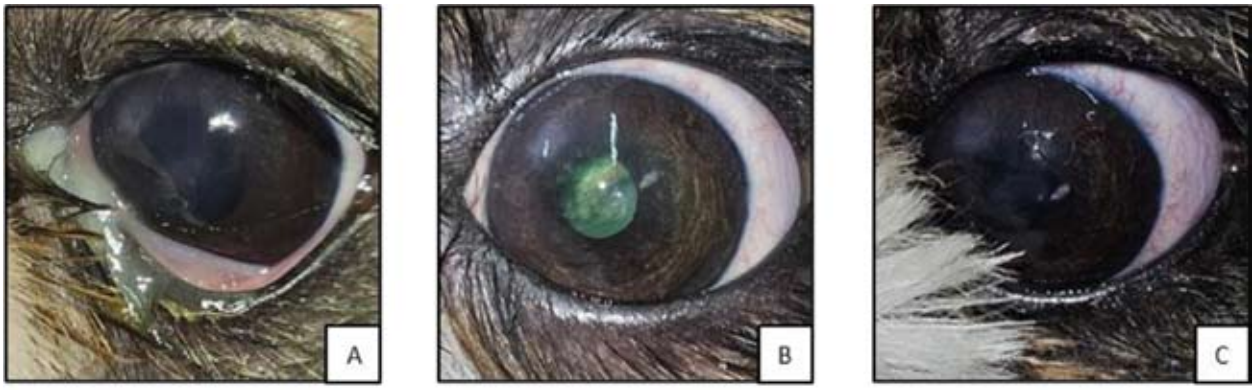

Fig. 2. Dog P3: A-Day 0, mucopurulent discharge, B-Day 30, serous discharge, C-Day 60, serous discharge

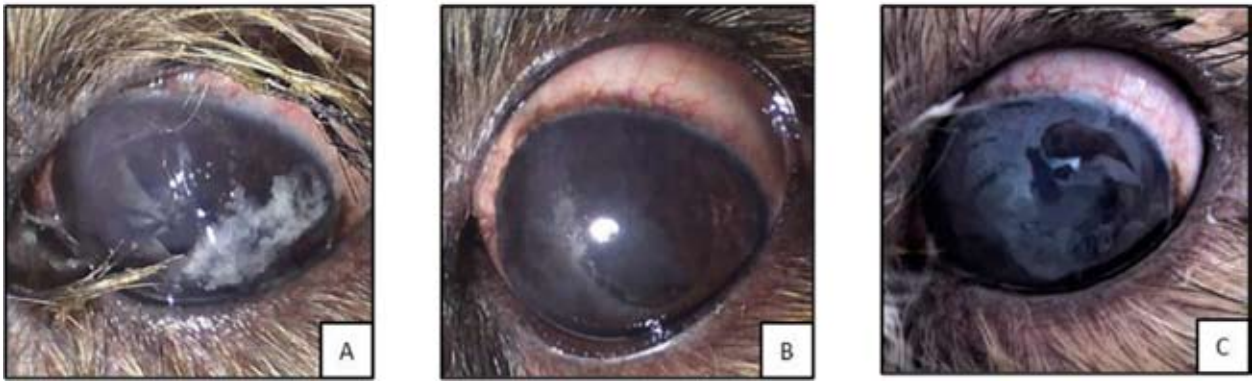

Fig. 3. Dog P5: A-Day 0, mucopurulent discharge, B-Day 30, mucoid discharge, C-Day 60, serous discharge

Table 4. Assessment of tear production

	Day 0	Day 30	Day 60	F value	p value
STT-I (mm/ min)	7.17 ± 0.65^{c}	12.00 ± 1.03^{b}	14.50 ± 1.52^{a}	16.625	0.001^{**}
STT-II (mm/ min)	2.17 ± 0.47^{b}	6.17 ± 0.47^{a}	7.00 ± 0.63^{a}	42.755	$<0.001^{**}$
TBUT (s)	6.33 ± 0.55^{c}	8.33 ± 0.95^{b}	9.50 ± 1.20^{a}	11.177	0.004^{*}

a, b, c Means with different superscript differ significantly at 1% level and 5% level**

Progressive reduction in inflammation and restoration of ocular surface integrity was consistent with findings by Avila et al*.* (2019) and Aswani (2022).

Significant improvement in tear production and tear film stability was observed across the study period in all the dogs (Table 4). Schirmer Tear Test-I values progressively increased from 7.17 ± 0.65 mm/min on day 0 to 14.50 ± 1.52 mm/min by day 60, while Schirmer Tear Test-II values, indicative of basal tear secretion, rose from 2.17 ± 0.47 mm/min to 7.00 ± 0.63 mm/min. This may be attributed to the regenerative properties of PRP, as platelets are known to release growth factors such as platelet derived growth factor (PDGF), Transforming growth factor- β (TGF-β) and Vascular endothelial growth factor (VEGF), which stimulate tissue repair, reduce inflammation and enhance lacrimal gland function (Tobita et al*.,* 2015; Mohammed et al*.,* 2022). This gradual stimulation of lacrimal gland regeneration and tear secretion aligns with previous studies demonstrating the efficacy of PRP in promoting ocular surface integrity and tear production in dry eye models (Anitua et al*.,* 2004; Castillo et al*.,* 2011; Sharun et al*.,* 2021). Similar findings were also noted by Aswani (2022), who reported a significant improvement in tear secretion following autologous PRP injection into the lacrimal gland, third eyelid and bulbar conjunctiva in canine KCS.

Tear Film Break-Up Time (TBUT) also improved significantly, increasing from 6.33 ± 0.55 seconds on day 0 to 9.50 ± 1.20 seconds by day 60 (Table 4). This enhancement is primarily attributed to the regenerative and trophic effects of platelet derived growth factors on the lacrimal gland (Castillo et al., 2011 and Dos Santos et al., 2021), leading to the restoration of aqueous tear quantity. The observed TBUT improvement, which indicates increased tear film stability, is therefore considered as a secondary benefit of the treatment. The significant increase in the aqueous volume (quantity) helps to restore overall ocular surface homeostasis, thereby stabilising the tear film and allowing the existing lipid and mucin layers to function more effectively. This is evidenced by human clinical studies utilising lacrimal gland PRP injection, which reported significant improvements in TBUT and

Lipid Layer Thickness (LLT) in their study (Avila et al*.,* 2019 and Mohammed et al., 2022).

Conclusion

The present study demonstrated that autologous PRP injection into the lacrimal gland is a safe, minimally invasive, and effective strategy for the management of canine KCS. The standardised preparation protocol ensured optimal growth factor concentration, leading to progressive improvements in tear volume, composition, and tear film stability, along with early resolution of ocular discharge and inflammation.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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