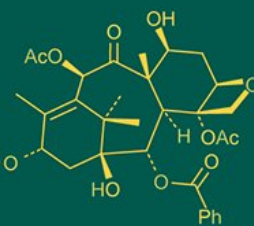
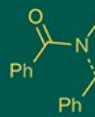


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Clinical outcomes of Cryotherapy with medial canthoplasty in the management of pigmentary keratitis in pugs: A prospective study

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Abstract

Aim: To study the efficacy and clinical outcomes of Cryotherapy with medial canthoplasty in the management of pigmentary keratitis (PK) in pugs.

Place and Duration of Study: Department of Veterinary Surgery and Radiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India during the year 2020-21.

Methodology: This study was conducted on six Pug breed of dogs (n=6 eyes) presented to the clinics with signs of reduced vision and difficulty in maneuverability. All the animals underwent a complete ophthalmic examination and were clinically diagnosed for Pigmentary Keratitis. Under general anaesthesia, Cryotherapy with medial canthoplasty was performed in all the cases and their clinical outcomes were assessed.

Results: The mean age of the study population was 6.33 ± 0.93 years. All the animals were males with four animals belonging to adult category and two animals belonging to senile category. Medial Caruncular Trichiasis (MCT) and Medial Entropion of Lower Eyelid (MELE) was observed in all six animals and Keratoconjunctivitis sicca (KCS) was observed in three animals. The preoperative mean Schirmer's tear test (STT) value, mean intraocular pressure (IOP) and mean pigmentation score was 12.67 ± 4.02 mm/min, 19.33 ± 2.09 mm Hg and 10.17 ± 1.76 . The menace response was positive in two animals and negative in four animals preoperatively. Cryotherapy with medial canthoplasty was performed in all the six animals and the postoperative mean pigmentation score on the 30th postoperative day was 6.58 ± 1.44 . The menace response was positive in four animals on the 30th postoperative day.

Conclusion: The early onset of favorable clinical outcomes such as improved vision and increased animal activity levels warrants the use of Cryotherapy with medial canthoplasty in the management of pigmentary keratitis in pugs in spite of the postoperative complications such as persistent pigmentation and recurrence of pigmentation.

Keywords: Eye, pigmentary keratitis, Cryotherapy, medial canthoplasty, pugs

Introduction

Ocular affections in dogs occurred at a rate of 6.62 percent and corneal affections were the third most common ocular ailments (Akinrinmade & Ogungbenro, 2016) [1]. Corneal affections were the most common ocular disorders reported in brachycephalic breed of dogs and pigmentary keratopathy accounted for the majority of corneal ailments (Amol, 2016) [4]. Pigmentary keratitis occurs from the centripetal migration of the melanocytic cells and deposition of melanocytic pigments in the corneal epithelium or deeper stroma usually starting from the limbal and perilimbal tissues accompanied by other signs of active keratitis such as corneal vascularization, stromal inflammatory cell infiltration and granulation tissue formation (Bellhorn & Henkind, 1966) [8].

Brachycephalic breeds such as Pugs, Pekingese, Shih Tzu and Lhasa Apso were more commonly affected with pigmentary keratitis and the Chinese Pugs were the most commonly reported breed of dog (Anoop *et al.*, 2016; Esson, 2015) [5,11]. The rate of incidence of superficial corneal pigment was higher in pugs compared to other brachycephalic breeds and the condition is affectionately termed as 'Pugmentary Keratitis' to mark the higher rate of incidence in pugs (Appelboom, 2016; Vallone *et al.*, 2017) [6,16].

Corneal pigmentation can be categorized as focal non-progressive and diffuse progressive forms and the former which occurs due to long term mechanical irritation is the most commonly observed form in brachycephalic breeds. Medical management of pigmentary keratitis mainly aims at curtailing the progression of the pigment by addressing the underlying causes or disorders such as tear film disorders and corneal inflammation by the use of topical corticosteroids, tear film replacements/stabilizers and lacrimostimulants (Esson, 2015) ^[11].

Medical management of pigmentary keratitis helps slow down the progression of pigmentary keratitis but to cease the further progression of pigmentation surgical intervention is required to eliminate the inciting causes of corneal irritation and inflammation such as medial canthi trichiasis, distichiasis, trichiasis and entropion. Surgical interventions such as superficial keratectomy and medial canthoplasty are usually performed to address the cases of chronic superficial keratitis with heavily pigmented cornea, alleviate the corneal irritation from medial entropion of lower eyelids, reduce palpebral fissure length and scleral show (Allgoewer & Sahr, 2014; Bettenay *et al.*, 2018; Wilkie & Whittaker, 1997) ^[3,9,17]. The higher water content of the melanocytes renders them highly cold sensitive compared to the other corneal cells and this differential cold sensitivity can be utilized for the selective destruction of the melanocytes by the use of soft cryogenic agents while causing less damage to other corneal cells (Azoulay, 2014) ^[7].

Considering the potential of soft cryotherapy in selective destruction of the melanocytes that contribute to the deposition of pigments in the corneal epithelium, this study was conducted to evaluate the efficacy of cryotherapy performed with medial canthoplasty in curtailing the progression of corneal pigmentation and thereby contributing to better vision and quality of life of the animals.

2. Materials and Methods

2.1. Study Population & Method

The study was conducted on six Pug breed of dogs (n=6 eyes) presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana with the presenting signs of deteriorating vision and difficulty in maneuverability. A detailed signalment and anamnesis were recorded in each case. All the animals underwent a detailed ophthalmic examination comprising gross ophthalmic examination, behavioral assessment of the vision, neuro ophthalmic tests or reflexes and special diagnostic procedures such as Schirmer's tear test (STT), fluorescein dye test, rose bengal dye test, tonometry, slit lamp biomicroscopy, ophthalmoscopy (Direct and Indirect) and ultrasonography, performed in a step-by-step approach to establish a diagnosis as described by Maggs, (2018) ^[13].

Routine preoperative blood work up was performed to assess the physiological status and suitability of animals for anesthesia and surgical intervention by collecting whole blood samples. Based on the age, the animals were categorized into young (0 to 3 years), adult (3 to 7 years) and senile (above 7 years) groups. The severity and pattern of pigmentation were assessed using the methods suggested by Maini *et al.*, (2019) ^[14] and Charbiwala, (2019) ^[10], respectively by photographing the eyes after rinsing them with sterile 0.9% normal saline solution.

2.2. Preoperative medicinal therapy

Preoperative medical management included topical ocular administration of lacrimomimetics (0.5% Carboxy methyl cellulose sodium), antibiotics (0.3% Gatifloxacin) and lacrimostimulants (0.1% Cyclosporine) till the scheduled date of surgery. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), systemic antibiotics and other supplements were included in the preoperative medications on case to case basis depending upon the results of the preoperative blood screening. To ensure ocular asepsis, 0.3% Gatifloxacin was administered on both eyes at an interval of 15 to 20 minutes starting from an hour prior to the commencement of the surgery.

2.3. Anaesthesia

All the animals were premedicated with Atropine @ 0.02 - 0.04 mg/kg body weight and Butorphanol @ 0.2 mg/kg body weight by intramuscular route. Acepromazine maleate @ 0.02 - 0.05 mg/kg body weight was included on case to case basis. Systemic antibiotics (Cefotaxime @ 25 mg/kg body weight) and NSAIDs (Meloxicam @ 0.2 mg/kg body weight) were also administered preoperatively by intravenous route. Induction of anaesthesia was performed by slow intravenous administration of Propofol @ 4 mg/kg body weight and Diazepam @ 0.5 mg/kg body weight and the surgical plane of anaesthesia was maintained using Isoflurane.

2.4. Patient positioning and preparation

On lateral recumbency, the head of the animals were conveniently positioned for the surgeon using padding materials under the head with the affected eye on the dorsal side. The periorbital area and eyelids were trimmed of the hairs and a solution of 5% povidone iodine was painted on the surgical site. The dorsal and ventral conjunctival fornix were flushed before the commencement of surgery using a solution of 0.5% povidone iodine.

2.5. Operating Procedure

Under general anaesthesia, the ventro-medially rotated eyeballs were retracted from the bony orbit and secured in position using traction sutures with 3-0 vicryl and a Lieberman speculum. Cryotherapy was performed on the eyes with the help of a commercially available kit (Compound W Freeze Off® Wart Removal Kit) using a single-use precision foam applicator tip (Fig. 1A & 1B). The aerosol canister contains a cryogenic mixture of dimethyl ether and propane and the cryogen is loaded onto the applicator tip by inserting the tip into the canister for 2 to 3 seconds (Fig. 1C).

The surgeon holds the tip for 15 seconds before applying it on the cornea, allowing the cryogen to evaporate and the temperature to drop to approximately -55 °C (Fig. 1D). The cryogen loaded tip was applied on the most pigmented area of the cornea and then gently rolled over the corneal surface, progressively covering the entire area of pigmentation (Fig. 1E & 1F). The cryotherapy procedure was performed in two freeze-thaw cycles [50 seconds of cryogen application followed by 120 seconds of thawing] (Fig. 1G). In cases of diffuse corneal pigmentation, the cryotherapy procedure was performed by dividing the corneal surface into two halves and performing two freeze-thaw cycles in each half separately.

All the animals underwent medial canthoplasty concurrently with cryotherapy. The medial canthus was rolled out in the direction of the nostrum to reveal the medial caruncle and the upper and lower nasolacrimal puncta were identified and cannulated with the help of a nasolacrimal cannula to prevent any inadvertent damage to the puncta and the ducts during surgery.

A triangular skin incision was made around the medial canthus to delineate the portion of medial caruncle and eyelid margins to be removed. A tenotomy scissor was used for the dissection and excision of the triangular tissue section taking care not to damage the puncta and ducts. Any remaining hair or its follicle was excised delicately and the surgical site was closed in two layers.

A simple continuous suture pattern was followed for the deep conjunctival layer using 6-0 vicryl with knots buried to minimize corneal irritation. The skin of the medial canthus was closed by performing a figure-of-eight suture pattern and the remaining skin section was closed with simple interrupted sutures using 5-0 Nylon, incorporating the tags of the figure-of-eight suture into the interrupted sutures to prevent corneal irritation.

2.6. Postoperative care

Postoperative care included topical ocular administration of lacrimomimetics (0.5% Carboxy methyl cellulose sodium, q1h for 5 days followed by q.i.d till 30th postoperative day), lacrimostimulant (0.1% Cyclosporine, q.i.d till 30th postoperative day) and antibiotic eye ointment (0.3% Tobramycin, b.i.d till 30th postoperative day). Topical corticosteroids (1% Prednisolone acetate) was included in the postoperative care after the 5th postoperative day when the fluorescein dye test results turned negative. Intramuscular administration of systemic antibiotics (Cefotaxime @ 25 mg/kg body weight, b.i.d. for 5 days) and NSAIDs (Meloxicam @ 0.2 mg/kg body weight, o.d. for 3 days) was also part of the postoperative care. The clinical outcomes were assessed in all the animals at regular intervals wherever possible with respect to disappearance of pigmentation, restoration of vision and complications. A positive menace response postoperatively was considered as a sign of restoration of vision provided that the corneal lesions did not affect the visual axis pathway of the animal. The data were analyzed using appropriate statistical methods wherever applicable.

3. Results

Mean age of the study population was 6.33 ± 0.93 years (4.5 to 10 years) and all the animals were male. Out of the six animals, four pugs (66.67%) were adults and two pugs (33.33%) were of senile category. Concurrent clinical findings observed in the animals in addition to various clinical signs such as conjunctivitis, episcleritis, corneal edema, corneal and conjunctival pigmentation, neovascularization and corneal fibrosis include Medial Caruncular Trichiasis (MCT) and Medial Entropion of Lower Eyelid (MELE) in all six animals (100%) and Keratoconjunctivitis sicca (KCS) in three animals (50%).

The mean pigmentation grading score of the study population was 10.17 ± 1.76 . Out of the six animals, moderate degree of pigmentation was observed in three animals (50%) and severe degree of pigmentation was observed in the remaining three animals (50%) (Fig. 2A & 3A). The pattern of pigmentation was patchy in three

animals (50%), diffuse in two animals (33.33%) and paintbrush in one animal (16.66%).

In the neuro ophthalmic reflexes, palpebral reflex was positive in all six animals, pupillary light reflex was positive in three animals and not appreciable in the remaining three animals due to the corneal pigmentation and menace response was positive in two animals and negative in four animals, respectively. The mean STT value of the animals was 12.67 ± 4.02 mm/min and the mean intraocular pressure (IOP) was 19.33 ± 2.09 mm Hg. Fluorescein dye test was negative in all six animals and rose bengal dye test was positive in four animals and negative in two animals. On ultrasonography, findings indicative of cataract in lens was observed in two animals and no other ultrasonographic structural abnormalities were observed (Table 1).

Cryotherapy was performed in all the six eyes with ease and no intraoperative complications were observed. Clinical outcomes observed on the 5th postoperative day differed based on the degree of pigmentation of the eyes. In the eyes which had a moderate degree of pigmentation, disappearance of the pigmentation was observed on the 5th postoperative day while persistence of pigmentation to an extent was observed in the eyes which had a severe degree of pigmentation (Fig. 2B & 3B). Stromal fibrosis in two eyes and corneal ulcer in one of the eyes (completely healed on 45th postoperative day) were observed with a positive fluorescein dye test in addition to other complications such as corneal opacity and neovascularization on the 5th postoperative day.

On the 15th postoperative day, neovascularization and corneal opacity to a mild extent and disappearance of corneal fibrosis were observed. Recurrence of pigmentation to a mild extent was observed in 2 eyes while progression of pigmentation was observed in 2 eyes that exhibited persistent pigmentation postoperatively (Fig. 2C & 3C). A positive menace response was observed in 2 out of the 4 eyes which had a negative menace response preoperatively. The menace response was negative in the other 2 eyes due to the prevailing cataract changes as suggested by the preoperative ultrasonographic findings (Table 2). A satisfactory level of healing was observed at the medial canthoplasty surgical site, with an average reduction of 0.5 cm in the unstretched palpebral fissure length.

On the 30th postoperative day, a lustrous cornea with a visible tapetal reflex was observed, though neovascularization and corneal opacity to a mild extent were still present. However, recurrence of pigmentation was observed in all six eyes with the progression of the pigmentation into the axial cornea in some eyes and intensification of pigmentation in the remaining eyes (Fig. 2D & 3D). On the 60th postoperative day, an increased corneal transparency with improved vision was observed, along with an increase in owner-reported activity levels of the animals (Fig. 2E & 2F). Though the progression of pigmentation was observed in these eyes, the rate of progression was observed to be slower with a mean pigmentation score of 6.58 ± 1.44 after 30th postoperative day.

4. Discussion

The majority of the animals included in this study were adults suggesting limited owner awareness of ocular pigmentation and delayed presentation of the animals at advanced stages of corneal pigmentation. All the animals in

the study population were males, indicating a higher prevalence of corneal pigmentation in males, which aligns the observations of Krecny *et al.*, (2015) ^[12] but contrasts with the observations reported by Azoulay, (2014) ^[7], Allgoewer & Hoecht, (2010) ^[2] and Petersen-Jones *et al.*, (2007) ^[15]. The entire study population exhibited MCT and MELE and half the study population exhibited KCS concurrently with pigmentation of cornea, consistent with the observations reported by Maini *et al.*, (2019) ^[14] and Krecny *et al.*, (2015) ^[12]. The mean STT value of the study

population was below the normal range differing from the observations of Maini *et al.*, (2019) ^[14]. The mean IOP of the study population was within the normal range, which aligns with the observations reported by Vallone *et al.*, (2017) ^[16] but contrasts with the findings of Anoop *et al.*, (2016) ^[5]. The postoperative findings observed in this study such as corneal opacity, corneal inflammatory changes, superficial corneal ulcers and recurrence of pigmentation after the 30th postoperative day were consistent with the findings reported by Azoulay, (2014) ^[7].

Table 1: Summary of the preoperative observations in animals

Case no	Age (years)	Sex	Eye	Etiology	Ocular Discharge	Extent of pigmentation	Pattern of pigmentation	Severity of pigmentation	Pigmentation Score	Duration of pigmentation	Menace response	PLR	Palpebral reflex	USG findings
A	4.5	Male	Left	MELE MCT	Serous	Medial	Paintbrush	Moderate	6	3 months	+ ve	+ ve	+ ve	NAD
B	5.5	Male	Left	MELE MCT	Serous	Medial	Patchy	Moderate	5	1 month	- ve	+ ve	+ ve	Cataract
C	10	Male	Left	MELE MCT KCS	Mucoid	Medial	Patchy	Severe	14	4 months	+ ve	NA	+ ve	NAD
D	6	Male	Right	MELE MCT KCS	Mucopurulent	Complete	Diffuse	Severe	14	4 months	- ve	NA	+ ve	NAD
E	8	Male	Right	MELE MCT KCS	Mucopurulent	Complete	Diffuse	Severe	14	5 months	- ve	NA	+ ve	NAD
F	4	Male	Right	MELE MCT	Serous	Medial	Patchy	Moderate	8	1 month	- ve	+ ve	+ ve	Cataract

Table 2: Summary of the postoperative observations in animals

Case no	Postoperative (Day 5)	Postoperative (Day 15)	Postoperative (Day 30)	Postoperative (Day 60)
A	Disappearance of pigmentation, corneal opacity, corneal ulcer, fluorescein (+ve), Menace (+ve)	Corneal opacity, corneal ulcer, neovascularization, faint re-pigmentation, fluorescein (+ve), Menace (+ve)	Corneal opacity, neovascularization, progressive pigmentation, fluorescein (+ve), Menace (+ve)	Progressive pigmentation, Menace (+ve)
B	Disappearance of pigmentation, corneal opacity, neovascularization, fluorescein (-ve)	Mild opacity, neovascularization	Mild opacity, neovascularization, re-pigmentation, Menace (-ve) due to cataract	-
C	Persistence of pigmentation, mild opacity and fibrosis, neovascularization, fluorescein (faint +ve), Menace (+ve)	-	Mild opacity, neovascularization, cornea lustrous, progressive pigmentation, fluorescein (-ve), Menace (+ve)	-
D	Persistence of pigmentation, mild corneal fibrosis, neovascularization, fluorescein (+ve), Menace (Mild +ve)	Persistence & progression of the pigmentation, Menace (+ve)	Mild opacity, neovascularization, cornea lustrous, tapetal reflection observed, progressive pigmentation, Menace (+ve)	Improved corneal transparency, progressive pigmentation, Menace (+ve)
E	Persistence of pigmentation, mild opacity and corneal fibrosis, neovascularization, fluorescein (+ve), Menace (Mild +ve),	Persistence & progression of the pigmentation, fluorescein (faint +ve), Menace (+ve)	Mild opacity, neovascularization, cornea lustrous, progressive pigmentation, fluorescein (-ve), Menace (+ve)	-
F	Disappearance of pigmentation, corneal opacity, neovascularization, fluorescein (-ve)	Mild opacity, neovascularization, re-pigmentation	Mild opacity, neovascularization, Progressive pigmentation, Menace (-ve) due to cataract	-

Plate 1: Cryotherapy Procedure

Fig. 1A: Applicator tip loaded into the handle **Fig. 1B:** Inserting the tip loaded handle into the canister



Fig. 1C: Press and hold the handle inside the canister for 3 seconds **Fig. 1D:** Handle removed from the canister & thawed for 15 seconds before application on the cornea



Fig 1E: Applying the cryogen loaded tip on the cornea for 50 seconds **Fig 1F:** The cryogen loaded tip is rolled slowly over the cornea



Fig. 1G: Thawing the cornea for 2 minutes before second freeze cycle

Plate 2: Preoperative and Postoperative eye images of Case D



Fig 2A: Pre-operative



Fig 2B: Post-operative - Day 5



Fig 2C: Post-operative - Day 15



Fig 2D: Post-operative - Day 30

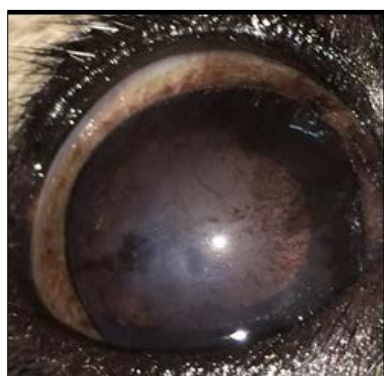


Fig 2E: Post-operative - Day 45

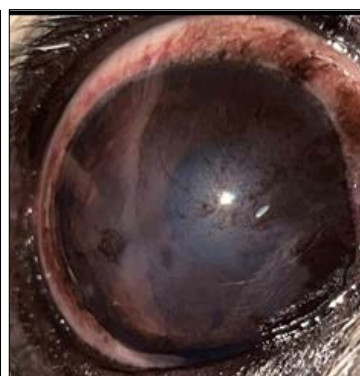
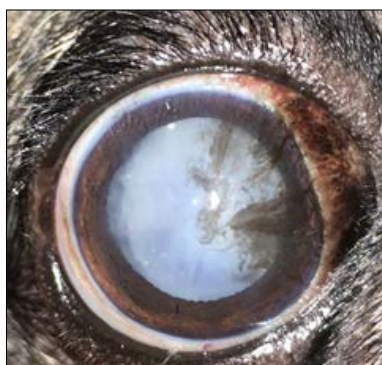
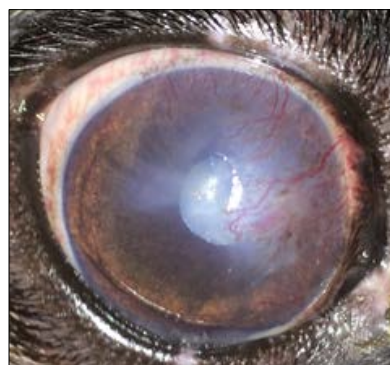
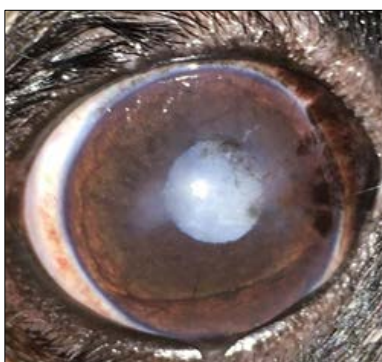
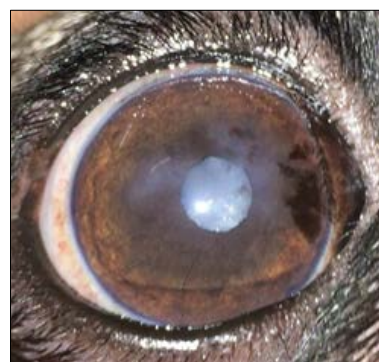


Fig 2F: Post-operative - Day 60

PLATE 3: Preoperative and Postoperative eye images of Case F**Fig 3A: Pre-operative****Fig 3B: Post-operative - Day 5****Fig 3C: Post-operative - Day 15****Fig 3D: Post-operative - Day 30****5. Conclusion**

Though the clinical outcomes of cryotherapy with medial canthoplasty in the management of pigmentary keratitis in pugs were limited by the persistent pigmentation and recurrence of pigmentation observed after 30 days of surgery, the earlier onset of favorable postoperative outcomes such as improved vision, increased activity levels of the animals along with a less severe postoperative inflammatory changes, lower mean postoperative pigmentation score and slower rate of postoperative pigment progression warrants the use of cryotherapy for effective management of pigmentary keratitis in pugs.

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7. Competing interests

The authors have no conflict of interest/ competing interests.

8. Authors' Contributions

Rajasekaran Tiruppur Manikumar & Shashi Kant Mahajan designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Nameirakpam Umeshwori Devi managed the analyses of the study. Jasmeet Singh Khosa & Arun Anand managed the literature searches. All authors read and approved the final manuscript.

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