CLINICAL STUDIES ON SURGICAL MANAGEMENT OF PIGMENTARY KERATITIS IN PUGS

Thesis

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(Minor Subject: Veterinary Anatomy)

By

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CERTIFICATE I

This is to certify that the thesis entitled, **"Clinical studies on surgical management of pigmentary keratitis in Pugs"** submitted for the degree of **M.V.Sc.,** in the subject of **Veterinary Surgery and Radiology** (Minor subject: **Veterinary Anatomy)** of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **T. M. Rajasekaran,** Registration No. **L-2019-V-104-M** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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ABSTRACT

The present study was conducted in two parts, viz. screening study and surgical study. The screening study was conducted on 200 eyes of hundred Pug breed of dogs presented for ailments other than ocular affections to study the prevalence of pigmentary keratitis (PK). Digital photographs of the eyes were used to study and score the corneal pigmentation (CP) and classify them into various patterns. CP was detected in a total of 96 Pugs (192 eyes) in the study population with a mean age of 5.27 ± 0.29 years. The severity of pigmentation was mild for 29 eyes (15.10%), moderate for 76 eyes (39.58%) and severe for 87 eyes (45.31%) with their mean pigmentation score being 2.98±0.21, 6.89±0.18 and 13.35±0.16 respectively. Detection of PK was not significantly associated with the sex, coat colour or the side of eye but was significantly associated with the age of the animal. The severity of CP was not significantly associated with the coat colour or the side of eye but was significantly associated with the age and sex of the animal. The surgical study was conducted on 12 eyes of 12 Pug breed of dogs presented primarily for ocular disorder with the history of poor vision and affected with PK. The animals were randomly divided into two groups based on the surgical technique adopted. The animals of the group I (n=6) underwent Superficial keratectomy (SK) along with Medial canthoplasty (MC) and the animals of group II (n=6) underwent Cryotherapy along with MC. The animals were followed postoperatively to study the outcome of the surgical protocol and their associated complications. The animals of group II exhibited a better visual and tectonic outcome with less severe complications compared to the animals of group I. In conclusion, Cryotherapy performed along with MC was a better technique compared to SK performed along with MC in effective surgical management of PK in pugs.

Keywords: Pigmentary keratitis, Corneal melanosis, Superficial keratectomy, Cryotherapy, Medial canthoplasty, Pugs.

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Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS

-	:	Dash
+ ve	:	Positive
- ve	:	Negative
%	:	Percentage
@	:	At the rate
+	:	Plus, the sign of addition
x	:	The sign of multiplication
<	:	Less than
=	:	Equal, the sign of equality
>	:	Greater than
±	:	Plus-minus
≤	:	Less-than or equal to
α	:	Alpha
β	:	Beta
$\chi 2$	:	Chi square test
am	:	Ante meridiem
ALT	:	Alanine Transaminase
b.i.d.	:	Twice in a day
BUN	:	Blood Urea Nitrogen
℃	:	Celsius
CCT	:	Central Corneal Thickness
Cm	:	Centimeter
CM	:	Corneal Melanosis
CP	:	Corneal Pigmentation
Cryo	:	Cryotherapy
CsA	:	Cyclosporine A
dL	:	Decilitre
DLC	:	Differential Leukocyte count

EDTA	:	Ethylene diamine tetra acetic acid
ESMC	:	Episcleral silicone matrix cyclosporine
Et al	:	et alia
ECM	:	Extracellular matrix
F	:	Female
Fig	:	Figure
g	:	Gram
GAG	:	Glycosaminoglycan
Hb	:	Hemoglobin
IVCM	:	*in vivo* confocal microscopy
IOP	:	Intraocular pressure
KCS	:	Keratoconjunctivitis sicca
Kg	:	Kilogram
L	:	Litre
LI	:	Labeling index
M	:	Male
m/s	:	Metre per second
MC	:	Medial canthoplasty
MCT	:	Medial caruncular trichiasis
MELE	:	Medial entropion of lower eyelids
mg	:	Milligram
MHZ	:	Mega Hertz
min	:	Minute
ml	:	Millilitre
mm Hg	:	Millimetres of Mercury
mm	:	Millimetre
NA	:	Not appreciable due to corneal pigmentation
NAD	:	No abnormalities detected
NCP	:	Nasal canthoplasty

NSAID	:	Nonsteroidal anti-inflammatory drug
o.d.	:	Once a day
pm	:	Post meridiem
PAS	:	Periodic acid-Schiff
PCV	:	Packed cell volume
PK	:	Pigmentary keratitis
PLR	:	Pupillary light reflex
RBC	:	Red Blood cells
RD	:	Retinal detachment
STT	:	Schirmer’s tear test
SCP	:	Superficial corneal pigment
SE	:	Standard error
SENCAR	:	Sensitivity to carcinogenesis
SK	:	Superficial keratectomy
SD-OCT	:	Spectral-domain optical coherence tomography
t.i.d.	:	Three times in a day
TEC	:	Total erythrocyte count
TLC	:	Total leukocyte count
TT	:	Topical therapy
µL	:	Microlitre
µm	:	Micrometre
µm/h	:	Micrometre per hour
U/L	:	Units per litre
USG	:	Ultrasonography
Yr	:	Year

CHAPTER I

INTRODUCTION

Current trends in the ocular disorders are more breed related owing mainly to the artificial selection and inbreeding of the pure breed of dogs (Rooney & Sargan, 2009). Ocular disorders affecting the dogs may average to 6.6 percent different eye disorders for each breed, but there are almost 18 or more disorders that may affect the brachycephalic breeds (Whitley et al., 1995). Various reasons reported for the higher incidence of ocular affections among the brachycephalic breeds are shallow orbit, prominent globe, macropalpebral fissure and lower craniofacial ratio with an absence of muzzle all contributing towards an increased scleral show compared to the other breed of dogs.

Corneal affections account for the majority of the ocular disorders as they act as a window for any traumatic injury to the eye. Amol (2016) stated that pigmentary keratitis accounted to about 61.72 percent of the total corneal affections in brachycephalic breeds. Brachycephalic breeds are more prone to corneal dry spot formation and exposure keratopathy as they exhibit lagophthalmos while sleeping (Maggs, 2018b).

Pigmentary keratitis also termed as corneal melanosis or corneal pigmentation is one of the most common ocular disorder reported in pugs that is characterised by progressive deposition of melanocytes and melanin pigment usually starting from the nasal quadrant of the cornea. Appelboam (2016) stated that the condition is affectionately termed as ‘pugmentary keratitis’ making reference to the higher incidence of this condition in pugs.

Pigmentary keratitis is not a ‘clinical diagnosis’ but a mere clinical sign to the underlying chronic inflammatory process (Maggs, 2018b). Migration of the melanocytes into the superficial corneal epithelium or more deeply into the corneal stroma occurs as a response to the chronic corneal irritation and inflammation incited by various ocular affections such as nasal fold trichiasis, medial caruncular trichiasis, distichiasis, ectopic cilia and insufficient tear production (either quantitative or qualitative) and entropion of the eyelids at the nasal canthus (Westermeyer et al., 2009).

Development of the corneal pigmentation may occur following corneal trauma as a part of the wound-healing response (Kaswan et al., 1989). Corneal vascularisation and fibrosis usually precedes and accompanies the corneal epithelial melanosis. The pigmentation of cornea is also reported frequently as a feature of various inflammatory corneal affections, such as chronic superficial keratitis (pannus), keratoconjunctivitis sicca (KCS) and chronic ulcerative/nonulcerative keratitis (Maini et al., 2019). A relatively higher incidence of cases of pigmentary keratitis without keratoconjunctivitis sicca warrants the presence of some other yet to be determined factors involved in the development of corneal melanosis in pugs (Krecny et al., 2015).

Corneal pigmentation can be categorized into focal non-progressive and diffusive progressive forms. The focal form which is usually caused by long term mechanical irritation is commonly seen in the brachycephalic breeds. Treatment of the corneal pigmentation is aimed at curtailing the progression of the pigmentation which is usually accomplished by addressing the root causes or disorders such as corneal inflammation, tear film disorders by the use of topical corticosteroids, tacrolimus, cyclosporine and/or tear film replacements/stabilizers (Esson, 2015).

Corneal pigmentation may progress leading to the loss of vision when there is a delay or insufficiency in the medical management. Though medical management has its own benefit in the treatment of pigmentary keratitis, the root causes of corneal irritation and inflammation such as entropion, distichiasis and trichiasis may require surgical correction in order to prevent the pigmentation from further progression. In cases of severe chronic superficial keratitis, the heavily pigmented cornea may be excised by performing superficial keratectomy (Wilkie & Whittaker, 1997).

Medial canthoplasty is usually performed as a corrective measure to address the brachycephalic ocular syndrome and medial entropion of lower eyelids in brachycephalic dogs (Bettenay et al., 2018). Medial canthoplasty when performed in cases of pigmentary keratitis can reduce the corneal irritation from entropion or trichiasis, reduce the palpebral fissure length and scleral show allowing for an improved blink response in the pugs thereby preventing the progression of corneal pigmentation (Allgoewer & Sahr, 2014).

The routine cryosurgery used in veterinary ophthalmology to treat conditions such as ectopic cilia and distichiasis was suggested as an alternate technique to address the corneal melanosis (Merideth & Gelatt, 1980). Cryotherapy for the treatment of corneal pigmentation is warranted due to the sensitivity of melanocytes to cold (Featherstone et al., 2009). The cold sensitivity of the melanocytes is heightened when compared to the other corneal cells due to their high water content. This cold sensitivity can be utilised for the selective destruction of melanocytes with comparatively less damage to the other corneal cells by the use of soft cryogenic agents (Azoulay, 2014).

Keeping in view the high incidence and clinical importance of corneal pigmentation and limited work at national level, there is need for further studies for their successful management. Therefore, the present study has been planned with following objectives:

1. To study the hospital occurrence of Pigmentary Keratitis in Pugs.
2. To evaluate and compare the efficacy of cryotherapy with medial canthoplasty and superficial keratectomy with medial canthoplasty for management of Pigmentary Keratitis in Pugs.

CHAPTER II

REVIEW OF LITERATURE

A comprehensive review pertinent to the surgical management of pigmentary keratitis is presented under the following headings and sub headings.

2.1 Corneal Anatomy and Physiology

2.2 Incidence of corneal affections

2.3 Corneal wound healing

2.4 Pigmentary keratopathy

2.5 Clinical diagnosis and evaluation of Pigmentary keratitis in dogs

2.6 Grading and scoring of corneal pigmentation

2.7 Therapeutic and surgical management of pigmentary keratitis

2.1 Corneal Anatomy and Physiology

Startup (1984) studied the anatomy of cornea and stated that the anterior one-sixth of the globe was occupied by the cornea and its radius of curvature was slightly greater than the rest of the globe. The transparent nature of the cornea which allows it to transmit light freely was dependant on its uniform structure, avascularity and its state of deturgescence.

Robert et al. (2001) reported in a study that cornea was one of the most highly differentiated connective tissue and has three types of cell-constituents, namely, epithelial cells, stromal keratocytes and endothelial cells. All these cells are engaged in the biosynthesis of a rich extracellular matrix (ECM) especially rich in collagen. The precise regulation of the diameter and orientation of fibres with remarkably uniform interfibrillar space contributes to the unique physical property of transparency of cornea.

Abrams et al. (2002) characterized the surface topographical features of epithelial and endothelial (Descemet's) basement membrane of the canine cornea in a study using electron microscopy and concluded that both the basement membranes were found to be made up of an intricate meshwork of pores and fibres. The

topographical features of the endothelial basement membrane were finer, less porous and relatively smaller compared to the epithelial basement membrane.

Maggs (2018b) stated that the normal cornea was devoid of blood vessels, but various stimuli, such as the stimulated lymphocytes or their elaborated lymphokines, may induce vascularization of the cornea.

Alario & Pirie (2014) compared the central corneal thickness (CCT) measurements obtained from normal canine corneas using an ultrasonic pachymeter and a spectral-domain optical coherence tomography device (SD-OCT). The mean CCT value for all eyes examined via ultrasonic pachymetry (velocity set at 1636 m/s) and SD-OCT was 598.54±32.28 μm and 587.72±32.44 μm, respectively.

Nautscher et al. (2016) compared the morphological features of cornea of different domestic animals using histological and immuno-histochemical methods and reported that corneas were composed only of four layers and that Bowman's layer was not detected using the PAS reaction. The study reported that corneal epithelium is composed of three layers specific to each species: superficial non-keratinizing stratified squamous cells, intermediate wing cells and basal cells. The study revealed that dogs and cats had the thinnest corneal epithelium among the domestic species and suggested the presence of differences in the maintenance of structural integrity and fluid balance.

2.2 Incidence of corneal affections

Akinrinmade & Ogungbenro (2015) conducted a retrospective study of ocular affections in dogs in Southwest Nigeria for a period of about 10 years (2003-13) and recorded that out of a total number of 3488 cases surveyed, a total of 231 cases were presented with ocular affections. The reported incidence of ocular affections in dogs was 6.62 percent and clinical categorization based on the anatomical location of ocular lesions revealed highest incidence was of eyelid and/or conjunctiva (58.01%) followed by the lens/globe (22.51%) and cornea (19.48%).

Amol (2016) studied the incidence of ocular affections in brachycephalic dogs belonging to eight different breeds and reported that out of 95 dogs (161 eyes), corneal affections (88%) constituted the major portion of ocular disorders reported in the study

and among the corneal affections, pigmentary keratopathy alone constituted 61.27 percent followed by corneal ulcers (26.05%).

2.3 Corneal wound healing

Startup (1984) studied the corneal physiology and reported that its avascular nature will reduce the surface temperature by 0.5 to 1 ℃ and in case of the brachycephalic breeds with large corneal surfaces, the lowered temperature and sensitivity may render the cornea with less resistance to infection and ulceration.

Whitley (2000) stated that corneal epithelium acts as a barrier to the microorganisms and the disruption of this layer generally precedes bacterial invasion. Corneal ulceration was reported as one of the most common ocular diseases in dogs. Uncomplicated superficial ulcers healed readily in a few days, with minimal scar formation, while the complicated and refractory ulcers took weeks or months to heal and impaired vision because of corneal scarring or fibrosis.

Miller (2001) reported that corneal epithelium has a crucial role in the wound healing and permanent adhesion of the epithelium to the underlying stroma completes the wound healing process. The adhesion process is completed within a week if the basement membrane is not disturbed at the time of injury and an injury to the basement membrane may delay the process of basement membrane regeneration and epithelial adhesion for as long as 6 weeks.

Robert et al. (2001) reported in the study that biosynthesis of collagen changes with age and in several pathological conditions such as corneal dystrophies and the refibrillation of wounded corneas does not follow the normal program of vectorial collagen synthesis and GAG-collagen interactions.

Bentley & Murphy (2004) noted that corneal wound healing was a complex process that involves the interplay between cellular components of the cornea, constituents of the extracellular matrix (ECM), a host of cytoactive factors, chemical characteristics of the extracellular milieu (pH, osmolarity) and biomechanical forces (eyelid movements). Corneal wound healing involves an initial lag phase, where no appreciable cell migration occurs, followed by a centripetal migration of the epithelial cells in a radial fashion at a constant rate of approximately 20-50 µm/h.

Netto et al. (2005) reviewed the wound healing response to various corneal surgical procedures and reported that the main tissues and organs which played a major role in the healing response were corneal epithelium, stroma, nerves, inflammatory cells and lacrimal glands. Cellular interactions such as keratocyte necrosis, keratocyte apoptosis, keratocyte proliferation, myofibroblast generation and migration of inflammatory cells were involved in extracellular matrix reorganization, stromal remodelling, wound contraction and other various other responses to surgical injury.

Carter (2009) reported that epithelial injury is followed rapidly by the production of fibrin and fibronectin by the basal epithelial cells and stromal keratocytes. The fibrin and fibronectin acts as a temporary scaffold or provisional matrix allowing for the epithelial cell migration and attachment across the wound surface. Integrin $\alpha_5\beta_1$ was found to be the major receptor for fibronectin facilitating attachment of the epithelial cells to the provisional matrix.

Ledbetter & Gilger (2013) observed that functional decompensation of the corneal endothelium and bullous keratopathy can result from various congenital and acquired ocular disorders including breed related endothelial dystrophy, trauma, uveitis, iris-to-cornea persistent membranes, glaucoma, endothelitis, toxic changes to endothelium and melting keratitis.

2.4 Pigmentary keratopathy

Maggs (2018b) describes pigmentary keratitis as a common ocular disorder involving the progressive deposition of pigment in the corneal epithelium and associated conjunctival surface. Corneal melanosis was described as a response to the chronic corneal and conjunctival irritation.

The centripetal migration of the melanocytic cells from the limbal and perilimbal tissues into the corneal epithelium or more deeply into the corneal stroma is usually accompanied by other signs of active keratitis such as corneal vascularization, stromal inflammatory cell infiltration and granulation tissue formation. Melanocytic pigment was seen deposited in the basal epithelial cells of the corneal epithelium and in the anterior stromal tissue (Bellhorn & Henkind, 1966).

Migration of pigment into the superficial cornea occurs after irritation or chronic inflammation of the cornea from a variety of causes, including medial lower entropion, distichiasis, medial caruncular trichiasis, ectopic cilia, nasal fold trichiasis and insufficient tear production (Westermeyer et al., 2009).

2.4.1 Incidence of Pigmentary keratitis

2.4.1.1 Age wise distribution

Labelle et al. (2013) concluded in the study that detection of corneal pigmentation had no significant association with the age, coat color, eyelid conformation or tear film characteristics of the dogs and though the severity of corneal pigmentation was not significantly associated with the age, it was significantly associated with the coat color and tear film characteristics.

Ledbetter & Gilger (2013) described the age of onset of corneal pigmentation as an important prognostic indicator of the ocular disorder. Young dogs in the age group of 1-5 years typically exhibit rapid and progressive pigmentation of the cornea while the animals affected later in the life (i.e. > 4-5 years of age) exhibit less severe lesions.

Anoop et al. (2016) studied the incidence of pigmentary keratitis in dogs and reported that the mean age of the animals affected was 33.13±3.12 months. The mean duration of the disease as noticed by the owner was 07.21±0.65 months which indicated that most of the owners were totally unaware about the condition of the eye.

2.4.1.2 Breed wise distribution

Bedford & Longstaffe (1979) described pigmentary keratitis/pannus as a breed specific disorder in German shepherd dogs and characterized it as a sub-epithelial invasion of the cornea by fibrovascular tissue accompanied by lymphoid cells and melanocytes.

Allgoewer & Hoecht (2010) reported a higher incidence of chronic superficial keratitis and subsequent pigmentation in German shepherds and Shepherd crosses.

Ledbetter & Gilger (2013) described a breed predilection for pannus/chronic immune-mediated superficial keratoconjunctivitis in German shepherds, Greyhounds, and Belgian Tervuren or Malinois.

Azoulay (2014) reported chronic superficial keratitis as an immune-mediated keratitis diagnosed commonly in German shepherds, greyhounds, Belgian shepherds and more rarely in beaucerons, collies, poodles and Siberian huskies.

Esson (2015) described that brachycephalic breeds such as Pugs, Pekingese, Shih Tzu and Lhasa Apso were commonly affected with pigmentary keratitis.

Amol (2016) studied the incidence of ocular affections in brachycephalic dogs belonging to eight different breeds and reported that out of 95 dogs (161 eyes), breed-wise incidence was maximum in case of pugs i.e. 83 dogs (142 eyes, 88%) followed by three cases in Shih Tzu (6 eyes, 3%), three in Lhasa Apso (5 eyes, 3%), two in boxers (2 eyes, 2%), one case in French bull dog (1 eye, 1%) and one in English bull dog (2 eyes, 1%).

Anoop et al. (2016) performed a study on 55 dogs of different breeds, from which 83 corneas were studied to evaluate the incidence of pigmentary keratitis in dogs. Out of the 55 animals studied, 51 were Chinese Pugs (92.7%), 2 were Lhasa Apso (4%) and 1 each from Cocker Spaniel (2%) and Bull Mastiff (2%).

Vallone et al. (2017) used *in vivo* confocal microscopy (IVCM) to characterize the canine superficial corneal pigment in a study on 57 brachycephalic breed of dogs. Among the 57 dogs included in the study, 32 were Pugs and 25 were non-Pug breeds. The presence of SCP was observed in 23 pugs (71.8%) and 10 non-pugs (40%).

2.4.1.3 Sex wise distribution

Labelle et al. (2013) reported that detection of corneal pigmentation and its severity had significant association with the sex of the dogs. The occurrence of moderate to severe corneal pigmentation was significantly higher in number in case of males compared to the females. The spayed female pugs were 3.69 times less likely to be affected with corneal pigmentation when compared to the intact female pugs.

Anoop et al. (2016) reported a higher incidence of pigmentary keratitis in females compared to the males.

2.4.1.4 Incidence between right and left eye

Labelle et al. (2013) performed a study to determine the prevalence of corneal pigmentation in pugs and reported that corneal pigmentation was detected in at least 1 eye of 243 (82.4%) of the 295 pugs (>16 weeks old) included in the study. The occurrence of pigmentary keratitis in the right and the left eyes were almost similar and no significant difference was detected.

Anoop et al. (2016) reported that left eye is more often affected with pigmentary keratitis compared to the right eye.

2.4.2 Etiology & Pathophysiology

Michaelson (1952) studied the proliferation of limbal melanoblasts into the corneal epithelium in response to standard corneal lesions made artificially by means of electrocautery at a critical distance of 2-3 mm from the limbus in the cornea of the 100 rabbit eyes. The study reported that 18 out of the 100 corneas (18%) had pigment that was easily observable within the triangular area of neovascularization with a magnification of 17x and 80 percent of the cornea had a gap in the limbal pigment band opposite the base area of pigment intrusion or neovascularization. The results of the study concluded that the proliferation of melanoblasts into the corneal epithelium from limbal pigment band is a normal response to the corneal injury.

Henkind (1967) experimented on the migration of limbal melanocytes into the basal layer of corneal epithelium after experimental insult to the cornea in the eyes of the guinea-pigs. The injury to the cornea was made either by topical application of a (0.1-0.01% saline) solution of colchicine once or twice daily for a week or by injection of a solution of alloxan into the anterior chamber of the eye. The study concluded that the melanocyte migration into the cornea was always preceded by the superficial corneal neovascularization and the melanocyte migration into the basal layer of corneal epithelium was as extensive as the extent of superficial corneal vascularization.

McCracken & Klintworth (1976) studied the ultrastructure of melanin pigmentation of the corneal epithelium produced experimentally in pigmented guinea pigs. The pigmentation of cornea was preceded by a leukocytic and vascular invasion of the cornea and by the development of abnormal corneal epithelium with numerous cells arrested in cell division. The migration of melanocytes into the corneal epithelium largely resulted in the pigmentation. Electron microscopy disclosed that melanin granules seen within the epithelial cells occurred as a result of the fusion of the membranes of the melanocytes and epithelial cells.

Bedford & Longstaffe (1979) studied the clinical and histopathological features of canine chronic superficial keratitis (corneal pannus) in a series of 84 German Shepherd dogs and based on the clinical features of the cornea studied they established four distinct stages of the disease and termed them as pannus tenuis (stage of cellular infiltration into corneal tissue), pannus vasculosus (stage of vascularization of corneal tissue), pannus en epaulette or pannus crassus (stage of organization of connective tissue elements within corneal tissue) and pannus siccus (stage of scar formation). The study also reported that the clinical features of the last two stages of chronic superficial keratitis was associated with deposition of pigment in the corneal epithelium.

Lavker et al. (1991) tested the hypothesis that a population pressure is created towards the central cornea because of the higher proliferation rate of limbal epithelium compared to the central corneal epithelium by measuring the relative proliferative rates of central corneal and limbal epithelium using 3H-thymidine autoradiographic techniques in New Zealand white rabbit and SENCAR mouse. The results indicated that the labeling index (LI) of the central corneal epithelium is actually higher than that of the limbal epithelium and suggests that population pressure in itself cannot be responsible for the centripetal migration of the corneal epithelium and brings forth the possibility that preferential desquamation of the central corneal epithelium may draw the peripheral cells towards the central cornea.

Van Der Woerdt (2004) reported that brachycephalic breed of dogs are prone to breed-related exophthalmos with a macropalpebral fissure which leads to chronic exposure keratitis and increased risk of proptosis in these dogs. The chronic exposure along with the common presence of nasal fold touching the cornea, caruncular trichiasis and other abnormalities in eyelid function and position can lead to excessive and

chronic irritation of cornea and conjunctiva contributing to the development of various consequences such as corneal ulceration, vascularization and pigmentation.

Krohne (2008) studied the medial canthus syndrome in dogs and grouped the abnormalities into two larger categories, viz. the abnormalities seen in brachycephalic and shallow-orbit breeds and the abnormalities found in mesocephalic breeds with laxity of the eyelid margin. The study reported that chronic corneal and conjunctival irritation often caused by the anatomic eyelid abnormalities may lead to disorders such as pigmentary keratitis, epiphora and occasionally ulcers.

Westermeyer et al. (2009) performed a study to evaluate the breed predisposition and clinical features of congenital keratoconjunctivitis sicca in dogs and reported that clinical signs such as different degrees of conjunctivitis, mucoid or mucopurulent ocular discharge, pigmentary keratitis, corneal ulceration and blindness are a sequel to the congenital alacrimalia in dogs.

Labelle et al. (2013) conducted a study to determine the clinical features of corneal pigmentation in pugs and reported that the severity of the corneal pigmentation and not the presence of corneal pigmentation had significant association with the presence and severity of the corneal vascularization. The study also suggested that pigmentary keratopathy may be a genetic disease in pugs, the severity of which may be exacerbated or modified by other comorbid conditions such as medial entropion, lower tear production or corneal trauma, rather than a disease caused per se by tear film disorders or adnexal conformational abnormalities.

Stades & Woerdt (2013) described that the hairs on the caruncles, the nasal fold and the medial entropion are potential sources of irritation to the medial quadrant of the cornea, the conjunctiva including the nictitating membrane, resulting in corneal edema, vascularization, excessive lacrimation, slight blepharospasm, pigmentation and other corneal defects. These sources of irritation along with the prominent eyes and associated lagophthalmos in the shallow-orbit breed of dogs may result in drying of the axial cornea and the erosion of corneal epithelium leading to the final stage of medial pannus formation and associated pigmentation covering the entire corneal surface.

Plummer (2015) reported that inability to effectively blink in brachycephalic breed of dogs may predispose their eyes to chronic exposure, which can lead to a wide

variety of corneal problems such as corneal ulceration and erosion, vascular keratitis, pigmentary keratitis and corneal fibrosis.

Anoop et al. (2016) performed a study on 55 dogs of different breeds, sex and age from which 83 corneas were studied to evaluate the etiology and progression of pigmentary keratitis in dogs. Out of 55 animals, 29 animals (53%) were affected with keratoconjunctivitis sicca (KCS) followed by 13 animals (24%) affected with entropion, 6 animals (11%) with periorbital dermatitis and subsequent entropion, 5 animals (9%) with excess nasal fold and subsequent trichiasis and 2 animals (4%) with trichiasis.

Amol (2016) reported that out of the 81 pug corneas with pigmentation studied vascularization was noticed in 42 corneas (56.8%), while mild form of fibrosis was present in 67 corneas (90.54%) out of the 74 eyes of pugs. The presence of vascularization and fibrosis could not be determined in the remaining 7 corneas of the pugs with pigmentation. The study also reported that out of the 87 eyes, distichiasis was present in 68 (78.2%) eyes, nasal fold trichiasis was seen in 21 (24.14%) eyes and 2 eyes had the presence of ectopic cilia. The study on the affections of nasolacrimal system reported KCS in 35 eyes of 20 dogs, while all the eyes diagnosed with KCS had the presence of pigmentary keratopathy, however, not all the clinical cases of pigmentary keratopathy had keratoconjunctivitis sicca.

Vallone et al. (2017) conducted a study using *in vivo* confocal microscopy (IVCM) to characterize canine superficial corneal pigment (SCP) in brachycephalic dogs. The study included 57 client-owned brachycephalic dogs from breeds predisposed to SCP. To determine the presence or absence of SCP, a complete ocular examination, including slit-lamp bio-microscopy, was used, followed by IVCM examinations. Clinical and IVCM abnormalities were recorded and statistically compared between dogs with and without SCP using a standardized scoring system. For analysis, dogs were divided into two groups: Pugs and non-Pug breeds. Of the 57 dogs included in the study, 32 were Pugs and 25 were non-Pug breeds. SCP was diagnosed in 23 (71.8%) of Pugs and 10 (40%) of non-Pugs. Superficial epithelial pigment, basal epithelial pigment, Langerhans cells, anterior stromal dendritic cells, epithelial disorganization, and vascularization were the six out of the 13 IVCM features evaluated that were significantly associated with SCP in both the group of dogs. The

study also reported that SCP was significantly associated with superficial epithelial leukocytes in Pugs only. The study concluded that IVCM characterized the SCP in dogs using the microscopic features of chronic inflammation and that the abnormalities were superficial and largely confined to the corneal epithelium. In brachycephalic dogs, superficial pigment appeared morphologically as a result of the centripetal corneal migration of the microanatomical features normally confined to the perilimbal region of the cornea.

2.5 Clinical diagnosis and evaluation of Pigmentary keratitis in dogs

2.5.1 Anamnesis and signalment

A thorough ophthalmic examination should always be preceded by a general anamnesis and a comprehensive physical examination as many of the ophthalmic disorders are a manifestation of systemic ailments. Neurological examination is warranted to rule out any affections of nervous system if any neuro-ophthalmic abnormalities such as blindness, strabismus, anisocoria are observed (Maggs, 2018a)**.**

Ophthalmic examination should always be performed in a consistent and sequential manner following a step-by-step approach to ensure that no abnormality is missed. Accurate history taking is very crucial and information regarding the breed, age, sex, general health, current medication, vision, signs of ocular pain, duration and progression of the problem should be obtained. Ocular examination if performed in a consistent step-by-step approach with patience is very rewarding as a definitive diagnosis is most often possible (Mitchell, 2011).

2.5.2. Ophthalmic examination

2.5.2.1 Gross examination

The examination of the adnexa of the eyes should evaluate the eyelids for conformation, abnormalities (entropion, ectropion), abnormally placed hairs (distichiasis, trichiasis, ectopic cilia), periocular dermatitis and masses. The function of the eyelids and its neurological integrity can be assessed by examining the palpebral reflex and menace response (Moore & Constantinescu, 1997).

Felchle & Urbanz (2001) stated that any deviation of the cornea from its normal transparent state is indicative of a disease process and opined that the examination of the cornea must always be performed under adequate illumination and magnification.

Moore (2001) described that the location, colour, shape and pattern of corneal lesion were helpful in determining the underlying cause.

Michau et al. (2003) stated that techniques such as slit lamp bio-microscopy, direct ophthalmology and fluorescein staining can be employed for the diagnosis of corneal lesions.

2.5.2.2 Vision assessment and neuro-ophthalmic examination

a. Menace response

Menace response is a learned response performed to assess both the visual status and eyelid function by making a threatening gesture with the hand or fingers in the direction of the eye being tested. To avoid creating excessive air currents that can cause a false-positive response the hand or the fingers can be moved from the temporal region or the ear towards the eye being tested (Scagliotti, 1999).

A negative menace response usually indicates blindness, although the animals with cerebellar lesions and a normal vision may also exhibit a negative menace response (Mitchell, 2011).

b. Pupillary light reflex

Pupillary light reflex (PLR) is a subcortical reflex performed to assess the integrity of the sensory and motor functions of the eyes and to diagnose the conditions which interfere with PLR such as synechiae, retinal degeneration and optic neuritis (Moore, 2001).

Thompson et al. (2010) noted that PLR is not an assessment of the vision as the animals which are blind due to disorders such as cataract and occipital cortex lesions can have normal PLR while those animals with normal vision can have an absence of PLR due to conditions such as iris atrophy. The knowledge of the neuroanatomical pathway of the PLR can help localize the lesion and a totally unresponsive pupil can be

seen in disorders such as generalized retinopathy, glaucoma and in any lesion involving the pupillary sphincter muscle.

c. Palpebral reflex

Mitchell (2011) described that evaluation of the menace response should always be preceded by assessment of palpebral reflex to ensure the ability of the animal to establish a normal blink response. The palpebral reflex evaluates the integrity of the sensory (Trigeminal nerve) and the motor (Facial nerve) pathways. An abnormal result indicates either a poor sensation or more often facial nerve paralysis.

Featherstone & Heinrich (2013) stated that brachycephalic breed of dogs and cats exhibit lagophthalmos (the inability of the eyelids to cover the eyes completely while blinking).

d. Dazzle reflex test

Dazzle reflex test is a subcortical reflex used to check for potential vision in cases with some opacity of the ocular media (e.g., cataracts and hyphema) and to assess the function of the visual pathway from the eye to the rostral colliculus. A negative dazzle reflex is a poor prognostic indicator for vision as it is also a precortical reflex and does not involve the visual cortex (Mitchell, 2011).

e. Cotton ball tracking test

Maggs (2018a) stated that cotton ball tracking test formed the basis of vision testing in small animals. For this a small piece of cotton ball was dropped 20 to 30 cm in front of the animal in its field of vision on each side. It was mentioned that most dogs and cats followed the object to the floor, especially in the first one or two attempts.

f. Maze test

Turner (2008) emphasized on the importance of maze test to assess the visual capability in animals. A mixture of solid objects (e.g. bins) and open objects (e.g. chairs) were chosen and randomly placed in an unfamiliar room. To perform the test the animal was held at one corner of the room while the owner stood at the other side of the room and called out to the pet. The test was performed in both bright (to test photopic vision) and dim (to test scotopic vision) lighting levels and the objects were

moved about in between test. It was mentioned that normally sighted animals negotiated the room confidently, but visually impaired were hesitant and slow, and many bumped into objects.

2.5.2.3 Special diagnostic procedures

a. Schirmer's tear test (STT)

Schirmer (1903) devised STT more than a century ago as a basic tool for the assessment of tear production and has since been used widely both in human and veterinary ophthalmology as the standard test to evaluate quantitative tear production.

STT can be performed by placing a 5×35 mm of Whatman filter paper no. 41 in medio-ventral palpebral cul-de-sac of an un-anesthetized eye for one minute and recording the length of wetting of the strip. The test can be performed in dogs using the same paper used for humans but the duration of test has been limited to one minute to make it more practical (Gelatt et al., 1975).

Rubin et al. (1965) clinically estimated the lacrimal function in dogs and found that the STT-1 (without topical anaesthesia) value in normal dogs was 20±4mm/min.

Gelatt et al. (1975) evaluated the tear formation in dogs using a modified Schirmer tear test and found that the STT-2 (after topical anaesthesia) value in normal dogs was 11.6±6.1mm/min.

Hirsh & Kaswan (1995) performed a comparative study of the Schirmer tear test strips in dogs to estimate the tear production and categorized the STT measurements observed as moderately low (11-14mm/min) and low (≤10mm/min).

Berger & King (1998) studied the fluctuation and variation in the tear production of dogs by evaluating the daily STT-1 and weekly STT-1 and STT-2 values conducted on healthy dogs. The study concluded that STT-1 and STT-2 values in normal dogs do fluctuate both on a daily and weekly basis, however, significant biological fluctuations were seen only on a week-to-week basis and not daily. Dogs with higher body weights were reported to have significantly higher STT values.

Tyagi (2009) reported that STT values have no significant difference with the time of day in animals.

Hartley et al. (2006) studied the effect of age, gender, weight and time of day on tear production in normal dogs and concluded that the tear production in dogs decreases with the ageing. The study reported that greatest difference in the STT values was seen between the 10.00 a.m. and 04.00 p.m. STT measurements, but this still amounted only to 0.7 mm which is clinically non-significant.

b. Fluorescein dye test

Moore (2001) described that precorneal tear film disorders, corneal epithelial defects, corneal ulcers and nasolacrimal duct obstructions can be detected by performing fluorescein dye test using sodium fluorescein strips.

Felchle & Urbanz (2001) stated that the fluorescein stained cornea can be examined under cobalt blue filter of an ophthalmoscope in a dark room.

Moore (2003) noted that the classical fluorescein stained appearance of the epithelial lip which borders the corneal defects can be used to diagnose indolent corneal ulcers or the chronic corneal epithelial defects.

c. Rose Bengal dye test

Maggs (2018a) stated that Rose Bengal dye stains dead and devitalized cells and is retained by corneal epithelium which is dysfunctional or eroded to less than its full thickness. It is very useful in diagnosing conditions such as qualitative tear film disorders, KCS or the herpes virus infection where there is a surface injury to the corneal epithelium. It is even retained by surface squamous cells that have altered surface characteristics or altered mucin coating.

d. Tonometry

Moore (2001) stated applanation tonometry as the method of choice for measuring intraocular pressures in veterinary patients and reported that the normal intraocular pressure in dogs and cats depends on the breed and species. The normal IOP in dogs and cats was stated as approximately 20±5 mm Hg and any value less than 10 mm Hg or a difference of 5 mm Hg or more between the IOP of the 2 eyes was suggestive of anterior uveitis.

e. Slit lamp bio-microscopy

Maggs (2018a) stated that slit lamp bio-microscopy can be used to study the topographical detail such as corneal curvature, anterior chamber depth, lens position and to assess the anterior chamber contents.

Featherstone & Heinrich (2013) described slit lamp bio-microscopy as the most versatile diagnostic tool for the veterinary ophthalmologist allowing magnified three dimensional examination of adnexa, cornea, anterior chamber, lens and vitreous.

f. Direct and Indirect ophthalmoscopy

Beranek & Vit (2007) described that direct ophthalmoscopy is mainly used for the examination of ocular fundus which includes the optic disc, the retinal vessels, tapetum lucidum and nigrum even though it can be used for anterior eye structures.

Maggs (2018a) described that the lenses of the direct ophthalmoscope can be used to examine structures other than fundus and noted that the 0 lens allows focus upon the retina, the +8 lens brings the focus forward to the posterior lens, the +12 lens brings the anterior lens into focus and the +20 lens will permit the examination of the cornea. The dioptre equivalent is the actual distance that the focal plane moves anteriorly and posteriorly with each dioptre and it varies with the species examined (Murphy & Rowland, 1987).

The advantage of indirect ophthalmoscopy is its ability to examine large areas of fundus due to its greater field of view while maintain a safe distance from the patient. The disadvantage is that it provides an inverted and reversed image of the fundus (Bowersox & La Croix, 2001).

g. Ocular ultrasonography

Eisenberg (1985) described ultrasonography of the eye and the orbit as a non-invasive imaging technique for qualitative and quantitative evaluation of the intraocular and orbital lesions.

Williams & Wilkie (1996) adopted the trans-corneal technique of ultrasonography of the eye after using topical anaesthesia (Proparacaine 0.5%).

Scotty (2005) reported that B-mode scan with a 5 or 10 MHz transducer was the most commonly practiced mode of ultrasonography in veterinary ophthalmology as it provides a two-dimensional real time image of the eye and the orbit.

The indications for the ultrasonography include any anterior opacity that preclude the ophthalmoscopy of the deeper structures and to diagnose various ocular disorders such as cataract, lens luxation, retinal detachment, vitreal opacities and haemorrhage (Gilger, 2006).

Joy et al. (2011) performed a study on the eyes of 63 dogs presented with history of vision loss and those that underwent surgery for cataractous lens extraction by extra-capsular technique using real time B-mode ultrasonography. Retinal detachment (RD) was confirmed in 18 cases out of 65 from the ultrasound findings and it was categorized into complete RD in nine cases, bullous RD in one case and focal RD in remaining eight cases. The study concluded that ultrasonographic examination findings was found to be useful in evaluation and categorization of retinal detachment.

Tavana & Peighambarzadeh (2014) performed trans-corneal ultrasonography using 7.5 – 10 MHz transducer in both eyes of 10 mixed breed dogs to study the normal qualitative and quantitative ultrasonographic findings. Mean ± standard deviation measurements of the anterior–posterior length of the eye axis, thickness of the lens and depth of the anterior chamber were reported as 19.41±0.78, 5.71±0.45 and 8.63±0.35 mm, respectively.

Smith et al. (2018) measured the optic nerve sheath diameter in healthy dogs using a transpalpebral ocular ultrasonographic approach with the help of 6-13 MHz linear array ultrasound transducer.

2.6 Grading and scoring of corneal pigmentation

Yi et al. (2006) reported that in 23 dogs with epiphora the corneal changes such as corneal neovascularization, corneal pigmentation and edema were found predominantly in the nasal/inferior quadrant, adjacent to the hairs growing at the caruncle and/or on the eyelids at the medial canthus of the eye.

Labelle et al. (2013) performed a study to determine the prevalence of corneal pigmentation in pugs and used digital photography of the eyes to grade the corneal pigmentation. The pigmentation of eye was graded as absent, very mild (corneal pigment < 2-mm diameter originating at the nasal canthus of the corneal limbus), mild (pigment covering <25% of the corneal surface), moderate (pigment covering 25 to 50% of the corneal surface) or severe (pigment covering > 50% of the corneal surface).

Anoop et al. (2016) performed a study on 55 dogs of different breeds, sex and age from which 83 corneas were studied and was divided into four quadrants to assess the progression of pigmentation and into 24 sectors to assess the pigmentation grading, extent of pigmentation and mean pigment density. Among 83 corneas, 40 (35%) showed pigmentation in all the sectors with the medio-ventral quadrant of cornea being the most affected quadrant in the study with varying mean pigment density and pigmentation score. The values of pigmentation grading ranged from 8 to 72 with a mean value of 32.59±2.27 in 83 corneas. The values of extent of pigmentation ranged from 4 to 24 with a mean value of 15.67±0.83. The values of mean pigment density ranged from 0.3 to 3 with a mean value of 1.37±0.07.

2.7 Therapeutic and surgical management of pigmentary keratitis

2.7.1. Therapeutic management

Kaswan & Salisbury (1990) conducted a study evaluating a new approach in the management of keratoconjunctivitis sicca in dogs using ophthalmic cyclosporine, a relatively new and an investigational drug during the time of study. The study reported that 1.52 percent of all dogs presented to the program were diagnosed with keratoconjunctivitis sicca and pigmentary keratitis as a devastating consequence of keratoconjunctivitis sicca in exophthalmic breeds such as the Pug, Schnauzer and Dachshund. The study also reported that cyclosporine is a non-cytotoxic drug that decreases corneal and conjunctival inflammation and scarring and apparently increases tearing.

Lightowler et al. (1993) studied the lacrimomimetic effect of topical (2%) cyclosporine (formulated in oily base) in canine KCS and reported an observed increase in the values of the Schirmer tear test, marked improvement of the eyes and furthermore

an attenuation of the melanin pigment deposited on the cornea in all cases treated with cyclosporine A (CsA) thrice in a day (t.i.d.).

Berdoulay et al. (2005) conducted a study on 105 dogs diagnosed with keratoconjunctivitis sicca [Schirmer tear test (STT) ≤ 10 mm/min and clinical signs of dry eye] also including the eyes with marginally decreased STT (11 ≤ 15 mm/min) to evaluate the effect of (0.02%) tacrolimus in aqueous solution on tear production. The efficacy study reported that STT values were increased by ≥ 5 mm/min after 6 to 8 weeks of twice daily tacrolimus administration in 83 percent of the eyes with extremely low initial STT (≤ 2 mm/min). The study concluded that topical tacrolimus is a promising alternative to topical cyclosporine for the patients with less than optimal response to topical cyclosporine.

Maggs (2018b) reported that inflammatory response in pigmentary keratitis could be kept under control with the use of cortisone and suggested the use of antibiotics to control the concurrent infections of cornea. Steps must be taken to increase or replace tear production, if KCS is present. It can require continued life-long therapy to prevent further inflammation and pigment deposition.

Ofri et al. (2009) performed a study on 44 dogs with clinical signs of keratoconjunctivitis sicca and previously untreated with cyclosporine to evaluate and compare the efficacy of pimecrolimus eye drops in reducing the clinical signs of keratoconjunctivitis with that of cyclosporine A ointment. Two treatment groups were used and the dogs were randomly assigned to the groups and were medicated twice daily for 8 weeks. The study reported a mean increase in STT value of 9.2±1.6 mm/min in the pimecrolimus group and 5.8±1.1 mm/min in the cyclosporine group. The study concluded that 1 percent pimecrolimus oily eye drops were comparatively effective in alleviating the clinical signs of inflammation and controlling keratoconjunctivitis sicca in eyes than the cyclosporine ointment.

Hendrix et al. (2011) performed an investigation to compare the efficacy of topically applied ocular preparations of tacrolimus and cyclosporine, both diluted in olive oil, for treating KCS in dogs. The investigation was conducted for a period of 12 weeks involving 4 ophthalmic examinations as a two-phase, randomized, controlled, masked clinical trial. Phase I study involved daily ocular examinations and evaluated

ophthalmic 0.03 percent tacrolimus in normal dogs while Phase 2 study evaluated and compared the efficacy of tacrolimus in treating KCS with (2%) cyclosporine. The study reported that there was no statistical difference in combined clinical scores for ocular discharge, conjunctival hyperaemia, corneal pigmentation, corneal vascularization or keratinization over the study period for the cyclosporine or tacrolimus group though both the groups had an increase in their mean tear production and STT values at fourth visit compared to first visit. The study concluded that both the drugs were effective in increasing the STT values in dogs naïve to lacrimostimulants and added that tacrolimus was effective in cases nonresponsive to cyclosporine.

Esson (2015) described that therapeutic aspect of pigmentary keratopathy is mainly aimed at addressing any underlying disorders including corneal inflammation and/or tear film disorders, typically using topical corticosteroids, CsA, tacrolimus, and/or tear film replacements/stabilizers.

Tilley et al. (2021) suggested treatment of chronic superficial and pigmentary keratitis with topical (1%) prednisolone or (0.1%) dexamethasone, (1 to 2%) Cyclosporine and lubricants/artificial tears every 8 to 12 hours.

Amol (2016) reported that based on the severity of pigmentary keratopathy, various concentrations of CsA eye drops can be prescribed for lifelong topical medication. In cases where the pigment covers < 25 percent of the cornea (mild cases), 0.05 percent CsA eye drops or eye ointment can be prescribed b.i.d. In moderate cases *i.e.* pigment covering 25-50 percent of cornea, 0.5 percent CsA formulated in corn oil can be used b.i.d. after autoclaving. In cases where the pigment covers 50-75 percent of cornea (severe cases), 1 percent CsA can be used b.i.d. In cases of complete pigmentation where the pigment covers 100 percent of cornea, 1 percent CsA formulated in corn oil can be used b.i.d. after autoclaving initially and later the dose can be increased to 2 percent after evaluation and confirmation of no side effects. Eye lubricants were prescribed irrespectively in all cases t.i.d.

2.7.2 Surgical management

Baker & Formston (1968) conducted a study to evaluate the transplantation of the parotid duct in the treatment of keratoconjunctivitis sicca in dogs. 26 eyes from 17 dogs were treated surgically and two cases had post-operative complications. In one

dog it was post-operative xerophthalmia due to unforeseen transplanted papilla obliteration and duct ligation. In the other dog it was uncontrolled epiphora due to compromised lacrimal drainage. In other patients, the therapeutic effect of the parotid duct transplantation was observed within 2 days of operation. The rapid elimination of the mucopurulent ocular discharge was the most significant response. The therapeutic effects visibly noticed include reduction of cellular infiltration and edema of the cornea, healthier appearance of the conjunctiva, gradual regression of corneal vascularization and melanosis but complete resolution of the corneal affections was not observed.

Holmberg et al. (1986) performed an experimental and clinical study on cryosurgical treatment of pigmentary keratopathy in dogs. Liquid nitrogen spray was used to expose 1 cm diameter area of the central cornea to a double freeze cycle in forty normal dogs and their postoperative sequelae and histopathologic results were evaluated. All the eyes exhibited corneal edema and epithelial damage as a consistent clinical abnormality following cryosurgery but the clinical signs alleviated after 3 weeks returning the cornea to a normal or near normal condition. Nine clinical cases of corneal pigmentation due to pannus in German shepherd dogs were subsequently treated with cryosurgery and similar postoperative complications were recorded. The study concluded that cryotherapy is an effective and repeatable method of decreasing corneal pigmentation and restoring vision in dogs with pigmentary keratitis.

Yi et al. (2006) performed a retrospective study to evaluate the surgical outcome and the complications in 23 dogs that underwent medial canthoplasty as a treatment for epiphora. The study reported that epiphora was most prevalent in the Shih Tzu breed of dogs while reporting it in other breeds such as Pekingese, Maltese, Toy poodle and Pugs. 21 (91.3%) dogs had medial canthal trichiasis and 19 (82.6%) dogs had medial canthal entropion as an etiology for epiphora while pigmentary keratitis was observed in 9 (39.1%) of the dogs along with other ocular abnormalities. The study concluded that tear staining had resolved in all dogs after 2 weeks of surgery and epiphora did not recur in any of the dogs during the follow-up period.

Featherstone et al. (2009) conducted a study in fourteen dogs with unilateral canine limbal melanoma to evaluate the efficacy and complication rate of partial lamellar resection followed by cryotherapy and adjunctive grafting. The study reported early complications such as anterior uveitis, corneal ulceration, dyscoria, marked

corneal granulation tissue at the graft margin, corneal lipidosis and corneal edema while recording marked corneal lipidosis as the only late complication. The study concluded that cryotherapy following partial lamellar resection is a highly effective, simple, minimally invasive and inexpensive adjuvant management option for canine limbal melanoma.

Stades & Woerdt (2013) described that potential sources of irritation to the medial quadrant of the cornea such as the hairs on the nasal fold can be surgically removed, wherein, the fold is lifted and excised with large scissors and wound is closed with 5-0 absorbable sutures.

Allgoewer & Sahr (2014) recorded the preliminary results of the study conducted for the evaluation and comparison of the long-term effect of nasal canthoplasty (NCP) with or without soft cryotherapy followed by topical therapy (TT) with immunosuppressive agents such as cyclosporine with or without tacrolimus and dexamethasone to topical therapy (TT) as a sole therapeutic modality for the management of corneal melanosis in pugs. The animals in the Group 1 (n=18) were treated with TT only, the animals in the Group 2 (n=21) underwent NCP followed by TT and the Group 3 (n=23) underwent NCP, soft cryotherapy and TT. In Group 1, (2/18) animals were observed with reduced pigmentation, (8/18) showed no improvement and the other (8/18) exhibited progressive pigmentation after 2.3 years (mean follow up time). In Group 2, (7/21) animals had reduced pigmentation, (4/21) showed no improvement and (9/21) were observed with progressive pigmentation after 2.9 years. In Group 3, (17/22) animals exhibited a reduction in pigmentation, (5/22) showed no improvement and none of them exhibited progressive pigmentation after 1.9 years. These preliminary results from the study affirm that pigmentary keratitis in pugs was better managed with NCP and TT than when managed with TT alone. Soft cryotherapy had a positive impact to NCP and TT when pigmentation was extensive but re-pigmentation may occur over time.

Azoulay (2014) conducted a study to evaluate the efficacy of soft cryotherapy in addressing pigmentary keratitis in dogs when other conventional therapy has been unsuccessful. Soft cryotherapy was performed using a cryogen of (95%) dimethyl ether, (3%) isobutene and (2%) propane in 16 eyes of 9 dogs with unilateral or bilateral corneal pigmentation. The cryogen was applied in a double freeze-thaw cycle over the

pigmented areas of each cornea under anesthesia. The study recorded that most of the corneal pigment deposits were gone by 5-15 days after cryotherapy. Post-operative complications such as corneal edema, corneo-conjunctival inflammation and corneal ulcers were recorded which resolved by 1 month after the procedure. The study concluded that cryotherapy is a viable adjunct treatment modality for cases of refractory corneal melanosis but etiological treatment remains indispensable in preventing the rapid corneal re-pigmentation.

Barachetti et al. (2015) studied the episcleral silicone matrix cyclosporine (ESMC) implants for its use, tolerability and efficacy in treatment of dogs with KCS. ESMC implants (1.9 cm length, 30% wt/wt CsA in silicone; with approximately 12 mg of CsA loaded into them) were implanted in 27 eyes of 15 dogs with KCS responsive (good candidate) or not responsive (poor candidate) to topical CsA. The study reported that both groups showed a significant improvement in clinical signs such as conjunctival hyperaemia, corneal neovascularization, corneal opacity and ocular discharge and had a significant increase in STT values after placement of ESMC implants. The study concluded that ESMC implants were efficacious and well tolerated in dogs with KCS and further study is warranted to determine the optimal dose and the duration of efficacy of CsA.

Plummer (2015) reported that medial canthoplasty is a very effective surgical procedure in correcting various characteristics of brachycephalic ocular syndrome. By improving the globe coverage, the blink mechanism is effectively enhanced thereby reducing exposure and frictional irritation that can lead to ulcerative keratitis and corneal pigmentation.

CHAPTER III

MATERIALS AND METHODS

The technical program of the present study was divided into following parts:

3.1 Screening of Pugs : The screening study was conducted on 100 clinical cases of Pug breed of dogs presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana for ailments other than ocular affections.

3.2 Clinical study : The clinical study was conducted on 12 Pug breed of dogs presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana for the issue of corneal pigmentation.

3.1 Screening of Pugs

3.1.1 Research methodology and experiment design

The signalment and detailed anamnesis were collected from each case presented for ailments other than ocular affections. Each animal underwent a detailed physical examination to establish a diagnosis. The eyes of the pugs were rinsed with sterile 0.9% normal saline solution and pictures of the eyes were taken to study the extent of corneal pigmentation.

3.1.2 Grading of corneal pigmentation

The degree or extent of pigmentation of the corneal surface refers to the area of the corneal surface covered by the deposition of melanocytes and melanin pigments. The degree of corneal melanosis was assessed with the help of a grading system suggested by Maini et al. (2019). A circle was drawn with the limbus as the circumference and the corneal surface was divided into 12 ‘clock hours’ or sectors. The extent of pigmentation was assessed based on the number of ‘clock hours’ affected with melanosis and was graded as described in the (Table 1) & (Fig.1 to 5). The total score of the corneal pigmentation was then calculated and the corneal melanosis was categorised into various degrees as described in the (Table 2).

Table 1 : Grading of corneal pigmentation

Characteristics of pigmentation	Points awarded
Single line of pigment in a clock hour	½ Point
Pigment affecting a clock hour	1 Point
Pigment extending up to the resting pupil edge	1 Additional point
Pigment extending beyond the resting pupil edge, affecting the visual axis	2 Additional points
Pigment extending just beyond the limbus (limbal brush border)	1 Point per clock hour affected
The grey-white corneal lesions were considered a precursor to the corneal pigmentation and were allotted points similar to the corneal pigmentation.	

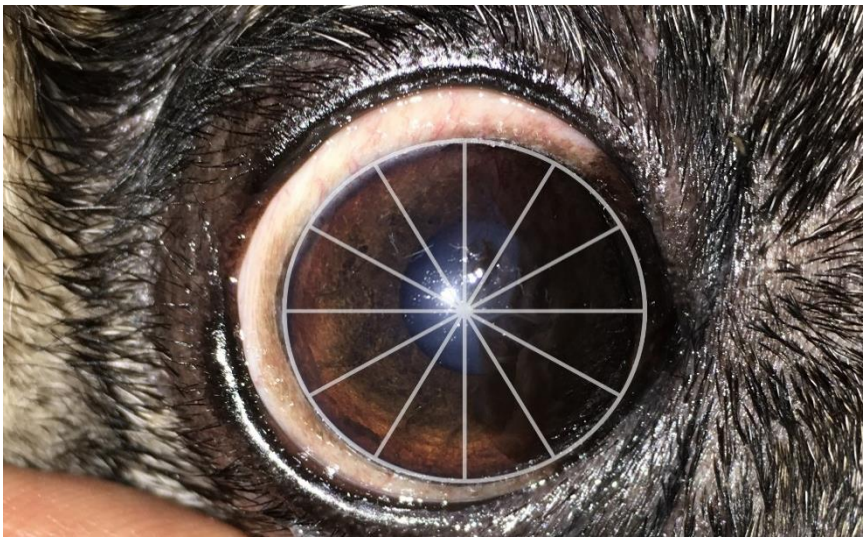
Table 2 : Degrees of corneal melanosis

Degree	Grading Score
Mild	0.5 – 4.5
Moderate	5.0 – 9.5
Severe	10.0 – 14.0

3.1.3 Pattern of corneal pigmentation

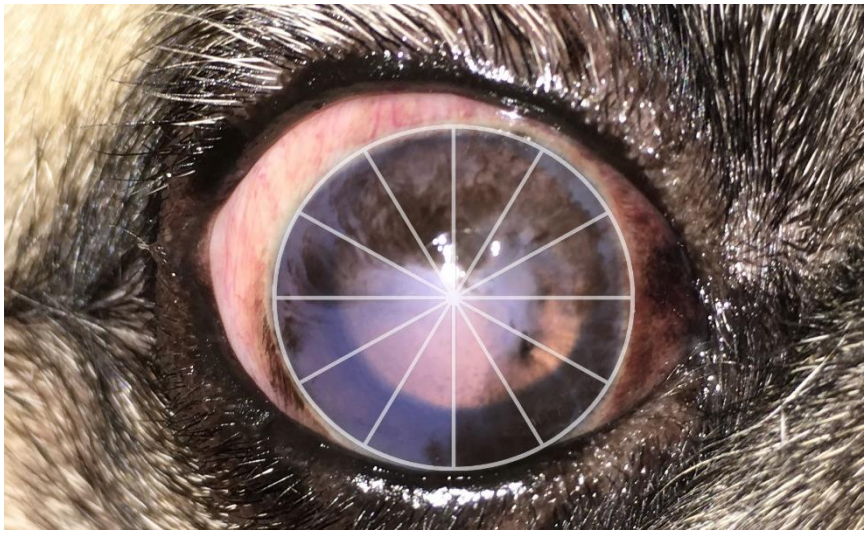
The pattern of corneal pigmentation was studied in all the eyes affected with pigmentary keratitis and was categorised as suggested by Charbiwala (2019) into ‘medial pyramidal’, ‘paintbrush’, ‘streak or limbal brush border’, ‘whorl’, ‘diffuse’, ‘patchy’ and ‘endothelial deposits’ (Fig.6 to 11). If an eye was presented with a mixture of patterns, the most prominent pattern among them was recorded.

Fig.1 : Grading of corneal pigmentation - Illustration 1



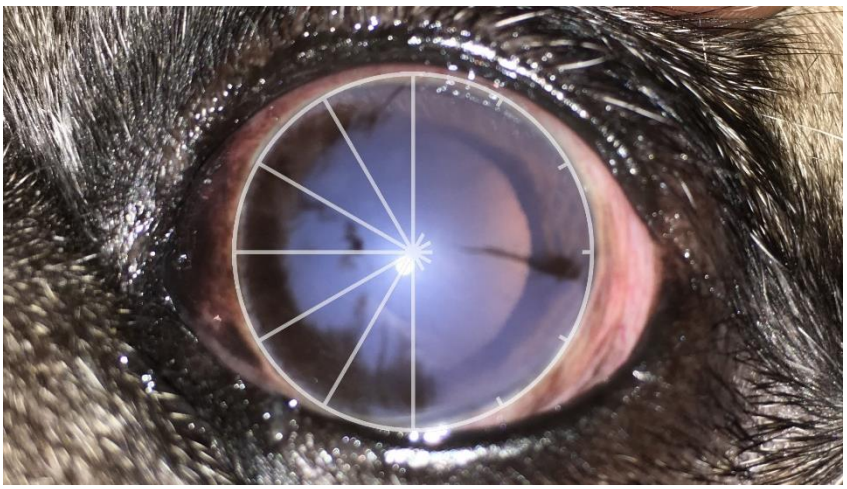
Note : Six clock hours were affected in this eye by corneal melanosis (6 points). Two additional points were given as the pigment can be seen extending beyond the resting pupil edge. Total = 8 points

Fig.2 : Grading of corneal pigmentation - Illustration 2



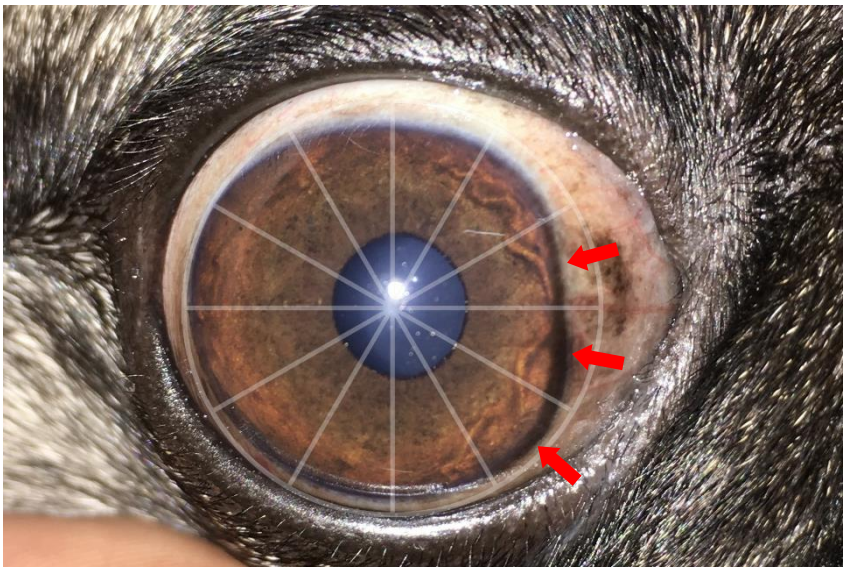
Note : 11 clock hours were affected in this eye by corneal melanosis (11 points). Two additional points were given as the pigment can be seen extending beyond the resting pupil edge. Total = 13 points

Fig.3 : Grading of corneal pigmentation - Illustration 3



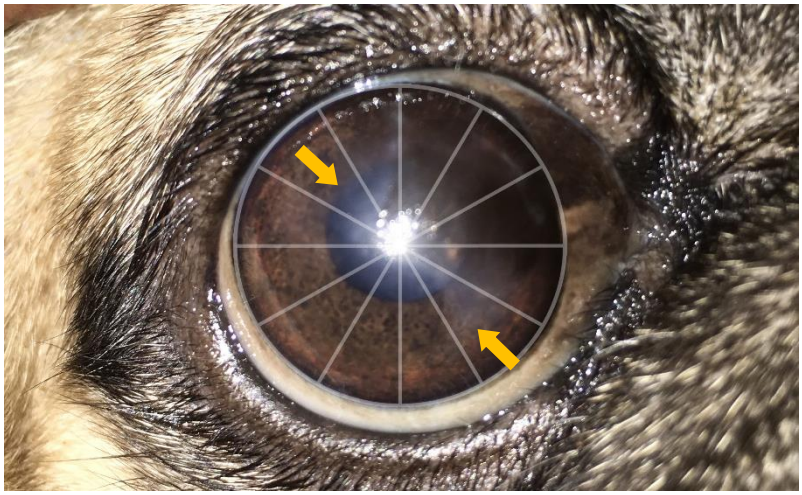
Note : 7 clock hours were affected in this eye by corneal melanosis near the medial canthus (7 points). 0.5 point was given to the single line of pigment near the temporal canthus. Two additional points were given as the pigment can be seen extending beyond the resting pupil edge. Total = 9.5 points

Fig.4 : Grading of corneal pigmentation - Illustration 4



Note : Corneal pigment (arrows) can be seen extending just beyond the limbus in this eye creating a 'limbal brush border' or 'streak' pattern of pigmentation. 3 clock hours were affected in this eye (3 points). Total = 3 points.

Fig.5 : Grading of corneal pigmentation - Illustration 5



Note : 5 clock hours can be seen affected with pigmentation (5 points). 2 additional clock hours can be seen affected with grey/white corneal lesions (arrows) which were considered as a precursor to the pigmentation and points were allocated similar to pigmentation (2 points). Two additional points were given as the pigment can be seen extending beyond the resting pupil edge. Total = 9 points

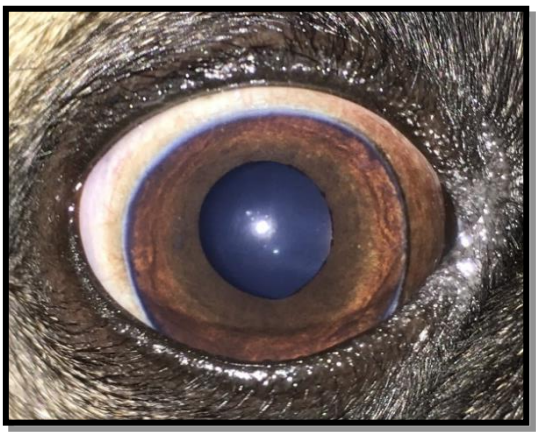


Fig.6 : Normal Cornea of a Pug

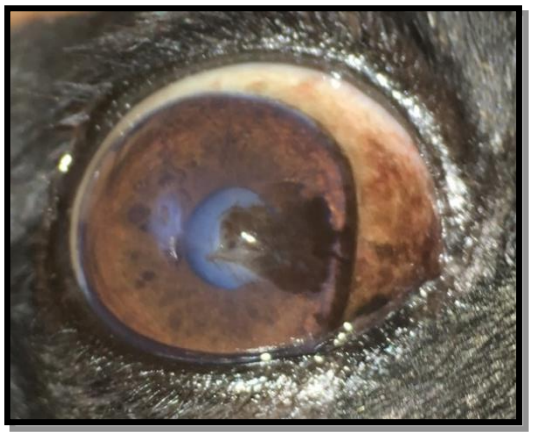


Fig.7 : Medial pyramid pattern of pigmentation

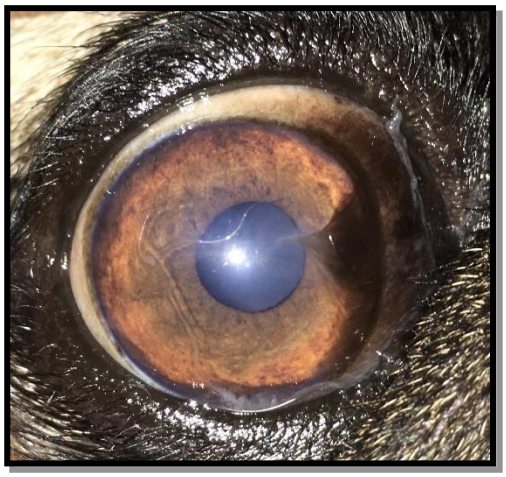


Fig.8 : Paintbrush pattern of pigmentation

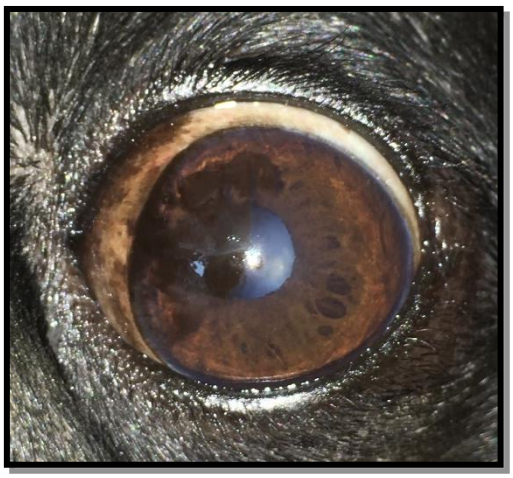


Fig.9 : Patchy pattern of pigmentation

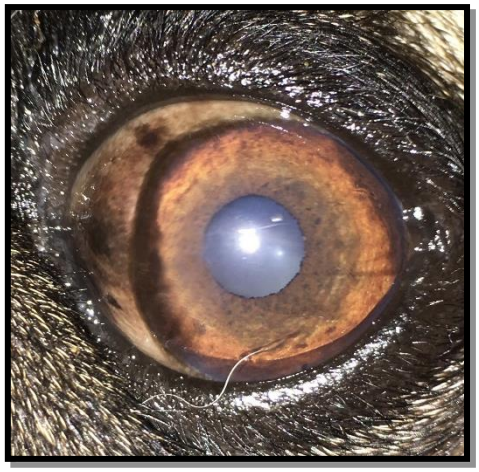


Fig.10 : Limbal brush border (or) Streak pattern of pigmentation

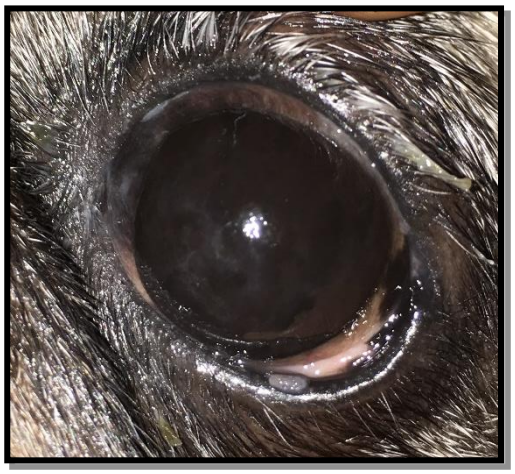


Fig.11 : Diffuse pattern of pigmentation

3.2 Clinical study

3.2.1 Research methodology and experiment design

The signalment and detailed anamnesis were collected from each case presented for corneal pigmentation. Each animal underwent a detailed physical examination and a thorough ophthalmic examination including specific ophthalmic tests on the day of presentation. Blood samples were collected for routine pre-operative blood work up to assess the complete blood count and biochemical profile. The animals presented were divided into following two groups (Table 3) based on the surgical technique adopted.

1. Group I (n = 6) : The animals of this group underwent Superficial keratectomy (SK) along with Medial canthoplasty (MC).

2. Group II (n = 6) : The animals of this group underwent Cryotherapy along with Medial canthoplasty (MC).

Table 3 : Group wise distribution based on the surgical technique adopted

Surgical procedure in different groups was performed as follow:

PIGMENTARY KERATITIS	
Group I (n = 6)	**Group II** (n = 6)
SUPERFICIAL KERATECTOMY with **MEDIAL CANTHOPLASTY**	**CRYOTHERAPY** with **MEDIAL CANTHOPLASTY**

a. Signalment & Anamnesis

Age, sex and other identification details were recorded and a detailed anamnesis regarding the onset of pigmentation, duration of the ailment, symptoms prevailing, changes in the vision and activity of the animal, previous medications administered and previous health related ailments were recorded.

b. Gross Ophthalmological examination

Ophthalmic examination was performed in a sequential manner in all the cases using standard eye examination procedures as described by Maggs (2018a). Initial ophthalmic examinations were conducted in a well-lit room and followed by examination in a dark room. All the animals were examined for their facial symmetry, visual activity, pain or irritation, changes in the appearance/colour of the eye, discharge, etc. The behavioural assessment of the vision was done by using cotton ball tracking test and maze test. All the parameters and the observations recorded were noted down in an ophthalmic examination sheet.

c. Cotton ball tracking test

A small piece of cotton ball was dropped 20 to 30 cm in front of the animal in its field of vision on each side. The dogs were observed for their ability to follow the cotton ball's trajectory to the floor. This was evident by the movement of the globe or the head with the fall of the object.

d. Maze test

Maze test or obstacle test was performed by placing a variety of obstacles of different sizes and shapes (Bins, cardboard boxes, etc) in the ophthalmic examination room. The patient was positioned on one side of the room with the clinician and the owner stays in the opposite side of the room. The owner was asked to call out the patient only once. Both the clinician and owner stood still during the test observing the progress of the patient through the obstacle course. The test was performed both in dark and lighted conditions to examine the scotopic and photopic vision respectively.

3.2.2 Neuro ophthalmic tests/reflexes: Visual activity of the eye was assessed using standard eye examination procedures as described by Maggs (2018a).

a. Palpebral Reflex : Palpebral reflex was tested by touching the eyelid skin at both the medial and lateral canthi. A normal palpebral reflex is seen as a complete closure of the eyelids in response to the stimulation of the eyelids at the canthus (Fig.12 & 13). This test was used to determine the integrity of the ophthalmic and maxillary branches of the Trigeminal nerve (5^{th} cranial nerve) and the auriculopalpebral branch of the Facial nerve (7^{th} cranial nerve).

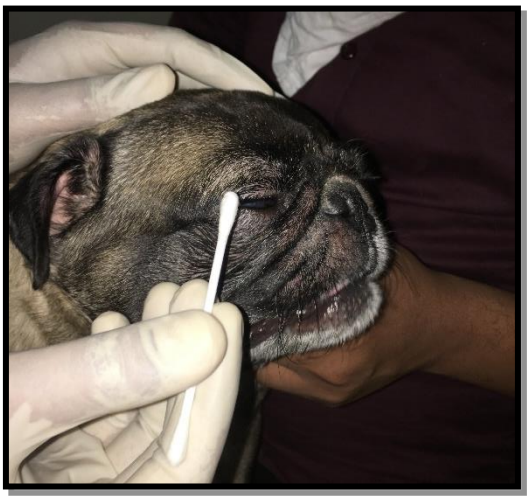


Fig.12 : Testing for palpebral reflex by touching the lateral & medial canthus

Fig.13 : Positive palpebral reflex indicated by the blink of eye

Fig.14 : Threatening gesture made for testing menace response

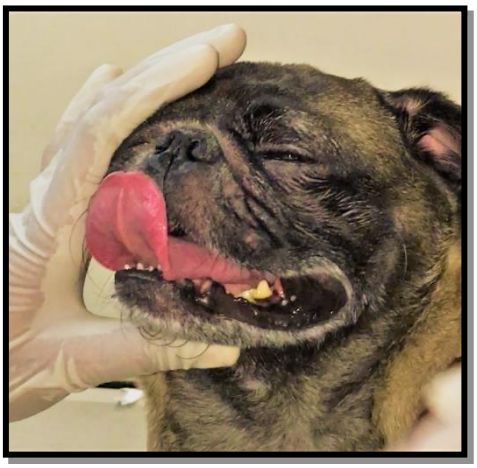


Fig.15 : Positive menace response indicated by the blink of eye

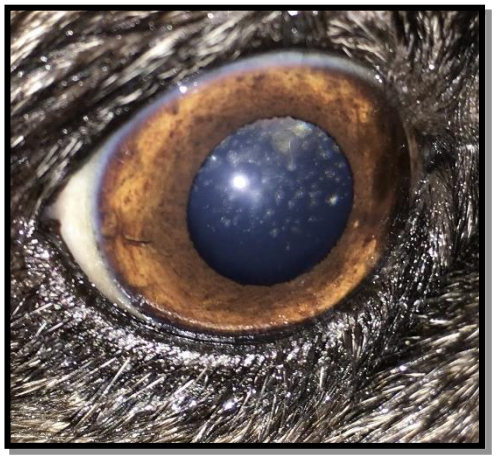


Fig.16 : Dilated pupil noticed under dim light condition

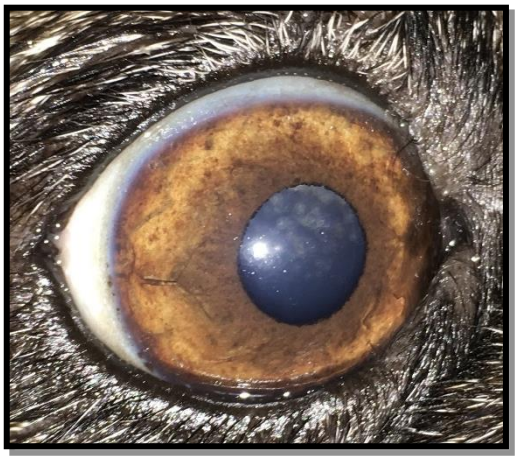


Fig.17 : Positive PLR indicated by relatively constricted pupil

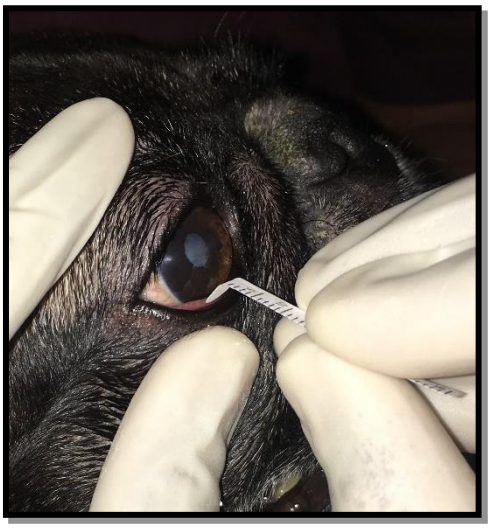


Fig.18 : Placement of Schirmer's tear strip in the eye

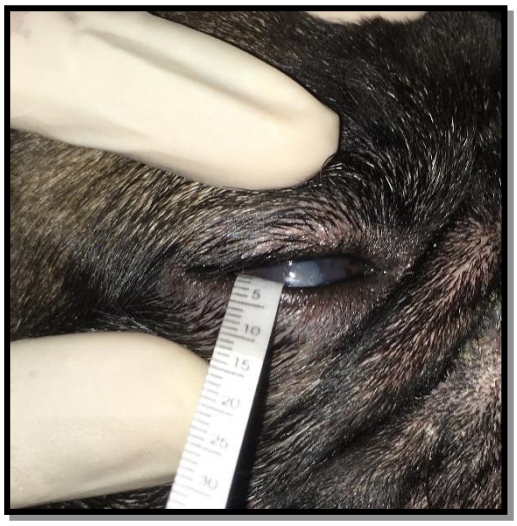


Fig.19 : Wetting of the Schirmer's tear strip by the tears

b. Menace Response : The test was performed by stimulating the eye in a visually threatening manner, most often by waving a hand in front of the eye. Each eye was tested individually with the non-tested eye covered (Fig.14 & 15). Care was taken to avoid the creation of air currents or any direct contact with the patient's periocular structures.

c. Pupil Symmetry: The resting pupil size, shape and symmetry of both the eyes were assessed by retro-illumination using a focal light source (direct or indirect ophthalmoscope). With both the eyes equally illuminated, the fundus reflex was used to assess and compare the pupil size, shape and symmetry.

d. Pupillary light Reflex (PLR): This test assess the reactivity of the pupils. The resting pupil sizes of both the eyes were noted and a focal source of bright light was applied to one eye and the speed and degree of pupil constriction was noted in the directly stimulated eye (Fig.16 & 17). The contralateral pupil was checked simultaneously to assess the consensual PLR which should be constricted relative to its resting state.

e. Dazzle Reflex: The test was performed by applying a bright source of light at one eye and the reflex was evident as a partial or complete closure of the eyelids on the illuminated side. This test was used along with the PLR and menace response to further localize some lesions.

3.2.3 Special Diagnostic Procedures : Specific diagnostic procedures like Schirmer's tear test, Fluorescein dye test, Rose Bengal dye test, tonometry etc. were performed on all the animals presented for corneal pigmentation using standard procedures as described by Moore (2001).

a. Schirmer's Tear Test

Schirmer's tear test (STT) was performed to measure the basal and reflex production of the aqueous portion of the pre-corneal tear film. Sterile, absorbent Whatman No.41 filter paper (5mm x 40mm) (Madhu Instruments Pvt. Ltd, New Delhi) printed with 1-35 in millimetre scale were used. The folded tip of the STT strip was hooked over the middle to temporal third of the lower conjunctival fornix for 60

seconds. The strip was then removed and the wetting of the strip was measured immediately as the tear production value in mm/min (Fig.18 & 19).

b. Fluorescein Dye Test

Fluorescein dye test was performed mainly to detect the corneal ulceration. It was performed by using sterile fluorescein impregnated paper strips (Madhu Instruments Pvt. Ltd, New Delhi). The strip was removed from the package, moistened with a drop or two of sterile normal saline or eye rinse and touched very lightly and briefly over the bulbar conjunctiva (Fig.20). Direct application of the fluorescein strip over the corneal surface should be avoided as it can cause artefactual stain retention. Excess fluorescein stain was rinsed with sterile saline after a minute or two and the eyes were examined with magnification under a blue light from the cobalt filter of a direct or indirect ophthalmoscope (Fig.21). Fluorescein stain is retained only by hydrophilic structures such as the pre-corneal tear film and corneal stroma and not by hydrophobic structures such as the corneal epithelium or the Descemet's membrane. Any damage to the corneal epithelium will result in stain retention by the corneal stroma which can be seen as a bright green fluorescence under blue light.

c. Rose Bengal Dye Test

The test was performed to diagnose the corneal surface injuries seen in conditions such as KCS and qualitative tear film abnormalities, where there is necrosis and desquamation of the corneal and conjunctival epithelium. Rose Bengal dye strip (Surgitech Innovation, Haryana) was used to detect the cornea in which the epithelium was dysfunctional or eroded to less than its full thickness because of its ability to stain the dead and devitalized cells, including the cells that have an altered superficial mucin layer. The test was performed in a similar manner as for the fluorescein dye test, but the eyes were examined for the retention of the stain using just white light and magnification (Fig.22 & 23).

c. Tonometry

Intraocular pressure was measured using a Tono-Pen (Accu Pen, Phoenixville Pike, 19355 USA). The intraocular pressure was measured after administering 1-2 drops of topical anaesthetic (0.5%) Proparacaine Hydrochloride Ophthalmic Solution (Paracain®, Sunways India Pvt. Ltd. Mumbai, India). The patient was restrained gently and the eyelids were held open with the non-dominant hand. The Tono-Pen was held in

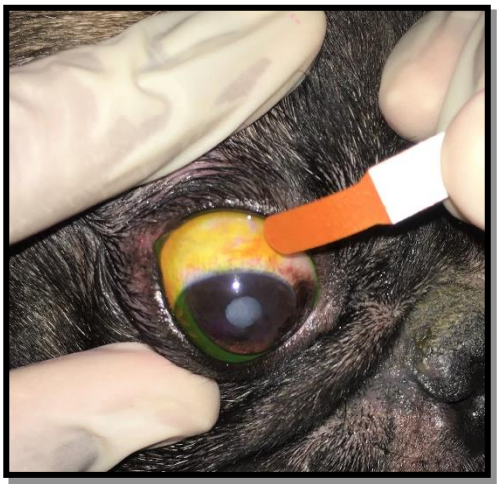


Fig.20 : Placement of fluorescein dye strip in the eye

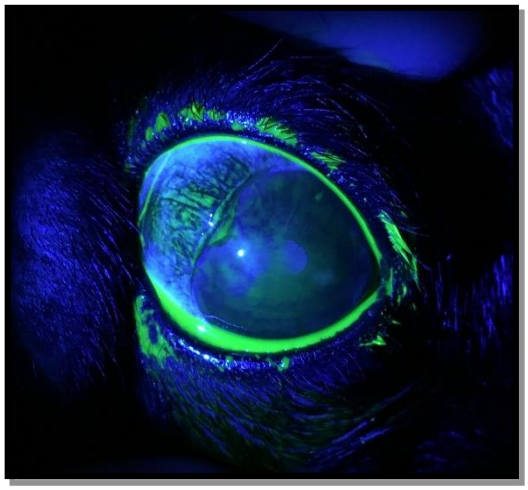


Fig.21 : Examination of fluorescein stained eye under cobalt blue light

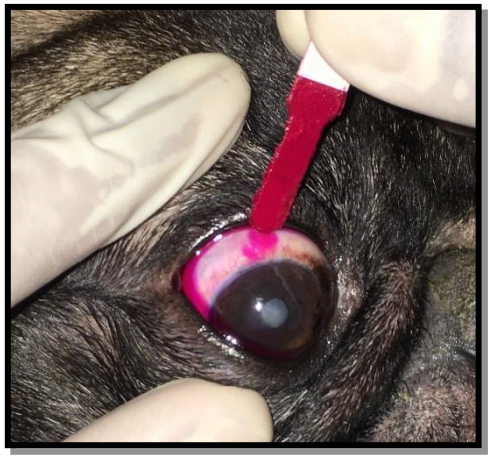


Fig.22 : Placement of rose bengal dye strip in the eye

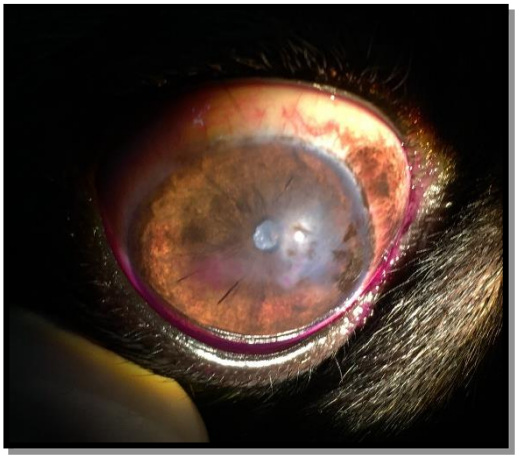


Fig.23 : Examination of rose bengal stained eye under diffuse light beam

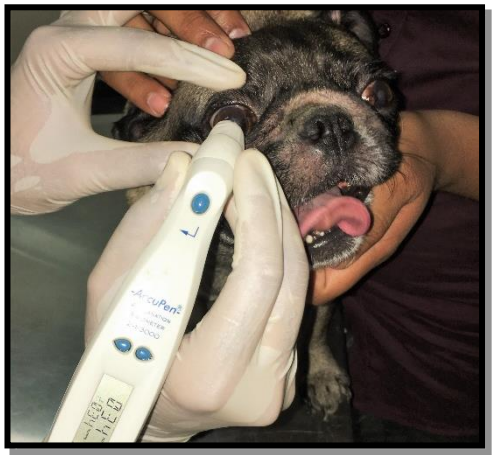


Fig.24 : Performing tonometry using tonopen

Fig.25 : Position of the tonopen tip relative to the corneal surface

Fig.26 : IOP reading taken by touching the corneal surface

the dominant hand in a particular “approach angle” so that the foot plate’s surface on the tip of the Tono-Pen is parallel to the corneal surface (Fig.24 & 25). The tip was gently touched on the cornea multiple times to get an average reading of the intraocular pressure indicated by an electronic ‘beep’ (Fig.26).

d. Slit Lamp Bio-microscopy

Slit lamp bio-microscope (Optitech: Tarun Enterprises Pvt. Ltd. Allahabad, India) was used to examine the anterior segment as well as the adnexal structures (eyelids, lacrimal apparatus, orbit and para-orbital areas) with illumination and magnification (Fig.27). The anterior segment was transilluminated with a slit beam and viewed from an angle of 45 degrees to study the topographical details such as the corneal curvature, anterior chamber depth and lens position (Fig.28). Diffuse illumination was used to examine the adnexal structures for abnormalities such as medial entropion of lower eyelid, medial caruncular trichiasis, trichiasis, distichiasis, etc.

e. Ophthalmoscopy (Direct and Indirect)

Ophthalmoscopy was performed in all cases of corneal pigmentation wherever possible after instilling tropicamide eye drops (Trophtha™, Ophtho Life Sciences Pvt. Ltd, Uttar Pradesh, India) to examine the posterior structures of the eye including the vitreous and the ocular fundus. Indirect Ophthalmoscopy was performed initially to take advantage of its greater field of view followed by closer examination of any region of interest with direct ophthalmoscopy making use of its higher magnification.

Indirect Ophthalmoscopy : A head mounted binocular indirect ophthalmoscope (Welch Allyn: State Road, New York, U.S.A) and a 20D dioptric lens was used to examine the fundus. The lens was held between the patient’s and the examiner’s eyes (Fig.30). The fundus was examined in quadrants with the optic disc as the reference point. The size, shape, colour and elevation/depression of the optic nerve was evaluated and the retinal blood vessels were observed for their size, congestion and haemorrhage and the tapetum for its hypo/hyper reflectivity.

Direct Ophthalmoscopy : Direct ophthalmoscope (Keeler: Clewer Hill Road, Windsor, U.K.) was used to examine any region of interest in the ocular fundus with higher magnification. The lenses -3D to 0 were used to examine the structures of

fundus, 0 to +8D were used for vitreous examination, +8D to +12D for the lens and lens capsule and +12D to +20D were used for iris, anterior chamber and cornea. The lenses in the direct ophthalmoscope were adjusted during the examination to estimate the depth below or height above of any lesion from the rest of the fundus (Fig.29).

f. Ultrasonography

B-mode ultrasonography was used as an inevitable diagnostic modality in all the cases included in the study because of the limited window available for the examination of the posterior structures of the eye due to the impediment by corneal pigmentation. A transcorneal approach was performed after administering 1-2 drops of topical anaesthetic (0.5%) Proparacaine Hydrochloride Ophthalmic Solution using 5-12/18 MHz probe of Philips Affinity 70 Ultrasound machine. The animal was restrained in sternal or lateral recumbency and the eyelids were retracted gently. A small amount of sterile Lignocaine Hydrochloride Gel (Lox-2% Jelly, Neon Laboratories Ltd, Mumbai, India) was used as a coupling gel on the transducer to obtain sufficient contact area and the transducer was placed directly on the cornea. The eyes were scanned both in transverse and longitudinal planes (Fig.31 & 32). The eyes were rinsed off with (0.9%) sterile normal saline solution after completion of the ultrasonography to remove the coupling gel.

3.2.4 Haemato-biochemical examination

Whole blood samples (at least 2 ml) were collected either from cephalic or recurrent tarsal vein for routine pre-operative blood work up from every case in ethylene diamine tetra acetic acid (EDTA) containing vials and clot activator vials.

The blood samples were assessed for Hemoglobin concentration (Hb), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Platelet count and Packed cell volume (PCV) using Fully Automated MYTHIC^{TM} 18 VET Hematology system (Orphée and Cormay diagnsotics, Switzerland). Leishman stained peripheral blood smears were used to estimate the Differential leukocyte count (DLC) manually.

The serum samples collected in clot activator vials were assessed for biochemical profiles such as liver function test, renal function test, total protein, serum albumin and blood glucose levels with an automated clinical biochemistry analyser

Fig.27 : Slit lamp biomicroscopy

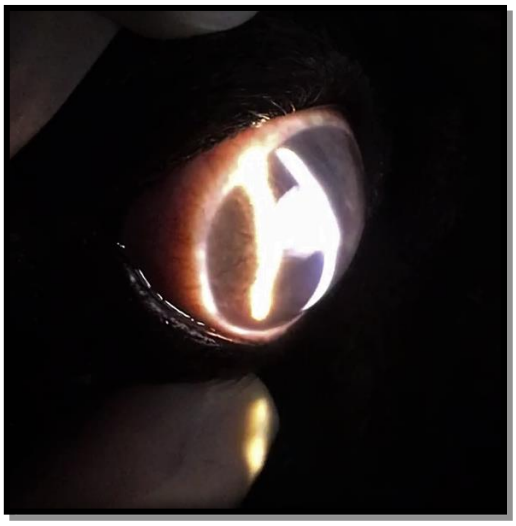


Fig.28 : Examination of anterior chamber using slit beam

Fig.29 : Direct ophthalmoscopy

Fig.30 : Indirect ophthalmoscopy

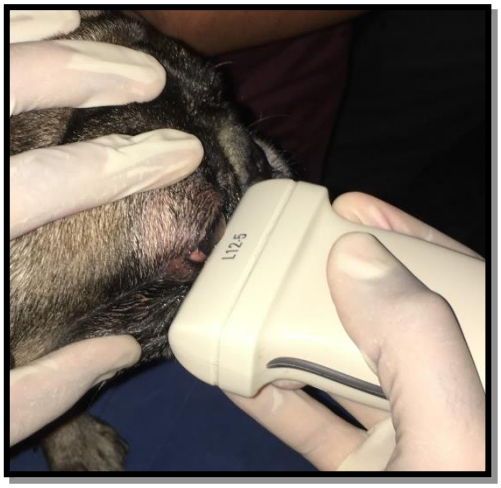


Fig.31 : Transcorneal USG of the eye in transverse plane

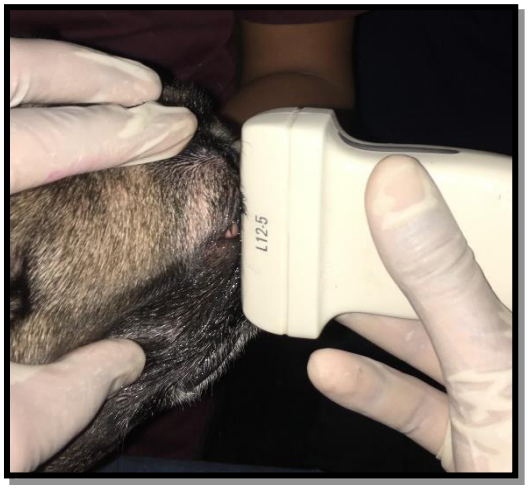


Fig.32 : Transcorneal USG of the eye in longitudinal plane

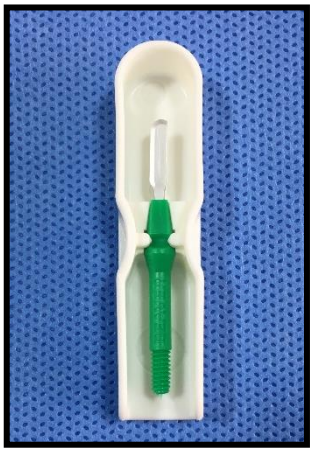


Fig.33 : Beaver blade No. 6400

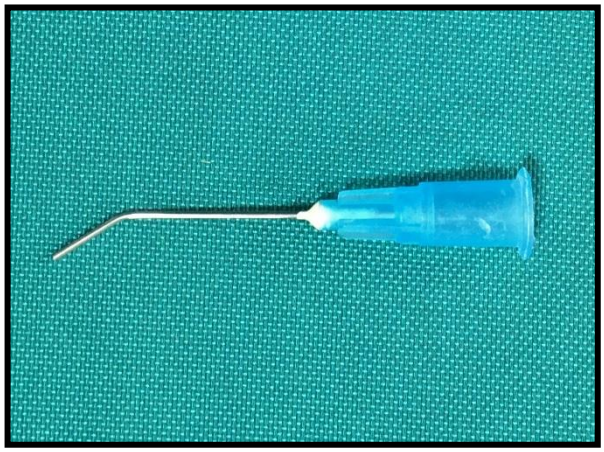


Fig.34 : Lacrimal cannula

(Vitros System Chemistry DT60 II, Ortho Clinical Diagnostics, Johnson and Johnson, USA) by using standard kits (Vitros Ortho Clinical Diagnostics, Mumbai).

3.2.5 Pre-operative medicinal therapy

All the animals diagnosed with corneal pigmentation were administered with topical intra ocular eye medications which included topical lacrimomimetic (0.5%) carboxy methyl cellulose sodium (Refresh Tears®, Piramal Enterprises Ltd, Madhya Pradesh, India), topical antibiotic eye drops (0.3%) gatifloxacin (Gatiquin, Aditi Pharmaceuticals Pvt. Ltd, Solapur, India), topical lacrimostimulant eye drops (0.1%) cyclosporine (Ophthocare, Corise Healthcare Pvt. Ltd, Maharashtra, India) from the day of presentation till the scheduled day of surgery. Based upon the results of pre-operative blood work-up, systemic antibiotics, non-steroidal anti-inflammatory drugs and other supplements were administered wherever required. On the day of surgery, gatifloxacin eye drops was administered on both the eyes at an interval of 15 - 20 minutes starting from 1 hour prior to surgery till the commencement of surgery to ensure ocular asepsis.

3.2.6 Anaesthesia

All the animals were premedicated with a combination of Butorphanol @ 0.2 mg/kg body weight (Butodol®, Neon Laboratories Ltd, Mumbai, India), Atropine @ 0.02 - 0.04 mg/kg body weight (Tropine*, Neon Laboratories Ltd, Mumbai, India) and Acepromazine maleate @ 0.02 - 0.05 mg/kg body weight (Ilium- Acepril-10®, Troy Laboratories Pvt. Ltd, Australia) by intramuscular route. Induction was done with Propofol® @ 4mg/kg body weight (Neorof®, Neon Laboratories Ltd, Mumbai, India) and Diazepam @ 0.5 mg/kg body weight (Lori®, Neon Laboratories Ltd, Mumbai, India) administered slowly by intravenous route and the anaesthesia was maintained with inhalant anaesthetic agent Isoflurane (Forane®, Aesica Queenborrough Ltd, UK). Acepromazine was either excluded or given at a lower dose depending upon the physical status of each animal on a case to case basis.

Other pre-operative medications include systemic antibiotics Cefotaxime @ 25 mg/kg body weight (Taxim®, Alken Laboratories, Mumbai, India) and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Meloxicam @ 0.2 mg/kg body weight (Melonex ™, Intas Pharmaceuticals Ltd., Ahmedabad, India) administered intravenously.

3.2.7 Patient positioning and preparation

Patients were placed in lateral recumbency with the affected eye on the dorsal side (Fig.36). Padding material was placed under the cranium of the patient to elevate and position the head according to the surgeon's convenience. Periorbital region and the eyelids were trimmed of the hairs and painted with 5 percent povidone iodine solution. 0.5 percent povidone iodine solution was also used to flush the dorsal and ventral conjunctival fornix just before the commencement of surgery.

Commercially available eye drape with operating window in the centre or sterile surgical drape was used for draping the eye during surgery. Lieberman speculum was used to hold the eyelids in position (Fig.37). Surgery was performed by the surgeon sitting in front of the patient.

3.2.8 Instruments Used During Surgical Procedure

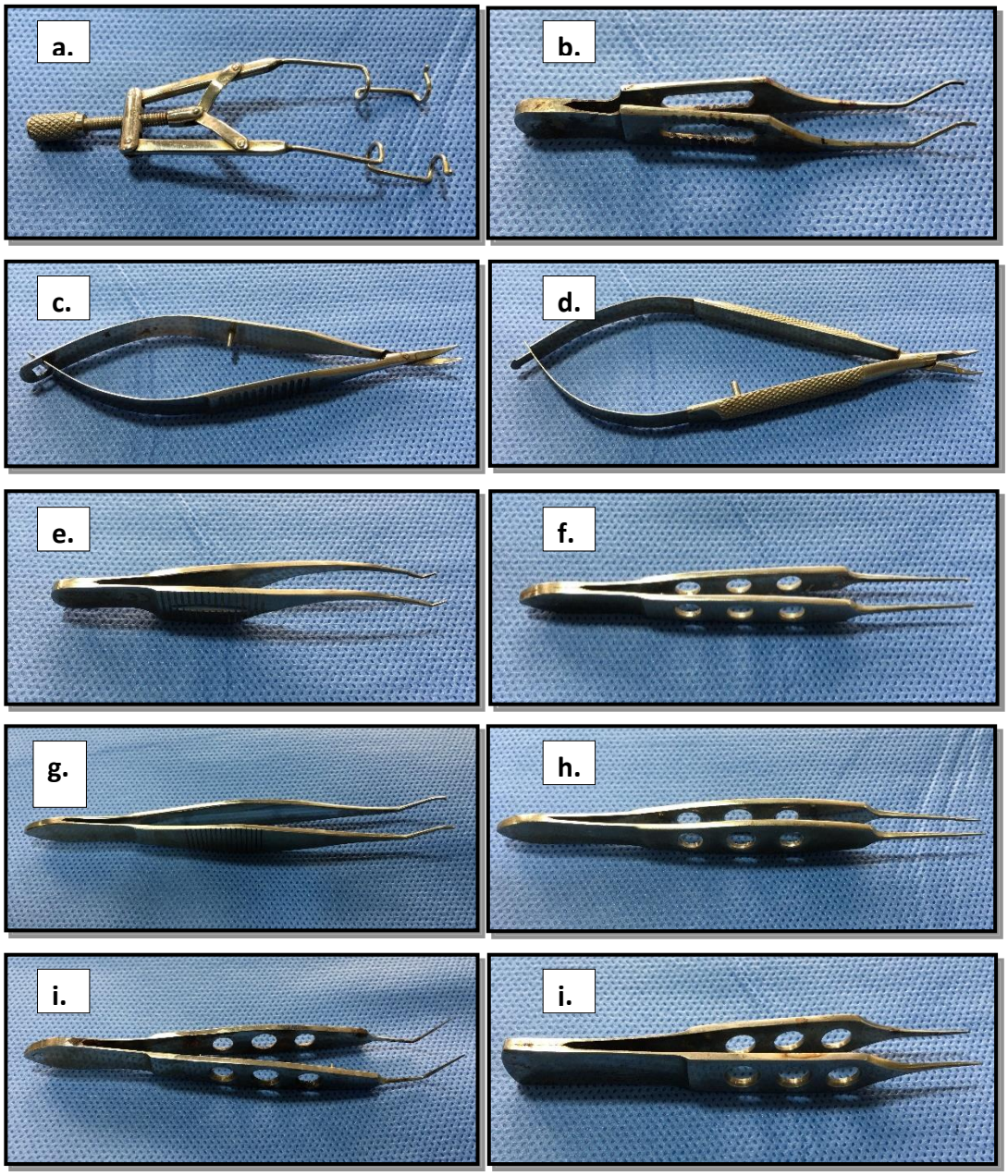
The following micro surgical instruments (Fig.35) were used during the surgical procedure for corneal pigmentation : Eye drape, Lieberman Eye speculum, Mosquito forceps, Beaver blade No.6400 (Fig.33), Lacrimal cannula (Fig.34), Lim's forceps, Colibri forceps, Troutman-Barraquer corneal utility forceps, McPherson straight tying forceps, McPherson angled tying forceps, Stevens tenotomy scissors, corneal scissors, Microsurgical needle holder and Coaxial operating microscope (Shin Nippon OP-2, Ohira Co. Ltd., Japan).

3.2.9 Operating procedures

a. GROUP I

SUPERFICIAL KERATECTOMY with MEDIAL CANTHOPLASTY (n = 6)

Superficial Keratectomy (SK) : Six eyes of the six animals in Group I were operated for pigmentary keratitis by performing superficial keratectomy in the entire cornea or only a part of it depending upon the extent of corneal melanosis. Superficial keratectomy involves the excision of corneal epithelium along with a variable amount of corneal stroma. Under general anaesthesia, the eye to be operated was secured in position with the help of a Lieberman speculum and two or more traction sutures made with Vicryl 3-0 to retract the ventro-medially rotated eyeballs from the bony orbit. Using a Beaver blade No.6400, an incision was made starting at the level of limbus



a. Lieberman Eye speculum

b. Colibri forceps

c. Corneal scissors

d. Microsurgical needle holder

e. Troutman-Barraquer corneal utility forceps

f. Toothed tying forceps

g. Lim's forceps

h. Tying forceps with platform

i. McPherson angled tying forceps

j. McPherson straight tying forceps

Fig.35 : Instruments used during surgical procedure

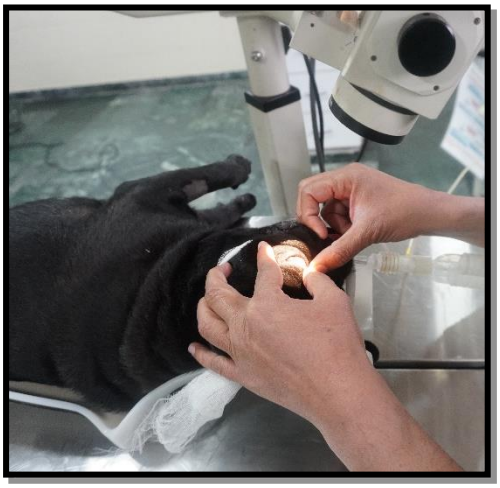


Fig.36 : Positioning the patient & setting up of operating microscope

Fig.37 : Eyeball held in position using Lieberman speculum & 3-0 vicryl sutures

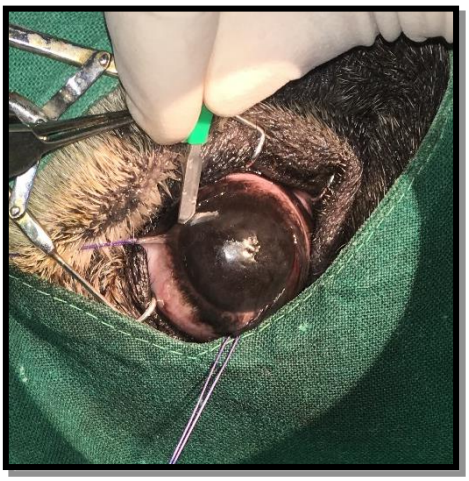


Fig.38 : Partial thickness corneal incision made at the limbus with Beaver blade No.6400

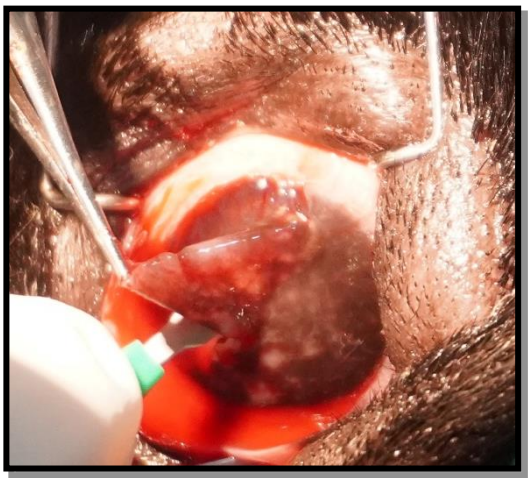


Fig.39 : Meticulous excision of pigmented corneal epithelium

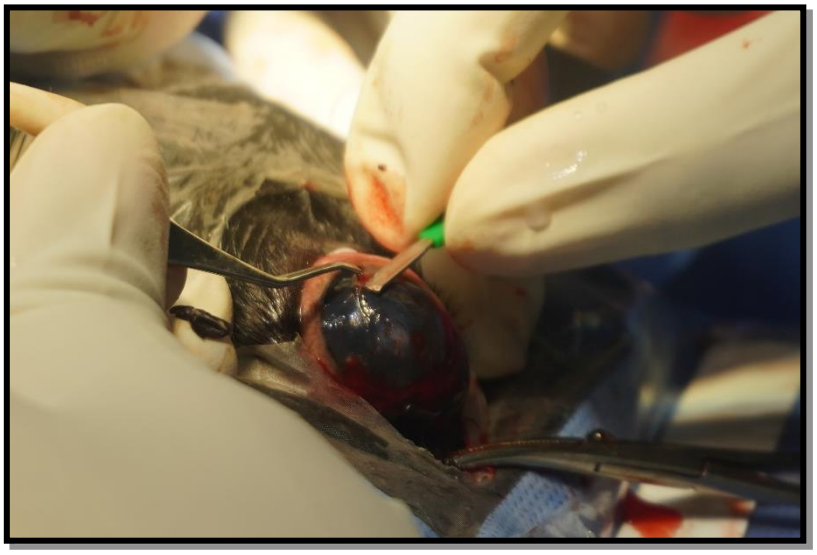


Fig.40 : Beaver blade held tangential to the corneal surface to prevent progressive deeper dissection

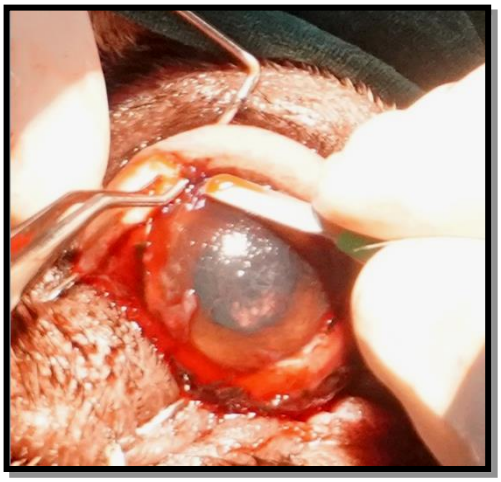


Fig.41 : Pupil visible as the pigmented epithelium is excised

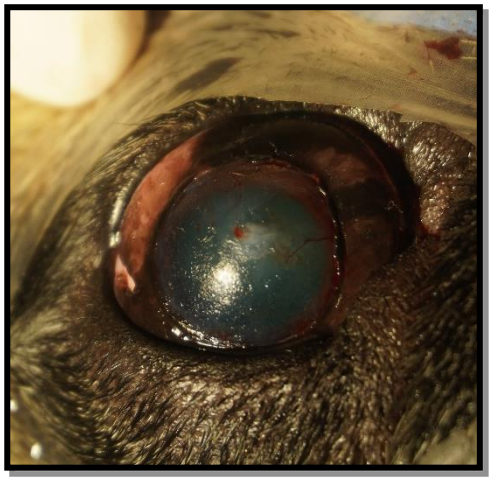


Fig.42 : Transparent cornea immediately after superficial keratectomy

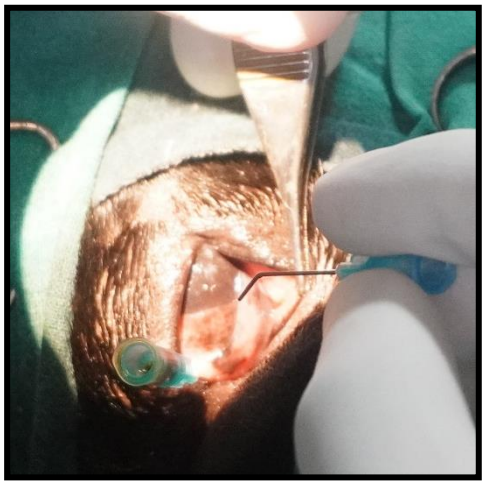


Fig.43 : Cannulation of lacrimal puncta

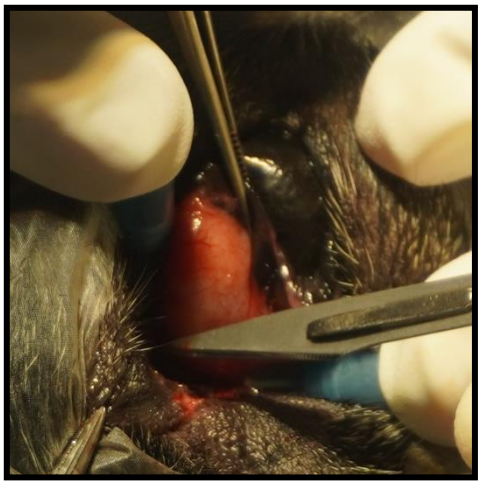


Fig.44 : Incision being made at the medial canthus

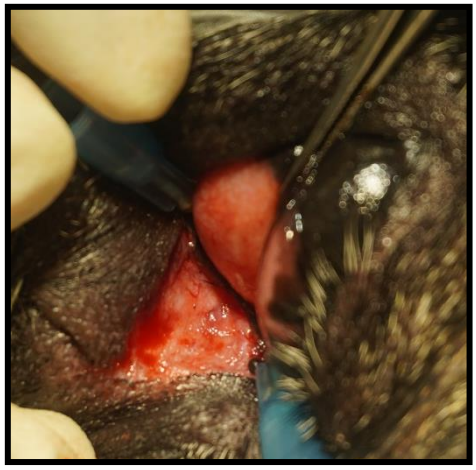


Fig.45 : Triangular area of tissue excised along with the caruncle

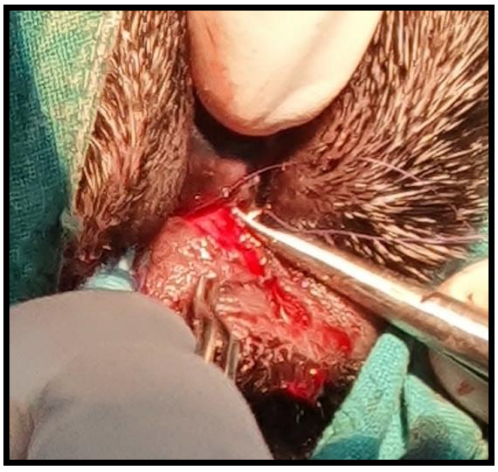


Fig.46 : Suturing the deep conjunctival layer with 6-0 vicryl

encircling the pigmented corneal surface (Fig.38). A continuous stream of (0.9%) normal saline was directed at the leading aspects of the corneal incision as it was performed. The depth of the incision was made sufficient enough to remove the base of the pigmented cornea. Once the circumscribing corneal incision was made, the edge of the superficial keratectomy section was grasped with the help of a colibri or lim's forceps and the pigmented corneal tissue was carefully dissected from the underlying stroma(Fig.39) with the Beaver blade held tangential to the corneal surface so that progressive deeper dissection into the stroma can be avoided (Fig.40). Care was taken to limit the dissection of the pigmented corneal tissue under one lamellar plane throughout the procedure though in some cases it was not possible due to the varying depths of pigmentation and stronger attachment of pigmented cornea to varying depths of the underlying stroma. Once the entire pigmented corneal surface was excised, the pupil can be visualised (Fig.41 & 42). The corneal surface was lavaged with a stream of (0.9%) normal saline and temporary partial tarsorrhaphy was performed using 2-0 Nylon leaving an area medially for the administration of topical medications. The tarsorrhaphy knot was kept on the upper eyelid to prevent the suture from irritating the cornea and from soiling with ocular discharge. The tarsorrhaphy sutures were removed after 15 days in all the animals and the surgical outcome was studied.

Medial Canthoplasty (MC) : Medial canthoplasty was performed in six eyes of the six animals in Group I along with superficial keratectomy. The medial canthus was rolled out in the nasal direction to reveal the medial caruncle. The upper and lower nasolacrimal puncta were identified at the medial canthus and cannulated with the help of a nasolacrimal cannula to prevent inadvertent damage to the puncta and the ducts during surgery (Fig.43). The cannulas were left in place for the duration of the procedure. The area of the tissue to be excised was identified and an incision was made in the skin around the medial canthus to delineate the portion of medial caruncle and eyelid margins to be removed (Fig.44). The triangular area of tissue section to be excised was undermined with the help of a tenotomy scissors taking sufficient care to avoid inadvertent damage to the puncta and the ducts (Fig.45). Any remaining hair or its follicles were excised delicately. The surgical site was closed in two layers. The deep conjunctival layer was closed with 6-0 Vicryl in a simple continuous pattern burying the knots so as to prevent its exposure and irritation to the corneal surface (Fig.46). The skin of the medial canthus was closed in a figure-of-8 suture pattern with 5-0 Nylon

(Fig.47). The remainder of the skin incision was closed in a simple interrupted suture pattern with 5-0 Nylon. The tags of the figure-of-8 suture were incorporated into the subsequent interrupted sutures to prevent the tags from irritating the corneal surface (Fig.48).

b. GROUP II

CRYOTHERAPY with MEDIAL CANTHOPLASTY (n = 6)

Cryotherapy (Cryo) : Six eyes of the six animals in Group II were operated for pigmentary keratitis by performing cryotherapy along with medial canthoplasty. Under general anaesthesia, the eyes were prepared for cryotherapy as for the superficial keratectomy and the eye was exposed and secured in position with the help of a Lieberman speculum. Cryotherapy was performed with the help of Compound W Freeze Off® Wart Removal Kit (Prestige Consumer Healthcare Inc., United States) (Fig.49). The cryotherapy kit contains an aerosol canister filled with a cryogenic mixture of dimethyl ether and propane and 8 single-use precision foam applicator tips with a handle (Fig.50). The precision foam applicator tip was inserted into the canister for 2 to 3 seconds and the cryogenic liquid was delivered to the applicator tip (Fig.51, 52 & 53). The surgeon waits 15 seconds for the cryogen to evaporate and to allow the temperature of the cryogen to drop down to approximately -55 °C before application on the cornea (Fig.54). The cryogen containing tip was applied on the cornea starting with the most pigmented area and progressively covering the entire lesion by rolling the foam tip slowly over the cornea (Fig.55 & 56). The cryotherapy procedure was done as two freeze-thaw cycles (50 seconds of cryogen application followed by thawing of 120 seconds) (Fig.57). In cases where the entire cornea was affected with melanosis, the corneal surface was divided into two halves and the cryotherapy procedure was performed as two freeze-thaw cycles in each half separately.

Medial Canthoplasty (MC) : Medial canthoplasty was performed in all six eyes of Group II that underwent cryotherapy following the same procedure as described in Group I.

3.2.10 Post-operative Care

Post-operative regimen for all the animals in both the groups included systemic antibiotics (Cefotaxime @ 25 mg/kg body weight, b.i.d. for 5 days) and NSAIDs

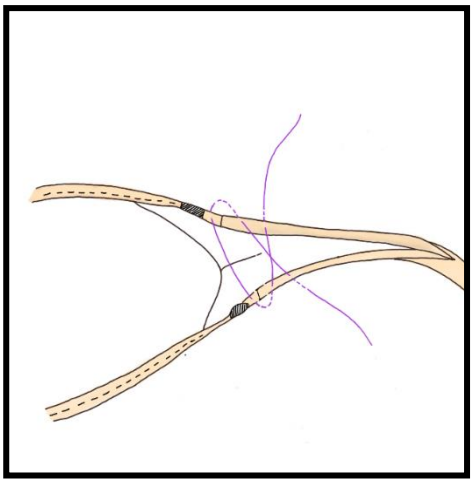


Fig.47 : Figure-of-8 suture pattern illustration

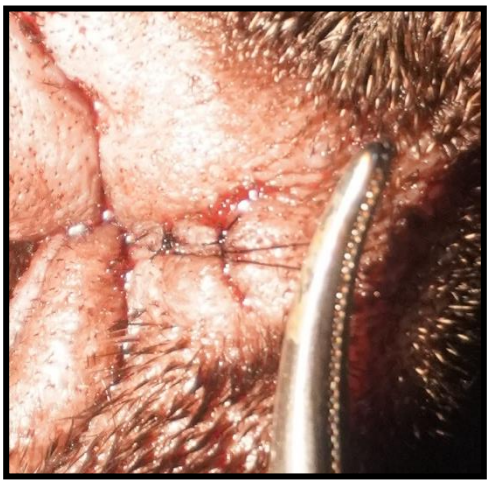


Fig.48 : The tags of figure-of-8 skin suture incorporated in the subsequent interrupted sutures

Fig.49 : Cryotherapy Kit (Content : Dimethyl ether & propane)

Fig.50 : Cryotherapy canister with handle & foam applicator tip

Fig.51 : Applicator tip loaded into the handle

Fig.52 : Inserting the tip loaded handle into the canister

Fig.53 : Press and hold the handle inside the canister for 3 seconds

Fig.54 : Handle removed from the canister & thawed for 15 seconds before application on the cornea

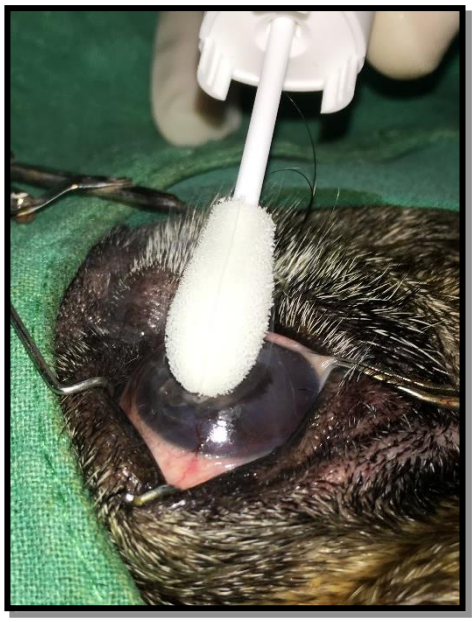


Fig.55 : Applying the cryogen loaded tip on the cornea for 50 seconds

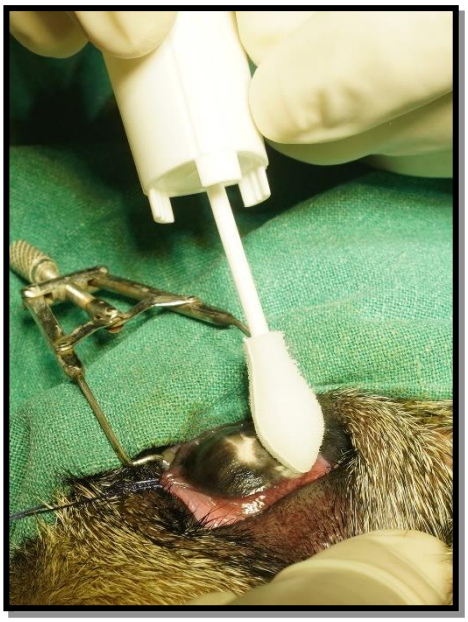


Fig.56 : The cryogen loaded tip is rolled slowly over the cornea

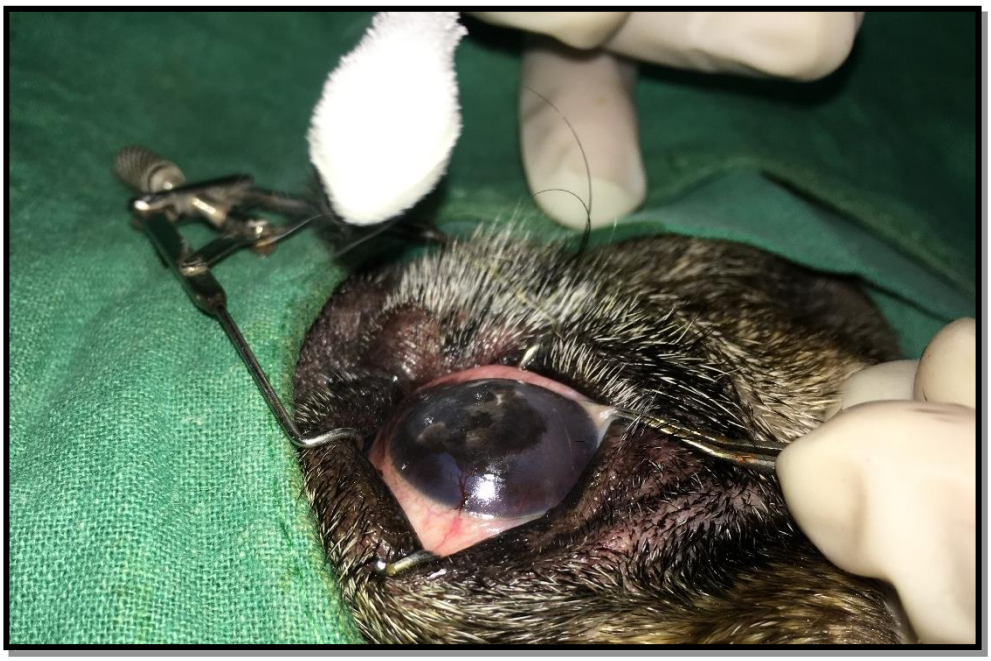


Fig.57 : Thawing the cornea for 2 minutes before second freeze cycle

(Meloxicam @ 0.2 mg/kg body weight, o.d. for 3 days) administered intramuscularly. The topical medication regimen varied between both the groups as described below

a. Group I

Topical NSAID 0.5% Ketorolac tromethamine (Ketodrops, Cipla Ltd, Mumbai, India) was instilled 4 times a day for 4 days. Topical lacrimomimetic eye drops (Carboxy methyl cellulose sodium) was instilled 4 times a day for 1 month post-operatively. Topical antibiotic eye drops (Gatifloxacin) was instilled on every 1 hour basis during the 1st post-operative week which was tapered to every 2 hour basis, 3 hour basis and 4 hour basis in the successive post-operative weeks. Topical lacrimostimulant eye drops (0.1% Cyclosporine) instillation was started when the fluorescein dye test result turned negative during the follow up.

b. Group II

Topical lacrimomimetic eye drops (Carboxy methyl cellulose sodium) was instilled on every 1 hour basis for 5 days followed by 4 times a day till 1 month post-operatively. Topical Lacrimostimulant eye drops (0.1% Cyclosporine) was instilled 4 times a day for 1 month. Topical antibiotic eye ointment (0.3%) Tobramycin (Nebracin, Sunways India Pvt. Ltd, Mumbai, India) was applied twice daily for 1 month post-operatively. Topical corticosteroids (1%) Prednisolone acetate (Gatiquin-P, Aditi Pharmaceuticals Pvt. Ltd, Solapur, India) instillation was started 5 days after surgery when the fluorescein dye test result turned negative.

3.2.11 Follow up

All the animals were followed up clinically in regular intervals wherever possible to evaluate the efficacy of the treatment protocol with respect to healing, restoration of vision and complications, if any. The vision of the animal was considered to be restored if the menace response turned positive after surgery and the corneal lesions did not affect the patient's visual axis pathway.

3.2.12 Statistical analysis

The data was analysed by using appropriate statistical methods wherever required.

CHAPTER IV

RESULT AND DISCUSSION

4.1 Screening Study

During the period of study, 100 clinical cases of Pug breed of dogs presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana for ailments other than ocular disorders were studied for the extent or degree of pigmentation using photography of the eyes taken after rinsing the eyes with sterile 0.9% normal saline solution.

4.1.1 Incidence of Pigmentary Keratitis

Out of the study population of 100 pug breed of dogs, a total of 96 dogs (96%) exhibited pigmentation of the eyes while only 4 dogs (4%) had a clear and transparent cornea as shown in the (Table 4) & (Fig.58). Of the 96 dogs which exhibited corneal melanosis, all of them (100%) exhibited bilateral pigmentation of the cornea to some extent or degree with varying pattern of pigmentation as depicted in the (Table 5).

Table 4 : Incidence of Pigmentary keratitis

Characteristics	No. of eyes	Incidence (%)
Pigmented Cornea	192	96
Transparent Cornea	8	4

Table 5 : Distribution of corneal melanosis on the basis of eye affected

Characteristics of affection		No. of dogs	Distribution (%)
Unilateral	Right eye	Nil	Nil
	Left eye	Nil	Nil
Bilateral		96	100

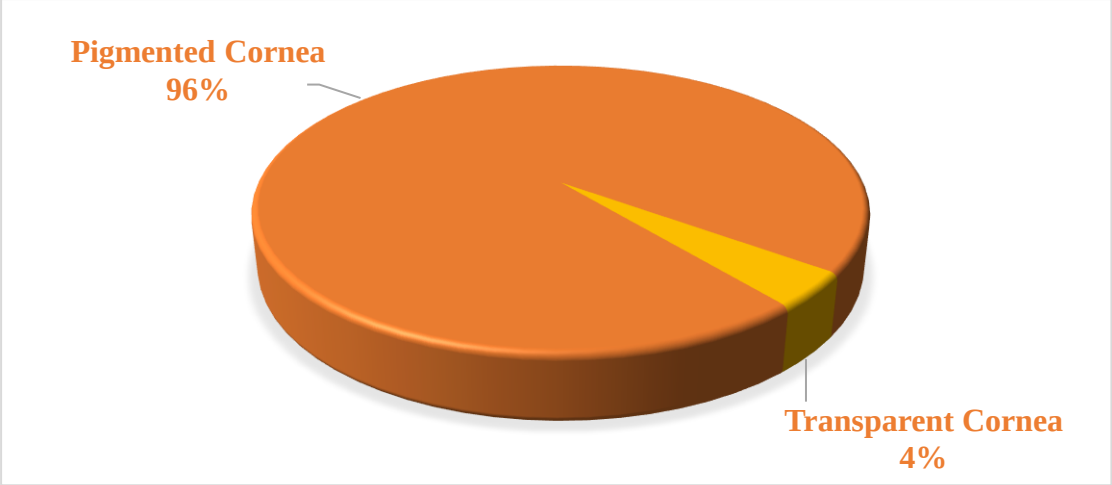


Fig.58 : Incidence of Pigmentary Keratitis

The results were in accordance with that recorded by Labelle et al (2013) that corneal pigmentation was noticed in at least 1 of the eyes of 243 Pugs (82.4%) of a study population of 295 Pugs. The results also coincided with the study done by Maini et al. (2019) which reported that pigmentary keratitis was detected in at least 1 of the eyes of 193 Pugs (91.9%) out of a total of 210 Pugs. The study also reported that 176 (91.2%) dogs out of the 193 dogs which exhibited corneal melanosis had bilateral pigmentation and 17 (8.8%) dogs had unilateral affection.

Krecny et al. (2015) reported incidence of corneal pigmentation in 101 eyes (39.14%) out of a total of 258 eyes studied for ocular abnormalities. Though most of the studies reported a higher incidence of corneal melanosis in Pugs, the differences in the incidence rate can be attributed to the differences in study population, genetic background, breeding patterns or any other yet to be determined factors.

4.1.2 Evaluation of severity of pigmentation

The photographs of the cornea were studied for the extent of pigmentation using the grading system suggested by Maini et al. (2019) and out of the 192 eyes studied, 29 eyes (15.10%) had mild degree, 76 eyes (39.58%) had moderate degree and 87 eyes (45.31%) had severe degree of pigmentation as shown in (Table 6) & (Fig.59). The mean score of pigmentation was 2.98±0.21, 6.89±0.18 and 13.35±0.16 for mild, moderate and severe degrees of pigmentation as mentioned in (Table 7) & (Fig.60).

Various studies conducted earlier reported results contradictory to the current study stating that corneal melanosis was typically mild to moderate in occurrence. Amol (2016) reported that out of the 87 eyes studied, 29 eyes (33.33%) had mild degree of pigmentation, 13 eyes each (14.94%) had moderate and severe degree of pigmentation while 32 eyes (36.78%) had complete corneal pigmentation. Maini et al. (2019) reported that out of 369 eyes, 171 eyes (46.3%) had mild degree, 184 eyes had moderate degree and 14 eyes (3.8%) had severe degree of corneal pigmentation.

Table 6 : Distribution of severity of pigmentation

Severity of Pigmentation	No. of eyes	Distribution (%)
Mild	29	15.10
Moderate	76	39.58
Severe	87	45.31

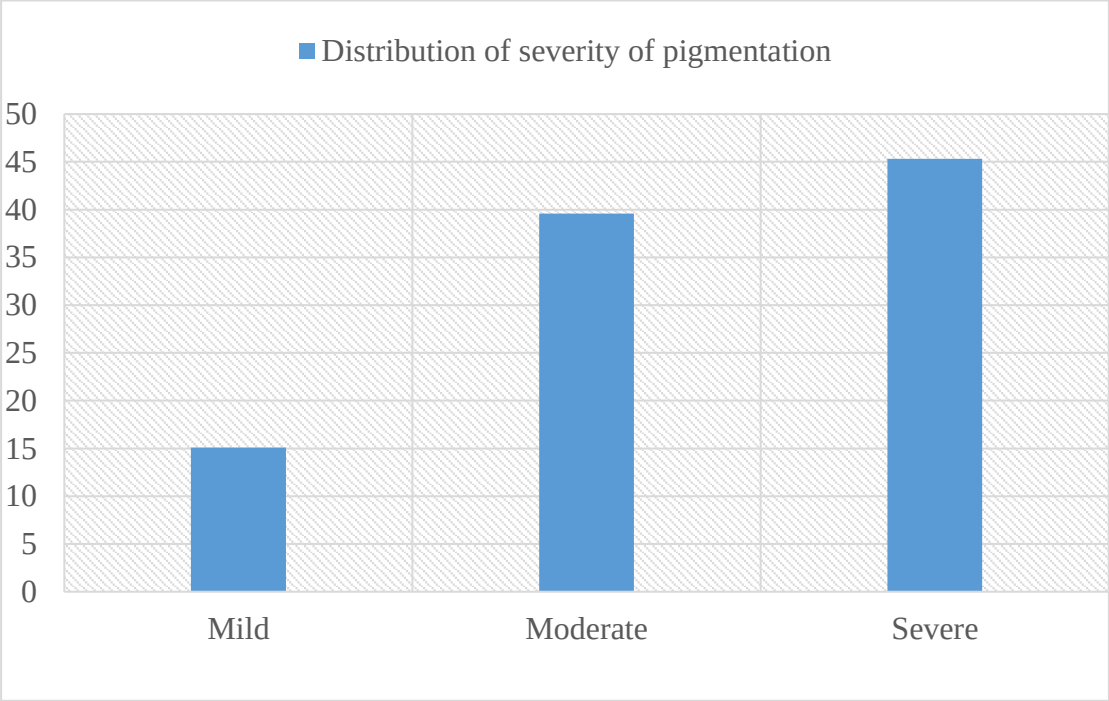


Fig.59 : Distribution of severity of pigmentation

Labelle et al. (2013) stated that out of the 467 eyes detected with corneal melanosis, 146 eyes (31.26%) exhibited very mild degree of melanosis, 216 eyes (46.25%) had mild degree, 58 eyes (12.42%) had moderate degree and 47 eyes

(10.06%) had severe degree of corneal pigmentation. These variations in the results with regard to the severity of corneal pigmentation may be attributed to the different standards used for the evaluation of the severity of pigmentation and the breeding pattern adapted in different geographical locations.

Table 7 : Mean pigmentation score of severity of pigmentation

Severity of Pigmentation	Mean Score ± SE
Mild	2.98±0.21
Moderate	6.89±0.18
Severe	13.35±0.16

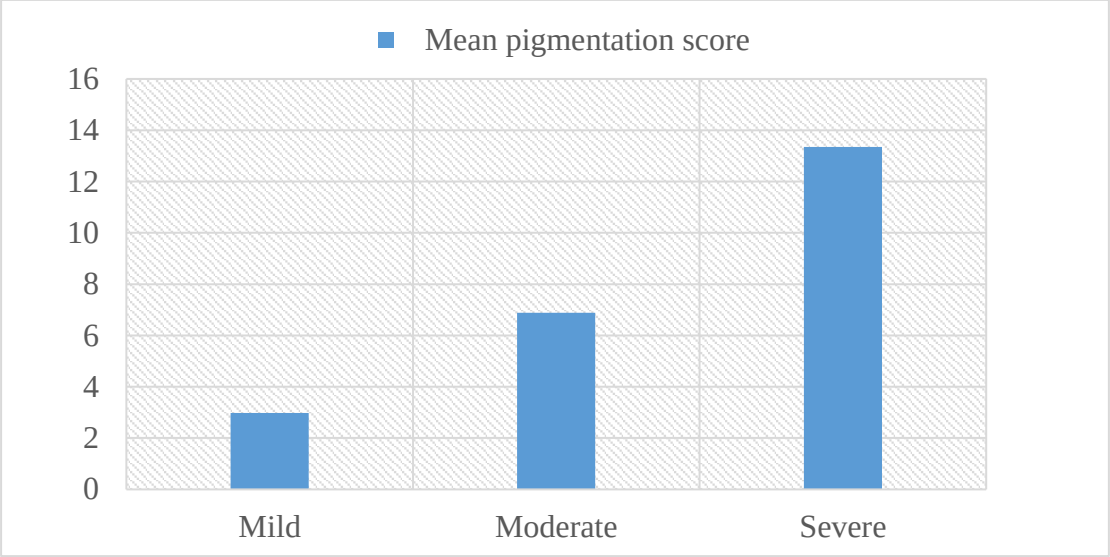


Fig.60 : Mean pigmentation score of severity of pigmentation

4.1.3 Age-wise distribution of pigmentary keratitis

The mean age of the animals in the study population was 5.27±0.29 years (range, 0.42 to 10.0 years). Corneal pigmentation was highly distributed in the adult age group (57.29%) followed by the senile group (29.17%) and the younger age group (13.54%) as mentioned in the (Table 8) & (Fig.61). Among the 4 animals that had a clear and transparent cornea, 3 were of younger age group and 1 was an adult.

However, contradictory results were obtained in various studies. Amol (2016) reported that pigmentary keratitis was found unilaterally in 6, 11 and 6 dogs in the age

group of < 1 year, 1-5 years and > 5 years respectively while it was found bilaterally in 8, 10 and 14 dogs in the age group of < 1 year, 1-5 years and > 5 years respectively. Corneal pigmentation was almost equally distributed among the adult and the senile group followed by the juvenile group.

Table 8 : Age-wise distribution of pigmentary keratitis

Age - Category	No. of animals	Distribution (%)
0 - 3 Years (Younger group)	13	13.54
3 - 7 Years (Adult group)	55	57.29
> 7 Years (Senile group)	28	29.17

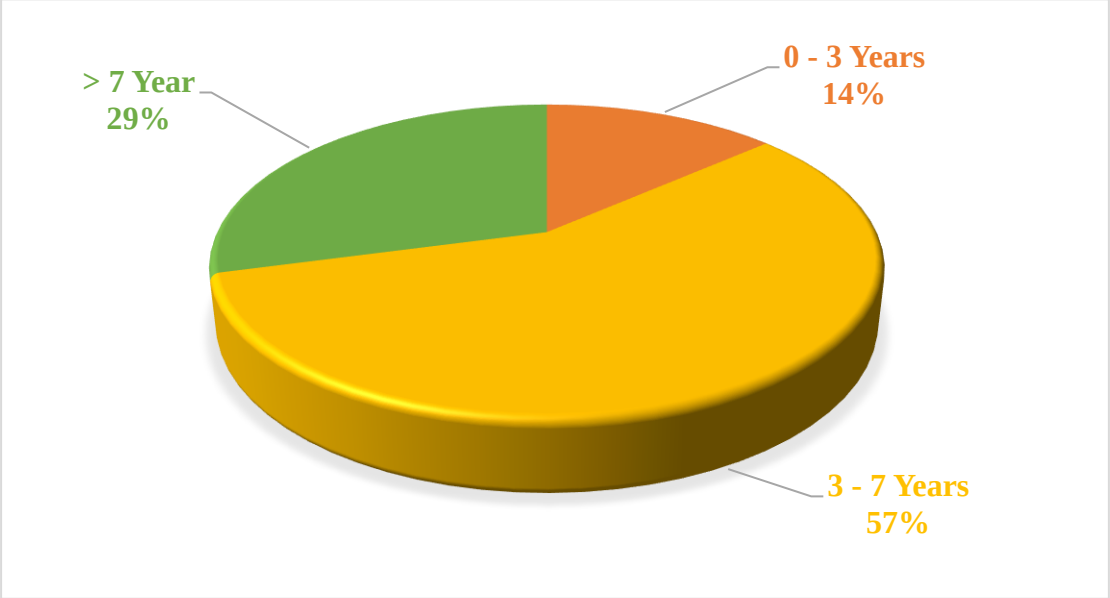


Fig.61 : Age-wise distribution of pigmentary keratitis

Anoop et al. (2016) stated that pigmentary keratitis was distributed highly in the younger animals aged between 1 to 3 years (53%) followed by the animals below 1 year of age (30.9%), animals above 5 years of age (15%) and animals between 3 to 5 years of age (4%).

These variations in the distribution of corneal pigmentation among various age groups can be attributed to the different standards used for the classification of age groups in various studies.

Table 9 : Age-wise distribution of severity of pigmentation

Age - Category	Severity of Pigmentation		
	Mild (%)	Moderate (%)	Severe (%)
0 - 3 Years	46.15	34.62	19.23
3 - 7 Years	10.97	36.58	52.44
> 7 Years	8.93	48.21	42.86

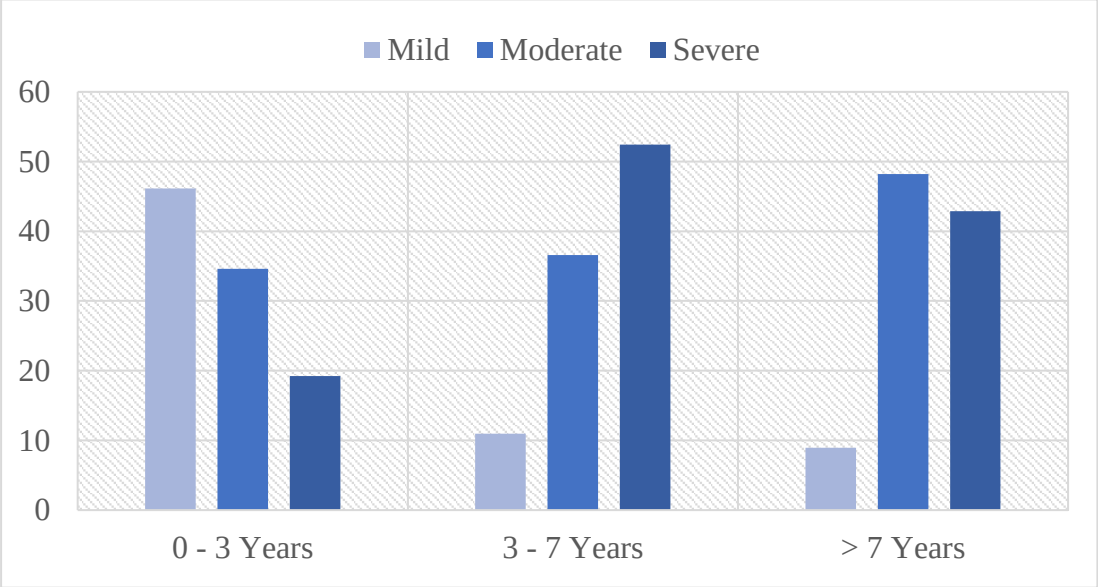


Fig.62 : Age-wise distribution of severity of pigmentation

The severity of pigmentation was mild to moderate in the younger age group while the severity was moderate to severe in the adult and senile age group as shown in the (Table 9) & (Fig.62). The mean pigmentation score of the younger, adult and senile age group was 5.15±0.87, 9.60±0.47 and 9.43±0.60 respectively. The increase in the severity of the pigmentation with the increase of age can be attributed to the persistent progression of the melanocytes as a response to the underlying chronic inflammatory process.

The detection of corneal pigmentation (P = 0.0177, 95% level of significance) and the severity of pigmentation (P <0.0001) in pugs was significantly associated with the age of the animal as shown in (Table 10). Maini et al. (2019) reported that detection of pigmentary keratitis in pugs and not its severity was significantly associated with increase age. The results were also contradictory to that stated by Labelle et al. (2013) that detection of corneal melanosis in pugs and its severity was not significantly associated with age. The variations reported in the relationship between age, detection of pigmentation and its severity may be attributed to the different study population with different median age.

Table 10 : Animal variables and their association with corneal pigmentation

Predictor	**Detection of pigmentation (P-value)**	**Severity of pigmentation (P-value)**
Age of the animal (years)	0.0177	0.00003
Sex of the animal (male vs female)	0.3393	< 0.00001
Coat colour (black vs fawn)	1.0	0.075
Side of the eye (left vs right)	1.0	0.766
The P-values were determined with $\chi 2$ of Fisher exact tests. Values of P < 0.05 are considered significant		

4.1.4 Sex-wise distribution of pigmentary keratitis

Among the study population, pigmentary keratopathy was highly distributed in the male population (54.17%) compared to the female population (45.83%) as shown in (Table 11) & (Fig.63).

Various studies reported contradictory results regarding the sex which had higher distribution of pigmentary keratitis. Krecny et al. (2015) stated that male animals had higher distribution while Azoulay (2014), Allgoewer & Hoecht (2010) and Petersen-Jones et al. (2007), reported that female animals had a higher distribution with respect to corneal melanosis compared to the male animals of the study population.

Table 11 : Sex-wise distribution of pigmentary keratitis

Sex of the animal	No. of animals	Distribution (%)
Male	52	54.17
Female	44	45.83

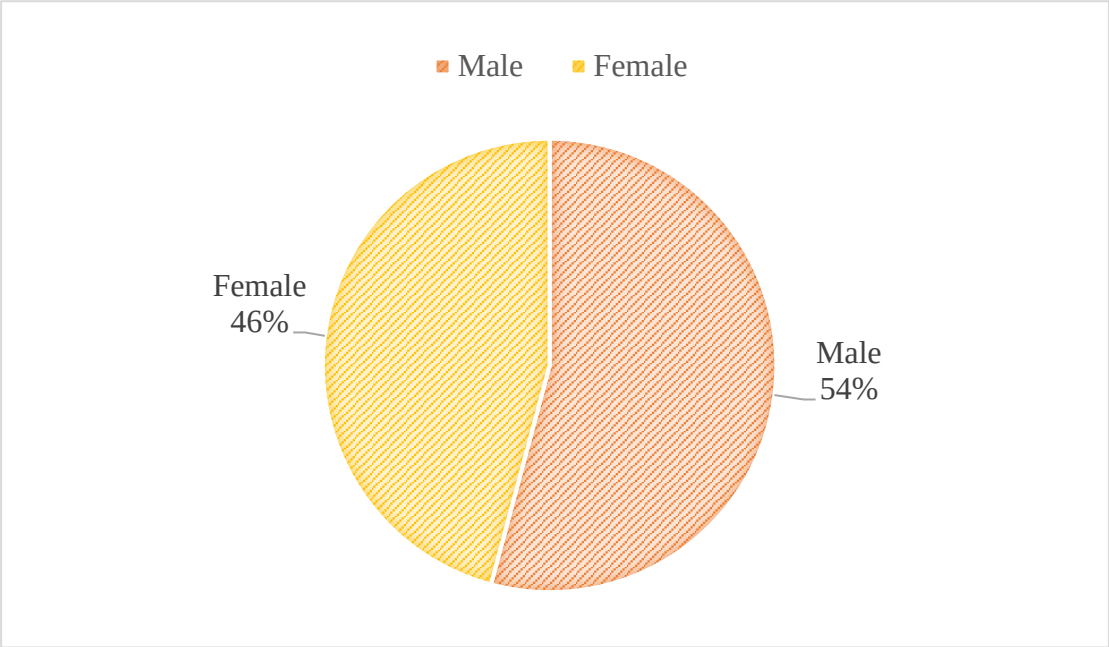


Fig.63 : Sex-wise distribution of pigmentary keratitis

The degree of corneal pigmentation was severe (58.65%) in the male animals while the female animals exhibited moderate (43.18%) degree of pigmentation as depicted in the (Table 12) & (Fig.64). The mean pigmentation score of the eyes in the male and female animals was 10.34±0.44 and 7.13±0.53 respectively.

Table 12 : Sex-wise distribution of severity of pigmentation

Sex of the animal	Severity of Pigmentation		
	Mild (%)	Moderate (%)	Severe (%)
Male	3.85	37.5	58.65
Female	28.41	43.18	28.41

The detection of corneal pigmentation (P = 0.3393) was not significantly associated with the sex of the animal while the severity of the pigmentation (P<0.00001) was associated with the sex of the animal. Labelle et al. (2013) stated that severity of corneal pigmentation was significantly associated with the sex of the animal and male animals exhibited moderate to severe pigmentation compared with the female animals which exhibited no pigmentation to very mild and mild corneal pigmentation. Maini et al. (2019) reported that no significant association was evident between the sex and the severity of pigmentation.

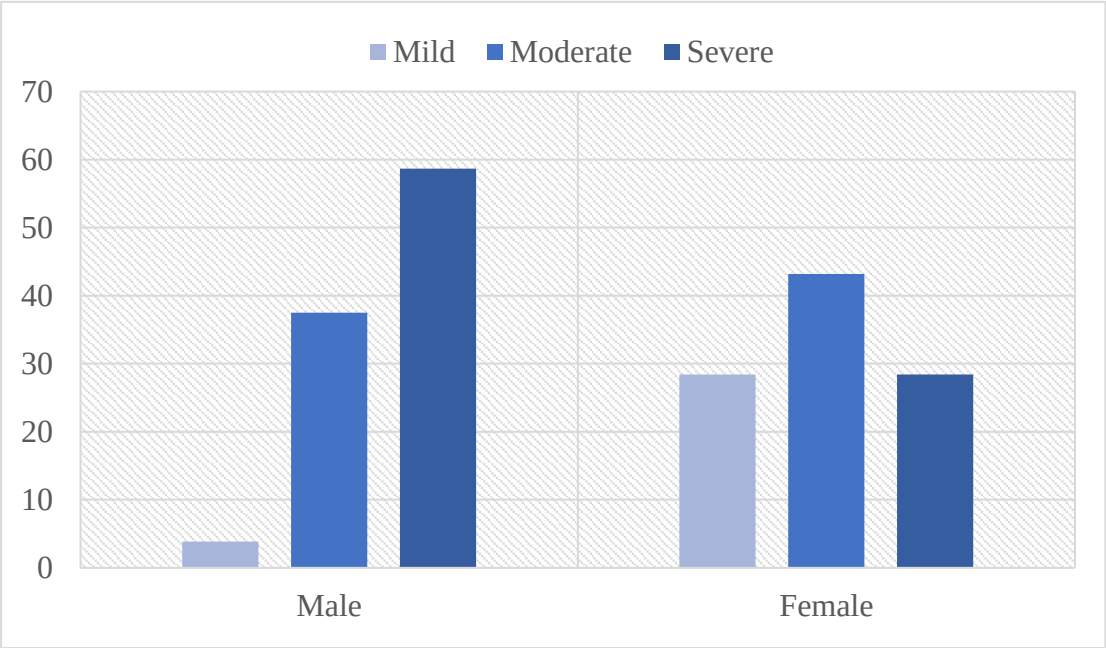


Fig.64 : Sex-wise distribution of severity of pigmentation

4.1.5 Coat colour-wise distribution of pigmentary keratitis

Among the study population, higher distribution of pigmentary keratitis was seen in the fawn coat colour (80.21%) followed by the black coat colour (19.79%) while the incidence of corneal pigmentation was above (95%) in both the coat colours as shown in the (Table 13).

The black coat colour animals exhibited higher percentage of moderate degree of pigmentation (52.63%) while the fawn coat colour animals had higher percentage of severe degree of pigmentation (49.35%) as described in the (Table 14) & (Fig.65). The mean pigmentation score of the eyes in black and fawn coat colour Pugs was 7.48±0.78 and 9.18±0.41 respectively.

Table 13 : Coat colour-wise distribution of pigmentary keratitis

Coat colour of the animal	No. of animals	Distribution	Incidence (%)
Black	19	19.79	95
Fawn	77	80.21	96.25

The detection of corneal pigmentation (P=1) and its severity (P=0.075) was not significantly associated with the coat colour of the animal. Labelle et al. (2013) reported that detection of corneal pigmentation was not significantly associated with coat colour of the animal but the severity of pigmentation was significantly associated with the coat

Table 14 : Coat colour-wise distribution of severity of pigmentation

Coat colour of the animal	Severity of Pigmentation		
	Mild (%)	Moderate (%)	Severe (%)
Black	18.42	52.63	28.95
Fawn	14.29	36.36	49.35

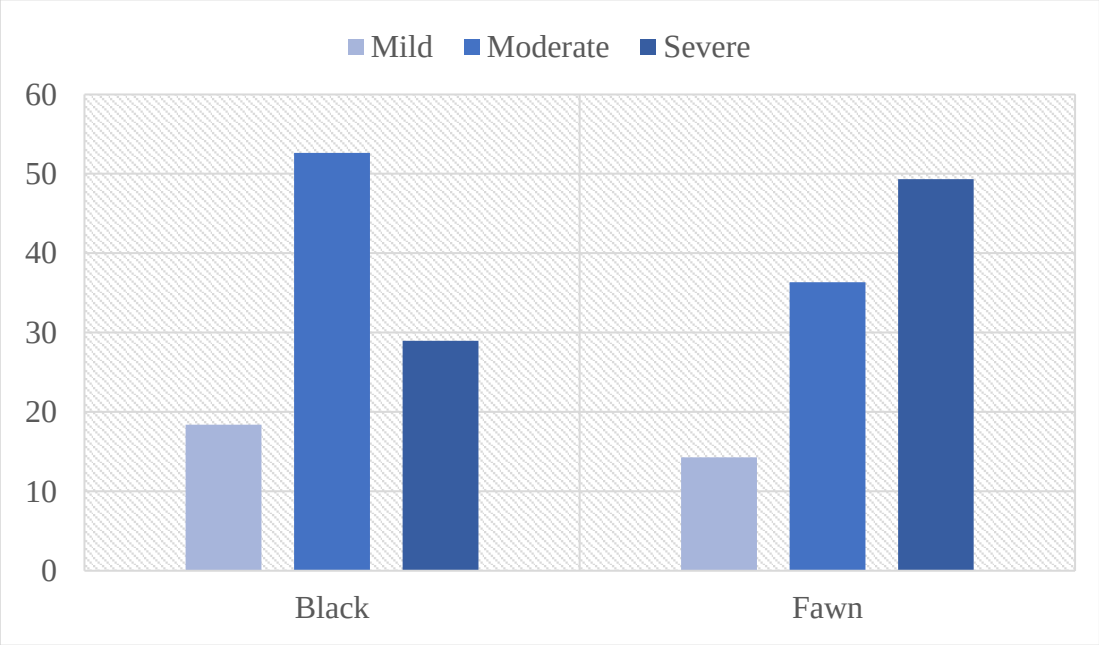


Fig.65 : Coat colour-wise distribution of severity of pigmentation

colour and the pugs with fawn coat colour were stated to exhibit a relatively severe degree of pigmentation compared with the black coat colour.

4.1.6 Distribution of severity of pigmentation based on the eye

The severity of pigmentation was moderate to severe in both the left and right eye of the animals included in the study population as shown in the (Table 15). The mean pigmentation score of the affected left and right eyes was 8.9±0.52 and 8.78±0.52 respectively. No significant association was evident between the severity of pigmentation and the side of the eye (P=0.766) affected.

Table 15 : Distribution of severity of pigmentation based on the eye

Eye of the animal	Severity of Pigmentation		
	Mild (%)	Moderate (%)	Severe (%)
Left	13.54	41.67	44.79
Right	16.67	37.5	45.83

4.1.7 Classification of corneal pigmentation based on patterns

The pattern of corneal pigmentation was studied in all the 192 eyes that exhibited corneal melanosis and was categorised as suggested by Charbiwala (2019).

Table 16 : Classification of corneal pigmentation based on patterns

Pattern of Pigmentation	Distribution (%)
Diffuse	34.38
Endothelial Deposits	Nil
Medial Pyramid	20.83
Paintbrush	10.94
Patchy	21.35
Streak	12.50
Whorls	Nil

Among the study population, diffuse pattern of pigmentation (34.38%) had the highest distribution followed by the patchy pattern (21.35%), medial pyramid pattern (20.83%), streak or limbal brush border pattern (12.50%) and paintbrush pattern (10.94%) as shown in (Table 16) & (Fig.66). Whorl pattern and endothelial deposit patterns were not identified in any of the study cases. In cases where the eye exhibited multiple patterns, the prominent one among them was considered for classification.

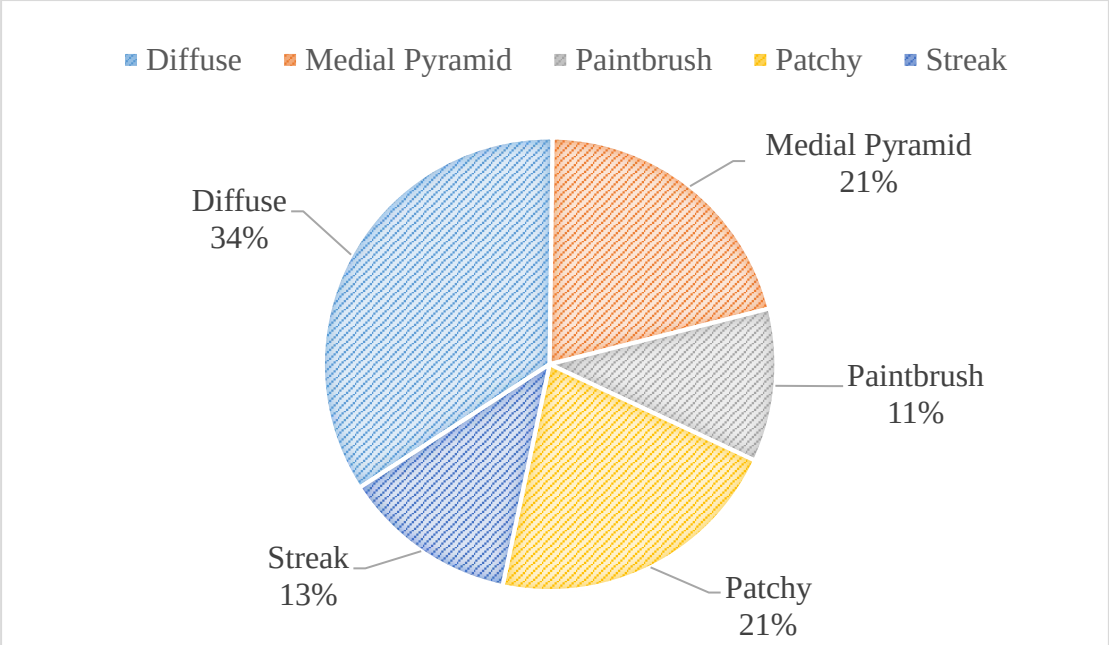


Fig.66 : Classification of corneal pigmentation based on patterns

Charbiwala (2019) reported results similar to the present study that majority of the cases had diffuse pattern (43.94%) of pigmentation followed by medial pyramid (31.82%), patchy (10.61%), whorl (4.54%), streak (4.54%), paintbrush (4.54%) and endothelial deposit (4.54%) pattern of pigmentation.

4.2 Clinical Study

During the entire period of study, 141 cases of canine referred to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana for primary ocular affections were studied by performing complete ophthalmological examination and the diagnosis was recorded.

4.2.1 Age-wise distribution of ocular disorders

Majority of the ocular disorders were distributed among the younger age group (0 – 3 Years old) followed by the adults (3 – 7 Years old) and the senile group (> 7 Years old) (Table 17) & (Fig.67).

The results were resemblant with that reported by Antonia et al. (2014) that younger dogs less than 2 years old are more susceptible (57.08%) to ocular affections. The results were similar to that reported by Akinrinmade & Ogungbenro (2015) that majority of the ocular disorders (68.80%) were recorded in animals less than 5 years old and analogous with that reported by Kumar et al. (2018) that dogs of younger age group (0 – 3 years) were presented with majority of ocular ailments (51.7%).

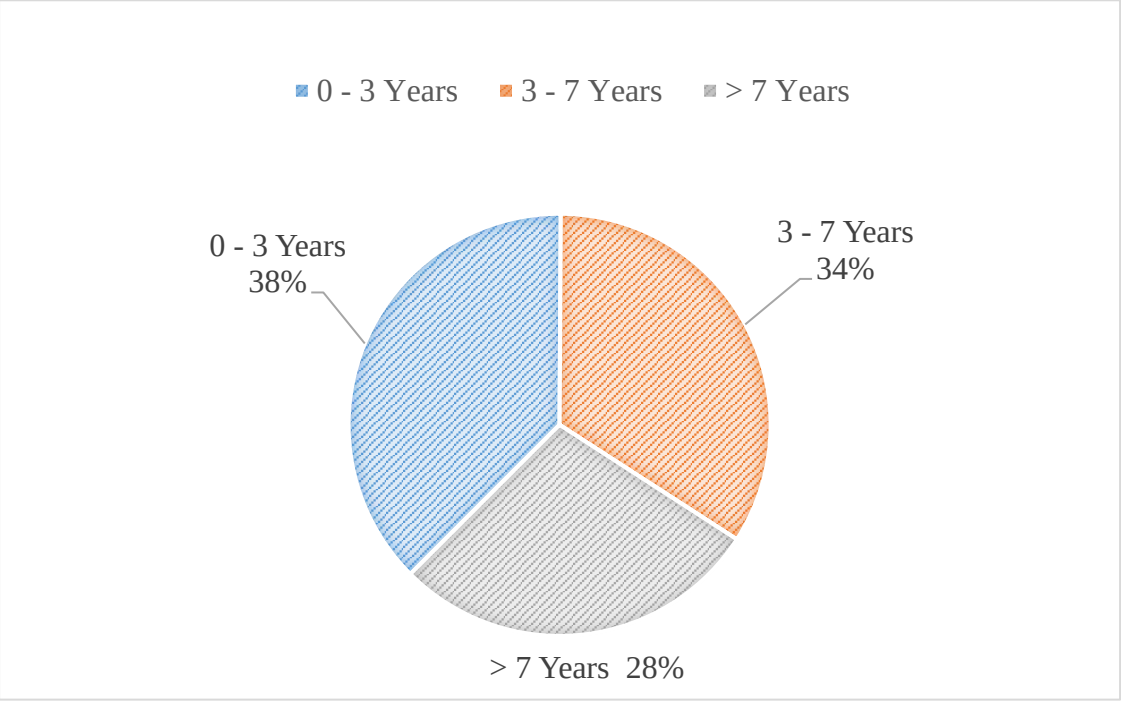


Fig.67 : Age-wise distribution of ocular disorders

The results were disparate from that reported by Tamilmahan et al. (2013) that adult dogs above 5 years of age represented the major share of ocular disorders (47.68%) followed by middle aged dogs (1-5 years) and young dogs (less than 1 year).

The higher occurrence of the ocular disorders in the younger age group may be due to their playful nature which makes them more prone for traumatic injuries and

partly due to the excess care and attention towards them by the owners leading to higher numbers being presented to the clinics.

Table 17 : Age-wise distribution of ocular disorders

Age category	Number of cases	Distribution (%)
0 – 3 Years (Younger group)	53	37.59
3 – 7 Years (Adult group)	48	34.04
> 7 Years (Senile group)	40	28.37

4.2.2 Sex-wise distribution of ocular disorders

Of the 141 animals presented for primary ocular disorders, majority of them (71.63%) were male animals while the rest (28.37%) were female (Table 18) & (Fig.68).

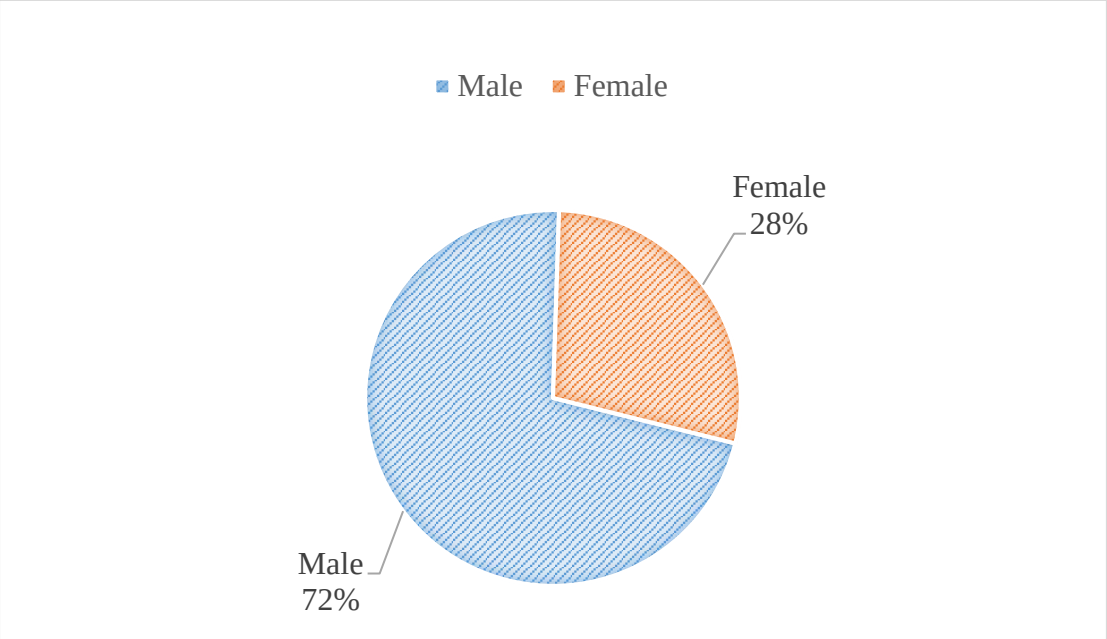


Fig.68 : Sex-wise distribution of ocular disorders

Table 18 : Sex-wise distribution of ocular disorders

Sex of the animal	Number of cases	Distribution (%)
Male	101	71.63
Female	40	28.37

The result were in accordance with that reported by Tamilmahan et al. (2013) that male animals (60.32%) were highly affected with ophthalmic diseases than female animals (39.29%). Kumar et al. (2018) also reported that male animals (65%) were more susceptible than female animals (35%) to ocular ailments. However the results were contradictory to that recorded by Akinrinmade & Ogungbenro (2015) that female animals (42.42%) were more often presented with ocular disorders than male animals (35.49%). The higher incidence of ocular affections in male dogs can be due to their general aggressive nature and feral attitude when compared with the female dogs.

4.2.3 Breed-wise distribution of ocular disorders

In the current study, the highest distribution of ocular disorders was among the Pug (31.21%) breed of dogs followed by Labrador Retriever (16.31%), Spitz (7.80%), Beagle and Chow Chow (4.96% each), American Bully and Cocker Spaniel (2.84% each), German Shepherd, Golden Retriever, Pitbull and Shih Tzu (2.13% each), Boxer, Bull dog, French Bull dog and Siberian Husky (1.42% each) and Bull terrier, Lhasa Apso, Rottweiler, Saint Bernard and Yorkshire terrier (0.71% each). Non-Descript dogs estimated around 11.35 percent of the total ocular disorders among canines (Table 19) & (Fig.69).

The result of the current study was in resemblance with that reported by Antonia et al. (2014) that Chinese pugs had the highest incidence (greater than 50%) of ocular affections. The result was also analogous with the study of Kumar et al. (2018) that reported maximum prevalence of ocular ailments among Pugs (28.3%).

The higher incidence of the ocular affections among pug breed of dogs may be due to lagophthalmos and macroblepharon but the factor that this breed of dog was seen as a favourite pet among the local population has to be considered.

The higher incidence may also be due to their pure breed characteristics as suggested by Whitley et al. (1995) that brachycephalic breed of dogs exhibit 18 or more ocular disorders when compared with the common average of 6 or more ocular disorders among the other breed of dogs.

Though pugs were reported to have more incidence of ocular ailments, various breeds have been recorded with highest prevalence of ocular disorders in various studies based on the geographical location. Kalaiselvan et al. (2009) reported that highest incidence of ocular affections was among Spitz breed of dogs.

Tamilmahan et al. (2013) stated that highest incidence was recorded among Spitz and German shepherd breed of dogs while Akinrinmade & Ogungbenro (2015) reported that Alsatian breed had the highest incidence (22.08%).

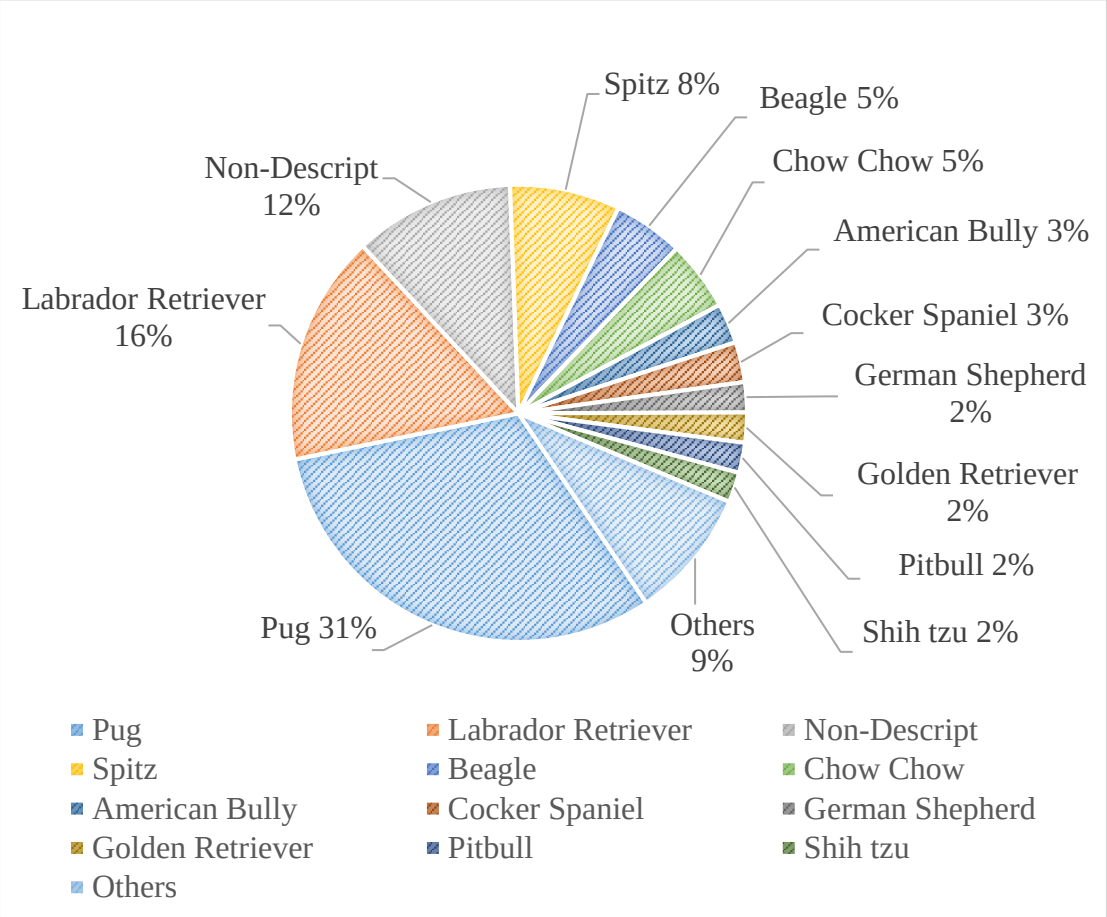


Fig.69 : Breed-wise distribution of ocular disorders

Table 19 : Breed-wise distribution of ocular disorders

Breed	Number of cases	Distribution (%)
Pug	44	31.21
Labrador Retriever	23	16.31
Non-Descript	16	11.35
Spitz	11	7.80
Beagle	7	4.96
Chow Chow	7	4.96
American Bully	4	2.84
Cocker Spaniel	4	2.84
German Shepherd	3	2.13
Golden Retriever	3	2.13
Pitbull	3	2.13
Shih Tzu	3	2.13
Boxer	2	1.42
Bull dog	2	1.42
French Bull dog	2	1.42
Siberian Husky	2	1.42
Bull terrier	1	0.71
Lhasa Apso	1	0.71
Rottweiler	1	0.71
Saint Bernard	1	0.71
Yorkshire terrier	1	0.71

4.2.4 Distribution of ocular disorders based on the eye affected

In the current study, bilateral ocular affections (65.96%) formed the major part of all ocular ailments followed by the left eye (19.86%) and the right eye (14.18%) (Table 20) & (Fig.70).

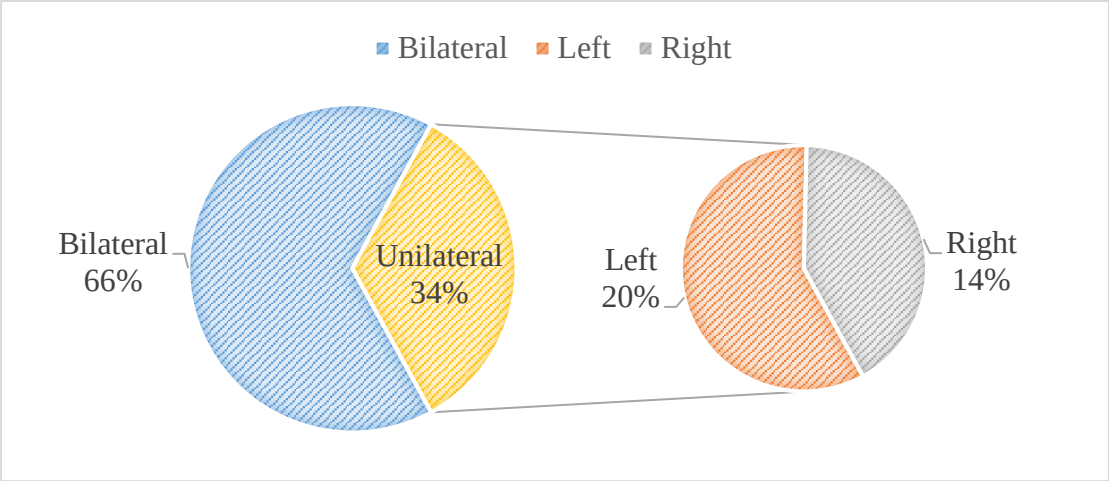


Fig.70 : Distribution of ocular disorders based on the eye affected

The results were concurrent with that reported by Akinrinmade & Ogungbenro (2015) that bilateral eye affections (54.98%) are more common than the disorders of right eye (21.11%) and left eye (13.42%) but were contradictory to that reported by Tamilmahan et al. (2013) that ocular affections had higher incidence in left eye (42.92%) compared to right eye (37.79%) and bilateral ocular affections (18.89%).

Table 20 : Distribution of ocular disorders based on the eye affected

Eye affected	Number of cases	Distribution (%)
Both eyes	93	65.96
Left eye	28	19.86
Right eye	20	14.18

Antonia et al. (2014) recorded that ophthalmic lesions were over presented in the right eye (45.72%) compared with the left eye (34.99%) and bilateral ocular affections (19.29%). These variations in the occurrence of ocular disorders between the right and left eyes may be due to chance and doesn't signify any predisposition of a particular side of eye to ocular ailments.

4.2.5 Distribution of ocular disorders based on anatomical location

The ocular disorders recorded in this study were categorised based on the anatomical structure involved as described in (Table 21) & (Fig.71). The corneal disorders (36.17%) constituted the major part of ocular affections followed by the lens (17.73%), orbit & globe (16.31%), eyelids (15.60%), conjunctiva (7.09%), fundus (5.67%) and uvea (1.42%).

The results were analogous with that reported by Tamilmahan et al. (2013) that corneal lesions (28%) had the higher incidence among ocular disorders followed by traumatic injuries. The results were also concurrent with those reported by Chakrabarti et al. (2014) that corneal disorders formed more than 50 percent of all ocular affections. Antonia et al. (2014) recorded that corneal affections formed the major part of the study on the occurrence of ophthalmic ailments in dogs.

Sale et al. (2013) reported that maximum cases of ocular affections involved the lens (34%) followed by cornea (28%). Eyelids (55.1%) were found to be the most commonly affected ocular structure in another the study by Martins & Barros (2014). The study by Akinrinmade & Ogungbenro (2015) stated that highest incidence of ocular affections was noted in the eyelids and conjunctiva (58.01%) followed by lens or globe (22.51%) and cornea (19.48%).

Table 21 : Distribution of ocular disorders based on anatomical location

Ocular affections	Number of cases	Distribution (%)
Cornea	51	36.17
Lens	25	17.73
Orbit & globe	23	16.31
Eyelids	22	15.60
Conjunctiva	10	7.09
Fundus	8	5.67
Uvea	2	1.42

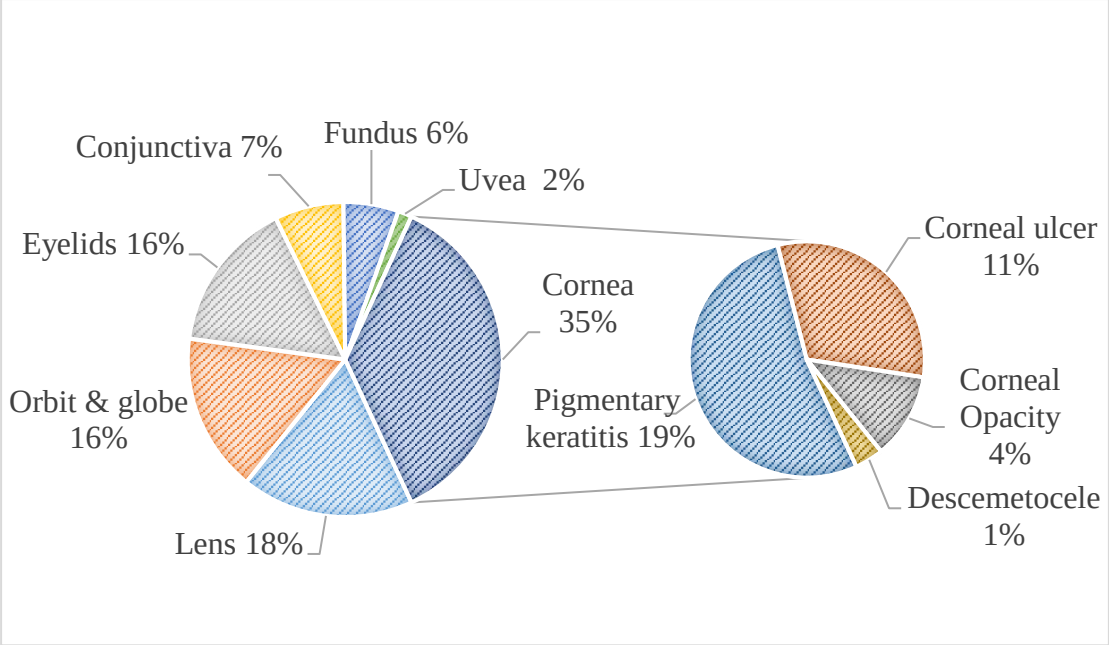


Fig.71 : Distribution of ocular disorders based on anatomical location

These variations recorded with the various ocular structures being presented with highest incidence of affections in various studies may be attributed to the difference of study population due to fondness among the local population for different breeds in different geographical locations along with the different standards of classification followed by different studies.

4.2.6 Pigmentary keratitis

Among the corneal disorders recorded in the study, pigmentary keratitis (52.94%) had the major distribution followed by corneal ulcers (31.37%), corneal opacity (11.76%) and descemetocele (3.92%) as shown in the (Table 22).

Table 22 : Distribution of corneal disorders based on the affection

Corneal disorder (n=51)	Number of cases	Distribution (%)
Pigmentary keratitis	27	52.94
Corneal ulcer	16	31.37
Corneal opacity	6	11.76
Descemetocele	2	3.92

Kumar et al. (2018) reported that pigmentary keratitis (21.7%) had the highest distribution among the ocular disorders recorded followed by corneal ulcer (21.7%) and corneal opacity (18.3%). Antonia et al. (2014) studied that among the disorders that affected the superficial cornea, ulcerative keratitis (18.66%) had the major distribution followed by corneal opacity (13.84%) and pigmentary keratitis (10.26%).

4.3 Surgical study

The study was conducted on 12 eyes of 12 pug breed of dogs presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana with the history of poor vision and pigmentary keratitis. All the animals underwent a complete ophthalmological examination to establish a diagnosis and the animals were selected randomly for the surgical treatment.

The study was conducted in two groups. In Group I, the animals were treated for pigmentary keratitis by performing Superficial keratectomy along with Medial canthoplasty. In Group II, the animals were treated for the same condition by performing Cryotherapy along with Medial canthoplasty as mentioned in the (Table 23).

Table 23 : Surgical procedures followed in different treatment groups

Group	Surgical Procedure	No. of cases
I	Superficial keratectomy with Medial canthoplasty (SK + MC)	6
II	Cryotherapy with Medial canthoplasty (Cryo + MC)	6

4.3.1 Age-wise distribution of animals operated for pigmentary keratitis

Mean age of the animals in the Group I that underwent superficial keratectomy along with medial canthoplasty was 5.33±0.25 years (4.5 to 6 years) while the mean age of the animals that underwent cryotherapy with medial canthoplasty was 6.33±0.93 years (4.5 to 10 years). Out of the 12 animals included in the study, 10 animals were of the adult age group (3 to 7 years) and 2 animals belonged to the senile age group (> 7 years) as shown in (Table 24) & (Fig.72).

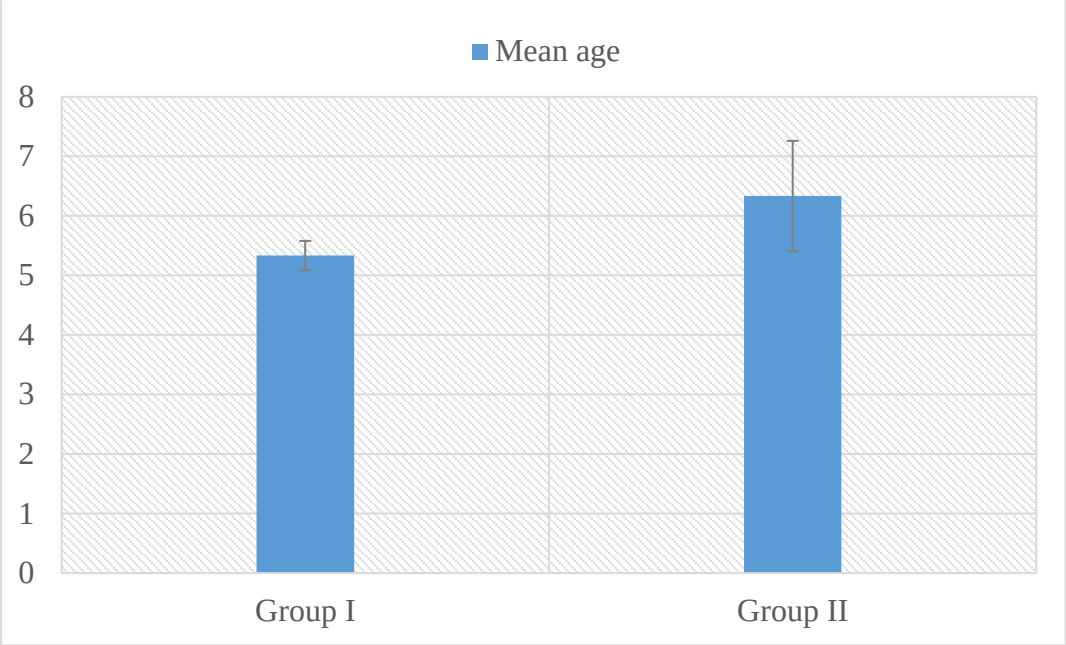


Fig.72 : Mean ± SE value of age of animals of different treatment groups

All the animals included in the study were above 4 years of age which indicated that owners were unaware of the condition in the early stages and the animals were most often reported only in the later stages of affection.

Table 24 : Age-wise distribution of animals operated for pigmentary keratitis

Age category	No. of cases in Group I	Distribution (%)	No. of cases in Group II	Distribution (%)
0 – 3 Years	-	-	-	-
3 – 7 Years	6	100	4	66.67
> 7 Years	-	-	2	33.33

4.3.2 Sex-wise distribution of animals operated for pigmentary keratitis

Out of the 12 animals operated in the study, 11 were male animals and 1 was a female animal. In Group I, 5 male animals (83.33%) and 1 female animal (16.67%) underwent surgery while in Group II all 6 animals that underwent surgery were male animals (100%) as mentioned in (Table 25).

Krecny et al. (2015) stated that male animals had higher distribution while Azoulay (2014), Allgoewer & Hoecht (2010) and Petersen-Jones et al. (2007) reported

that female animals had a higher distribution with respect to corneal melanosis compared to the male animals of the study population.

Table 25 : Sex-wise distribution of animals operated for pigmentary keratitis

Sex of the animal	No. of cases in Group I	Distribution (%)	No. of cases in Group II	Distribution (%)
Male	5	83.33	6	100
Female	1	16.67	-	-

4.3.3 Clinical signs associated with pigmentary keratitis

The animals in the present study exhibited clinical signs such as episcleritis, conjunctivitis, pigmentation of cornea and conjunctiva, corneal opacity, neovascularisation and corneal fibrosis with partial to total loss of vision in the affected eye. The ocular discharge varied from watery to mucoid and mucopurulent in the study population with majority of the animals exhibiting mucoid or mucopurulent ocular discharge.

4.3.4 Concurrent findings recorded in animals operated for pigmentary keratitis

The concurrent findings or the predisposing factors recorded in the present study were Medial Entropion of Lower Eyelid (MELE), Medial Caruncular Trichiasis (MCT) and KCS. Almost all the dogs involved in the study exhibited MCT while more than 80 percent of the dogs exhibited MELE. KCS was recorded in 40 percent of the animals in the study as shown in the (Table 26). This clearly shows that pigmentary keratitis has multiple causative factors.

Table 26 : Concurrent findings recorded in cases of pigmentary keratitis

Concurrent findings	No. of cases in Group I	Distribution (%)	No. of cases in Group II	Distribution (%)
MCT	6	100	6	100
MELE	4	66.67	6	100
KCS	2	33.33	3	50

Krecny et al. (2015) reported that all 130 cases of pugs (100%) studied had exhibited nasal entropion and bilateral macroblepharon. The study also stated that no

statistical significance was evident between the corneal pigmentation and nasal entropion while KCS was found to have a significant association with the presence of corneal pigmentation.

Maini et al. (2019) reported that detection of pigmentary keratitis in pugs and its severity had a significant association with the presence and the grade of MELE respectively. Various studies also suggested the involvement of additional yet to be determined factors in the development of corneal pigmentation.

4.3.5 Corneal pigmentation parameters observed in different treatment groups

The animals were studied for the corneal pigmentation and scored based on the grading system suggested by Maini et al. (2019). The mean pigmentation grading score of the animals in Group I was 14.0±0.00 and the mean score of the animals in Group II was 10.17±1.76 (Fig.73). All six animals in the Group I had a severe degree of pigmentation and with respect to Group II, 3 animals exhibited moderate degree and 3 animals exhibited a severe degree of pigmentation (Table 27).

The eyes were studied for the pattern of pigmentation and categorised into different patterns as suggested by Charbiwala (2019). Out of the 6 animals in Group I, 5 animals exhibited a diffuse pattern of pigmentation while 1 animal had a patchy pattern of pigmentation. In Group II, 3 animals had patchy pattern, 2 animals had diffuse pattern and 1 animal had paintbrush pattern of pigmentation (Table 27).

Table 27 : Corneal pigmentation parameters of different treatment groups

Parameter		**No. of cases in Group I**	**No. of cases in Group II**
Severity of pigmentation	Mild	-	-
	Moderate	-	3
	Severe	6	3
Pattern of pigmentation	Diffuse	5	2
	Paintbrush	-	1
	Patchy	1	3

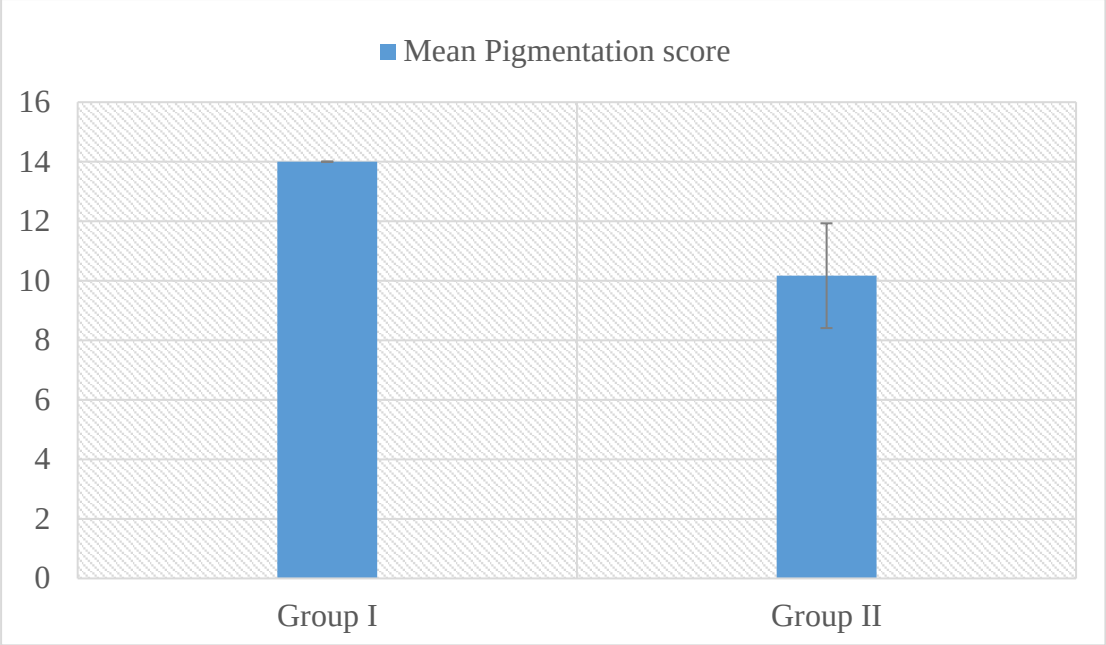


Fig.73 : Mean ± SE value of pigmentation score of different treatment groups

4.3.6 Neuro Ophthalmic tests/reflexes

Visual activity of the animals were evaluated using the basic visual functioning tests like menace response, palpebral reflex, pupillary light reflex (PLR) and the outcome of the tests were recorded.

a. Menace response : Out of the 6 eyes in Group I, pre-operatively menace response was exhibited normally in 3 eyes and was absent in the other 3 eyes. In Group II, menace response was normal in 2 eyes and was absent in the other 4 eyes (Table 28). This test was used to evaluate the functioning of the 2nd (optic) cranial nerve and 7th (facial) cranial nerve. The reflex closure of the eyelids to a threatening gesture was considered as a normal menace response. Mitchell (2011) had reported that absence of menace response in an animal is indicative of blindness, though the animals with cerebellar lesions and a normal vision may also exhibit a negative menace response clinically.

b. Palpebral reflex : Normal palpebral reflex was recorded in all 6 eyes of the Group I and Group II respectively (Table 28). This test evaluates the integrity of the ophthalmic and maxillary branches of the 5th (trigeminal) cranial nerve and the auriculopalpebral branch of the 7th (facial) cranial nerve. Featherstone & Heinrich (2013) stated that though this test doesn't evaluate the vision of the animals, it was

necessary to perform this test to evaluate the ability of the animals to establish a normal blink response before interpreting the results of menace response.

Table 28 : Neuro ophthalmic reflexes observed in different treatment groups

Group	Palpebral reflex		Menace response		Pupillary light reflex	
	Present	Absent	Present	Absent	Present	Absent
SK + MC	6	-	3	3	-	NA in 6
Cryo + MC	6	-	2	4	3	NA in 3
NA- Not appreciable due to corneal pigmentation						

c. Pupillary light reflex : Normal pupillary light reflex was recorded only in 3 eyes of the Group II while the PLR was not appreciable in the rest of the eyes due to the complete pigmentation of cornea (Table 28). It is a subcortical reflex performed to assess the normal functioning of the retina and it doesn't evaluate the vision of the animal. It should be noted that even completely blind animals may exhibit a normal PLR. Thompson et al. (2010) stated that PLR can be used to localise the lesion in some cases using the knowledge of the reflex neuroanatomical pathway.

4.3.7 Schirmer's tear test

The Mean ± SE STT value of the animals was 10.75±2.84 mm/min and 12.67±4.02 mm/min in Group I and Group II respectively (Table 29) & (Fig.74). The STT values were in the range of 3 to 25 mm/min. 2 eyes in Group I and 3 eyes in Group II had STT values less than 10 mm/min. Krecny et al. (2015) reported that STT values were in the range of 0 to 27 mm/min in 231 eyes of 117 pugs. KCS was diagnosed in a total of 39 eyes (15%) from 29 pugs. The study also stated that corneal pigmentation was detected in 117 eyes out of 192 eyes with normal STT values.

Labelle et al. (2013) reported that STT value was not significantly associated with the presence or detection of corneal pigmentation. The study also stated that STT values were lower in the pugs with severe corneal melanosis compared to the pugs with mild to moderate corneal pigmentation.

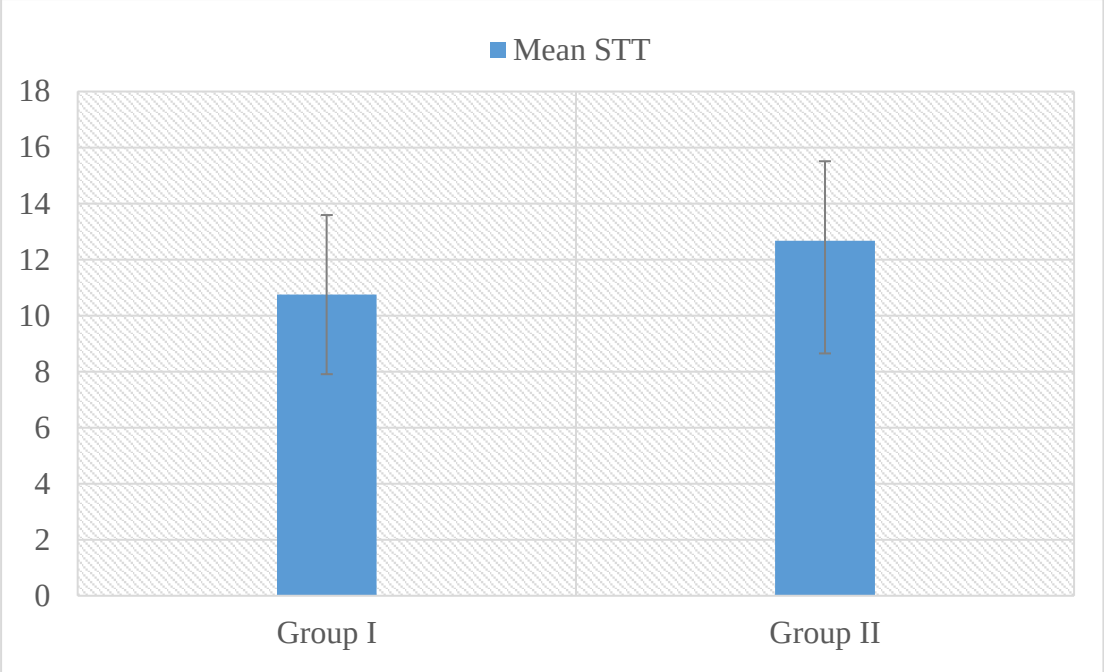


Fig.74 : Mean ± SE STT values of animals of different treatment groups

Maini et al. (2019) reported that STT values were in the range of 15.0 to 33.0 mm/min with a mean STT value of 21.10±3.24 mm/min of wetting in a total of 420 eyes of 210 pugs. The study reported that no significant statistical association could be identified between STT values and the detection or severity of pigmentary keratitis.

Table 29 : Mean ± SE STT values of different treatment groups

GROUP	SK + MC	Cryo + MC
STT (mm/min)	10.75±2.84	12.67±4.02

Various studies show that STT value has no significant association with the pigmentary keratitis and hence the STT values should be interpreted in light of the clinical signs observed in the animals.

4.3.7 Ocular staining tests

Ocular staining tests were performed with the help of Fluorescein and Rose Bengal dye strips and the outcome of the tests were recorded.

a. Fluorescein dye test : Fluorescein dye test results were negative in all 6/6 eyes in both Group I and Group II respectively (Table 30). This test was used to detect the

presence of ulcerative keratitis which accompanies corneal pigmentation. Labelle et al. (2013) used fluorescein dye test to detect the presence of any corneal epithelial damage and to assess the tear film breakup time in the eyes of the pugs studied for corneal pigmentation. Krecny et al. (2015) studied the ocular abnormalities in pugs and used fluorescein dye test to evaluate the status of the corneal epithelium.

Table 30 : Ocular staining test results in different treatment groups

Group	Fluorescein dye test		Rose Bengal dye test	
	Positive	Negative	Positive	Negative
SK + MC	-	6	5	1
Cryo + MC	-	6	4	2

b. Rose Bengal dye test : Rose Bengal dye test results were positive in 5/6 eyes and negative in 1/6 eyes in the Group I while the results were positive in 4/6 eyes and negative in 2/6 eyes in the Group II (Table 30). Kaswan & Salisbury (1990) suggested the use of rose bengal dye test in addition to other ophthalmological tests to establish and support a clinical diagnosis of KCS. Kim (2000) studied the mechanism of rose bengal staining and reported that positive results appear due to the tendency of the stain to appear whenever there is an abnormality of the preocular tear film leading to the poor protection of the surface corneal epithelium rather than by the lack of cellular vitality.

4.3.8 Tonometry

The Mean ± SE value of the intraocular pressure was 21.4±2.80 mm Hg and 19.33±2.09 mm Hg in the Group I and Group II respectively (Table 31) & (Fig.75). Anoop et al. (2016) recorded the intraocular pressure in the dogs affected with pigmentary keratitis and reported that the mean value of IOP was 40.64±2.38 mm Hg.

Table 31 : Mean ± SE IOP values of different treatment groups

GROUP	SK + MC	Cryo + MC
IOP (mm Hg)	21.4±2.80	19.33±2.09

The study also suggested that the increase in the IOP can be attributed to the increase in the central corneal thickness due to pigmentation or to the blockage of the aqueous humour filtering mechanism due to the deposition of pigment in the iridocorneal angle. Vallone et al. (2017) studied superficial corneal pigmentation in brachycephalic breeds and recorded that the mean IOP values for Pugs with corneal pigmentation was 17.8±3.2 mm Hg and 17.8±3.4 mm Hg in the right and left eye respectively.

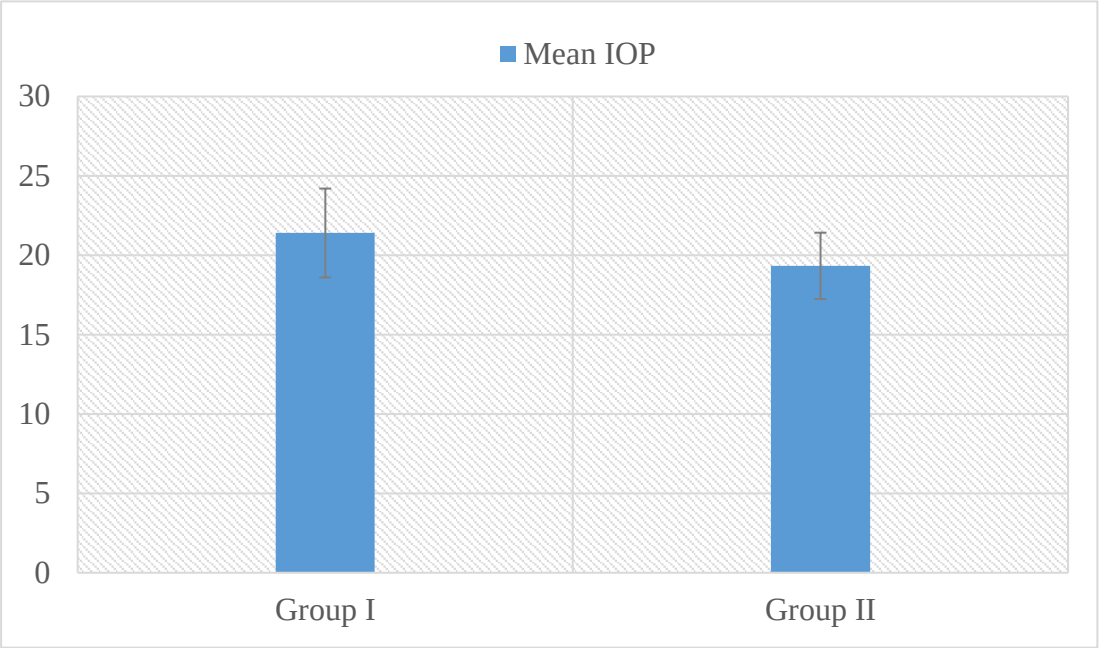


Fig.75 : Mean ± SE IOP values of different treatment groups

4.3.9 Slit lamp biomicroscopy

Slit lamp biomicroscope was used mainly to examine the adnexal structures using diffuse illumination and magnification as corneal pigmentation prevented the examination of the anterior segment in most of the cases. In the present study, 6/6 eyes in both Group I and Group II exhibited medial caruncular trichiasis while 4/6 eyes in Group I and 6/6 eyes in Group II had medial entropion of lower eyelid (Table 26).

Vallone et al. (2017) reported that 35 (83.3%) out of 42 pug eyes detected with SCP exhibited entropion while only 7 (31.8%) eyes out of the 22 pug eyes without SCP had entropion. The study also stated that among the non-pug brachycephalic dogs, 15 (83.3%) out of the 18 eyes affected with SCP exhibited trichiasis but only 10 (31.3%) out of the 32 eyes without SCP had trichiasis.

4.3.10 Ophthalmoscopy

Ophthalmoscopy was of limited diagnostic aid in the cases of corneal pigmentation due to the impediment of melanotic cornea from examining the fundus of the eye. However, ophthalmoscopy was performed in the cases of corneal pigmentation wherever possible to examine the posterior structures of the eye including the vitreous and the ocular fundus. Indirect Ophthalmoscopy was performed initially to take advantage of its greater field of view followed by closer examination of any region of interest with direct ophthalmoscopy making use of its higher magnification. In the present study, no abnormalities were observed in the cases in which ophthalmoscopy was performed.

4.3.11 Ultrasonography

Ocular ultrasonography was an indispensable diagnostic tool in the cases of corneal melanosis as it offered a diagnostic picture of the posterior segment of the eye. The animal was restrained in sternal or lateral recumbency and the eyelids were retracted gently. A transcorneal approach was performed after administering 1-2 drops of topical anaesthetic using 5-12/18 MHz probe. A small amount of sterile Lignocaine Hydrochloride Gel was used as a coupling gel on the transducer to obtain sufficient contact area. The eyes were scanned both in transverse and longitudinal planes.

1 eye out of the 6 in Group I and 2 eyes out of the 6 in Group II exhibited changes in the lens indicative of cataract. No ultrasonographic structural abnormalities were observed in the other eyes in both Group I and Group II respectively (Fig.76 & 77). Eisenberg (1985) reported that ultrasonography of the eye allows the examination of interior structures of the eye when the cornea is opaque to visible light. Joy et al. (2011) suggested that ocular ultrasonography is indicated prior to any ocular surgery to determine the outcome of the surgical procedures.

4.3.12 Haemato-biochemical parameters

Blood samples collected for routine pre-operative blood work up were analysed for various haemato-biochemical parameters and the following observations were recorded as mentioned in (Table 32 & 33) & (Fig.78).

Haemoglobin (Hb) : The Mean ± SE value of haemoglobin was 12.38±0.60 g/dL and 14.9±1.14 g/dL in Group I and Group II respectively. The values were within the normal range i.e. (12 – 18 g/dL).

Total Erythrocyte count (TEC) : The Mean ± SE value of erythrocytes was 6.02±0.20 ($\times 10^6$/µL) and 6.53±0.52 ($\times 10^6$/µL) in Group I and Group II respectively. The values were within the normal range i.e. (5.5 – 8.5 $\times 10^6$/µL).

Total Leukocyte count (TLC) : The Mean ± SE value of leukocytes was 18.71±2.38 ($\times 10^3$/µL) and 16.6±2.91 ($\times 10^3$/µL) in Group I and Group II respectively. The values were slightly elevated above the normal reference range i.e. (6.0 – 17.0 $\times 10^3$/µL) in Group I and within the reference range in Group II.

Thrombocyte count (Platelet count) : The Mean ± SE value of thrombocytes was 3.81±0.30 ($\times 10^5$/µL) and 5.67±0.14 ($\times 10^5$/µL) in Group I and Group II respectively. The values were within the normal reference range i.e. (2.1 – 6.2 $\times 10^5$/µL).

Packed cell volume (PCV) : The Mean ± SE value of packed cell volume was 39.18±2.12 (%) and 43.77±2.57 (%) in Group I and Group II respectively. The values were within the normal reference range i.e. (37 - 55%).

Table 32 : Mean ± SE values of haematological parameters

Parameter		**SK + MC**	**Cryo + MC**
Haemoglobin **(g/dL)**		12.38±0.60	14.9±1.14
TEC **(× 10^6/µL)**		6.02±0.20	6.53±0.52
TLC **(× 10^3/µL)**		18.71±2.38	16.6±2.91
Platelet count **(× 10^5/µL)**		3.81±0.30	5.67±0.14
PCV (%)		39.18±2.12	43.77±2.57
DLC (%)	**Neutrophils**	85.6±1.83	81.33±4.81
	Lymphocyte	11.2±2.33	9.9±2.96
	Monocyte	0.00±0.00	3.43±1.95
	Eosinophil	3.2±1.50	5.33±0.33
	Basophil	0.00±0.00	0.00±0.00

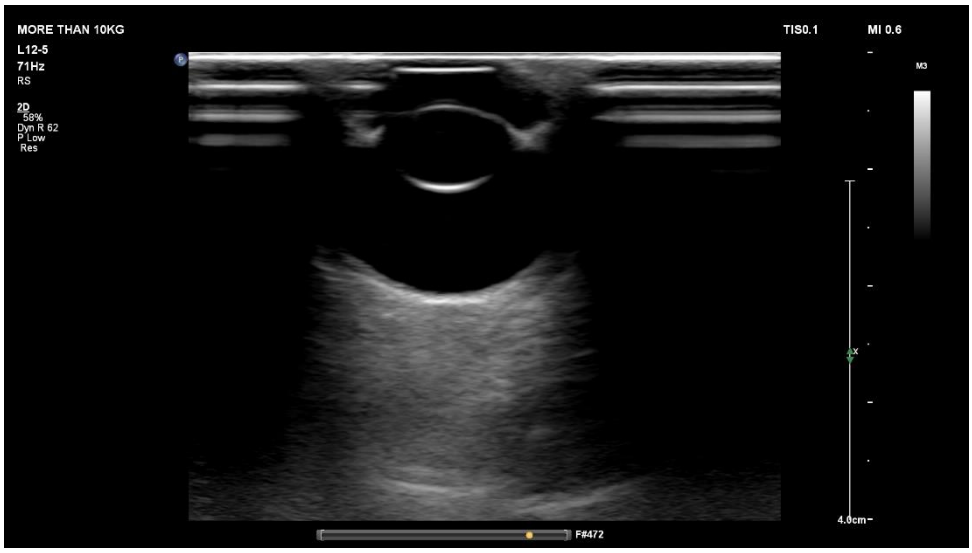


Fig.76 : Normal ultrasonographic image of the eye of a Pug

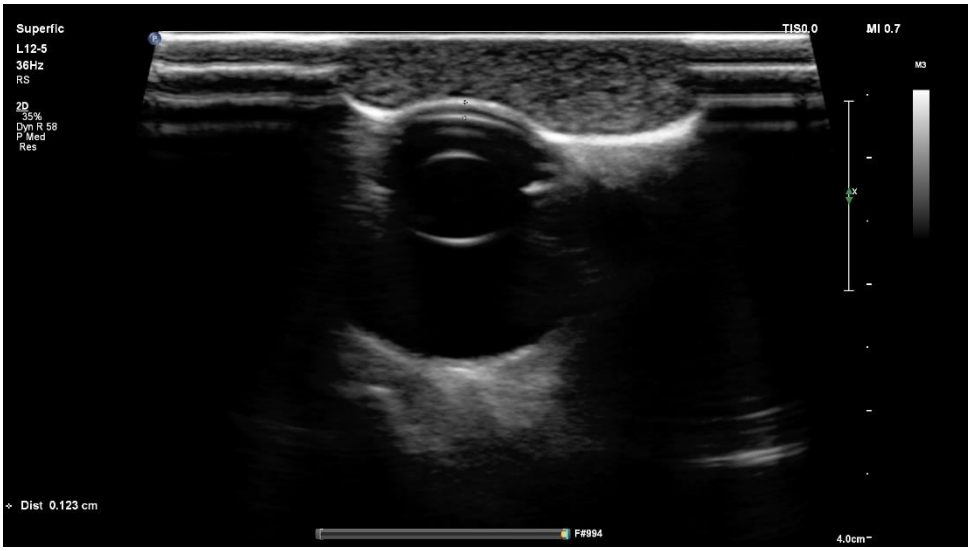


Fig.77 : Increased corneal thickness (0.123 cm) in the eye of a Pug with pigmentary keratitis

Neutrophils : The Mean ± SE value of neutrophils was 85.6±1.83 (%) and 81.33±4.81 (%) in Group I and Group II respectively. The values were elevated above the normal reference range i.e. (60 – 77%) in both the groups.

Lymphocytes : The Mean ± SE value of lymphocytes was 11.2±2.33 (%) and 9.9±2.96 (%) in Group I and Group II respectively. The values were within the normal reference range i.e. (12 – 30%) in both the groups.

Monocytes : The Mean ± SE value of monocytes was 0.00±0.00 (%) and 3.43±1.95 (%) in Group I and Group II respectively. The values were lower than the normal reference range i.e. (3 – 10%) in Group I and within the reference range in Group II.

Eosinophils : The Mean ± SE value of eosinophils was 3.2±1.50 (%) and 5.33±0.33 (%) in Group I and Group II respectively. The values were within the normal reference range i.e. (2 – 10%) in both the groups.

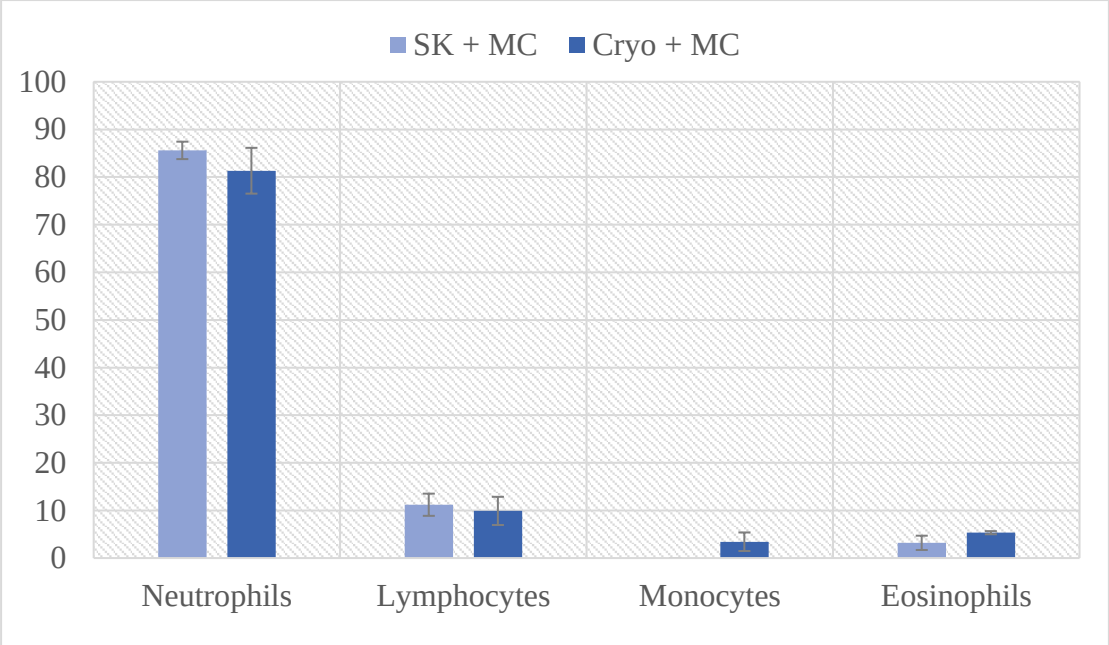


Fig 78 : Mean ± SE DLC values of different treatment groups

Total Protein : The Mean ± SE value of total protein was 7.225±0.05 (g/dL) and 6.55±1.15 (g/dL) in Group I and Group II respectively. The value was elevated slightly above the normal reference range i.e. (5.4 – 7.1 g/dL) in Group I and was within the reference range in Group II.

Serum Albumin : The Mean ± SE value of serum albumin was 3.4±0.15 (g/dL) and 2.75±0.05 (g/dL) in Group I and Group II respectively. The value was elevated slightly above the normal reference range i.e. (2.6 – 3.3 g/dL) in Group I and was within the reference range in Group II.

Blood Urea Nitrogen (BUN) : The Mean ± SE value of BUN was 13.8±1.83 (mg/dL) and 11.67±1.76 (mg/dL) in Group I and Group II respectively. The values were within the normal reference range i.e. (8.8 – 26 mg/dL) in both the groups.

Creatinine : The Mean ± SE value of creatinine was 0.98±0.07 (mg/dL) and 0.75±0.05 (mg/dL) in Group I and Group II respectively. The values were within the normal reference range i.e. (0.5 – 1.6 mg/dL) in both the groups.

Alanine Transaminase (ALT) : The Mean ± SE value of ALT was 37±1.92 (U/L) and 65.67±6.23 (U/L) in Group I and Group II respectively. The value was within the normal reference range i.e. (8.2 – 57 U/L) in Group I and the value was elevated slightly above the normal reference range in Group II.

Table 33 : Mean ± SE values of biochemical parameters

Parameter	**SK + MC**	**Cryo + MC**
Total protein **(g/dL)**	7.225±0.05	6.55±1.15
Serum Albumin **(g/dL)**	3.4±0.15	2.75±0.05
Blood Urea Nitrogen (BUN) **(mg/dL)**	13.8±1.83	11.67±1.76
Creatinine **(mg/dL)**	0.98±0.07	0.75±0.05
Alanine Transaminase (ALT) **(U/L)**	37±1.92	65.67±6.23
Total Bilirubin **(mg/dL)**	0.275±0.03	0.35±0.15
Blood Glucose **(mg/dL)**	98.8±10.32	83.67±3.84

Total Bilirubin : The Mean ± SE value of bilirubin was 0.275±0.03 (mg/dL) and 0.35±0.15 (mg/dL) in Group I and Group II respectively. The values were within the normal reference range i.e. (0.1 – 0.6 mg/dL) in both the groups.

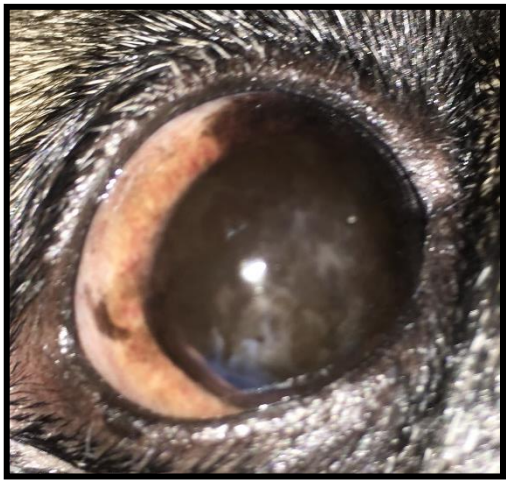


Fig.79 : Case (I-D) - Pre-operative

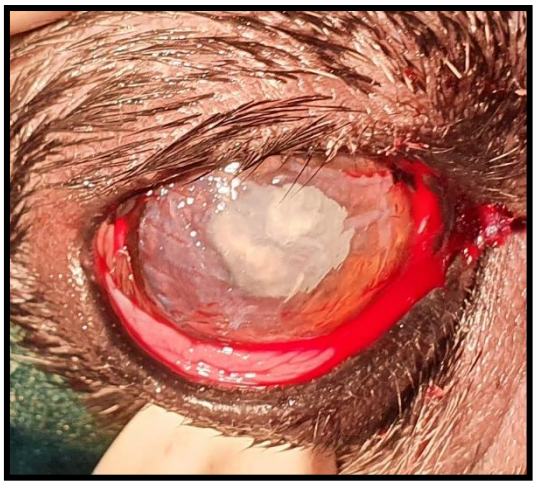


Fig.80 : Case (I-D) - Immediate Post-operative

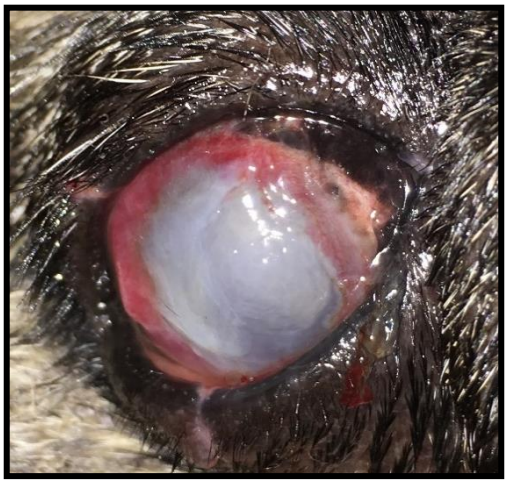


Fig.81 : Case (I-D) - 15th Day Post-operative

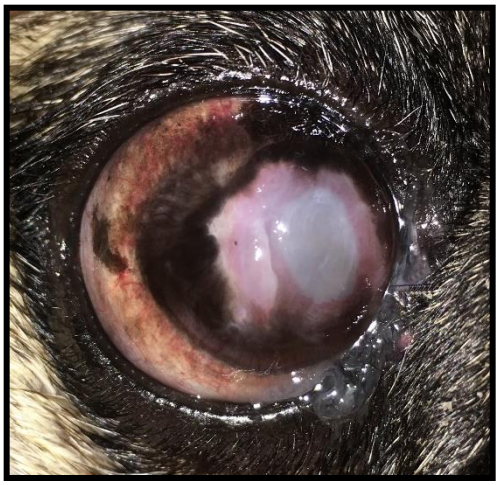


Fig.82 : Case (I-D) - 30th Day Post-operative

Blood Glucose : The Mean ± SE value of blood glucose was 98.8±10.32 (mg/dL) and 83.67±3.84 (mg/dL) in Group I and Group II respectively. The values were within the normal reference range i.e. (62 – 108 mg/dL) in both the groups.

4.4 Surgical management of Pigmentary keratitis

Pigmentary keratitis is not a clinical diagnosis but a mere clinical sign of chronic corneal irritation arising from various causes, each with a different line of treatment and prognosis (Maggs, 2018b). The surgical management of pigmentary keratitis is mainly aimed at correcting the underlying source of frictional irritation.

Ledbetter & Gilger (2013) suggested surgical correction of the source of corneal irritation before the surgical removal of pigmented corneal layers. Martin et al. (2019) reported that a combination of surgical procedures performed in brachycephalic breeds have shown to prevent the progression of corneal pigmentation. The surgical procedure usually aims at the correction of sources of corneal irritation such as entropion of lower eyelid and medial caruncular trichiasis by performing medial canthoplasty, reconstructive blepharoplasty and nasal fold resection.

4.4.1 Group I – Superficial keratectomy with Medial canthoplasty

Superficial keratectomy along with medial canthoplasty was performed in six eyes of six animals suffering from severe degree of pigmentary keratitis (Table 36) & (Fig.79, 83 & 87). Superficial keratectomy was a relatively easy procedure to perform in brachycephalic breeds due to their macropalpebral fissure and lagophthalmos compared to the medial canthoplasty.

Medial canthoplasty procedure was performed with difficulties in the pugs because of their severe brachycephalic nature owing to their lower craniofacial ratio. The surgeon found it difficult to find enough operating space at the medial canthus due to the prominent nasal folds and the condition such as medial entropion of lower eyelids. Amol (2016) reported that pugs had the lowest craniofacial ratio of 0.07 among the brachycephalic breeds and had a severely brachycephalic head shape associated with severe ocular affections. Cannulation of the lower punctum was the other difficulty experienced in some cases due to the inversion and displacement of the lower punctum due to the medial ventral entropion seen in the pugs (Table 34).

No such intraoperative complications were observed with the superficial keratectomy except for the fact that some cases had varying depths of corneal pigmentation and stronger attachment of the pigmented corneal epithelium to the underlying stroma making it not possible to limit the dissection of the pigmented corneal epithelium under one lamellar plane throughout the procedure (Fig.80, 84 & 88). Tarsorrhaphy sutures were removed after 15 days and the cornea was studied for its healing. Clinical examination 15 days after the surgery revealed opacity of the axial cornea with hyperaemic crests circumscribing the peripheral cornea at the level of the limbus (Fig.81 & 89).

The 30^{th} day post-operative examination revealed corneal ulceration along with corneal fibrosis/scarring, neovascularisation and hyperaemic crests. The recurrence of pigment was seen proceeding from the medial canthus and alongside the limbus circumscribing into the peripheral cornea 30 days after the surgical intervention in 4 eyes while complete corneal pigmentation along with the corneal ulcer wound was observed 45 days after surgery in 2 eyes out of 6 eyes (Fig.82, 85 & 90). Sufficient healing was noticed at the medial canthoplasty suture site 30 days after the surgery and the unstretched palpebral fissure length was recorded to be shortened by 0.4 – 0.5 cm compared with the contralateral eye. In the 4 eyes that were followed up 60 days after the surgery, the ulceration of cornea was seen healed completely with mild neovascularisation, opacity and fibrosis but the pigmentation was seen progressing into the axial cornea (Fig.91).

Corneal transparency was noticed after 4 months of the surgery with very mild neovascularisation and opacity and apparently with no corneal scarring or fibrosis (Fig.86 & 92). However, the progression of the corneal pigmentation continued covering more than 75 percent of the cornea. In cases which were followed up after 4 months of surgery, though the animal had a positive visual outcome, the pigmentation was seen covering more than 75 percent of the cornea (Table 35).

The mean pigmentation score observed 60 days after the surgery was 13.5±0.5 (Fig.93). Though positive visual outcome was recorded in few cases, the mean pigmentation score recorded postoperatively was almost similar to that observed preoperatively suggesting recurrence of pigmentation as the major postoperative complication of superficial keratectomy (Table 37).

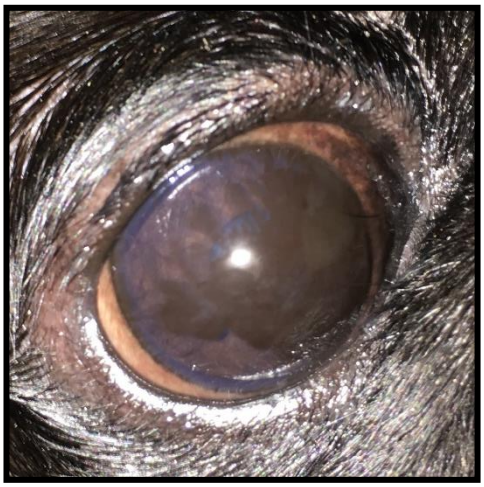


Fig.83 : Case (I-F) - Pre-operative

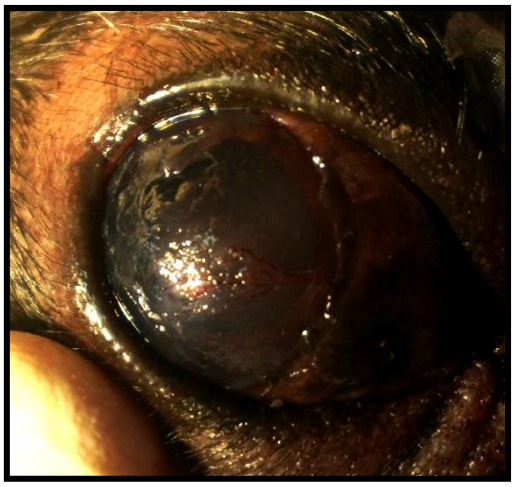


Fig.84 : Case (I-F) - Immediate Post-operative

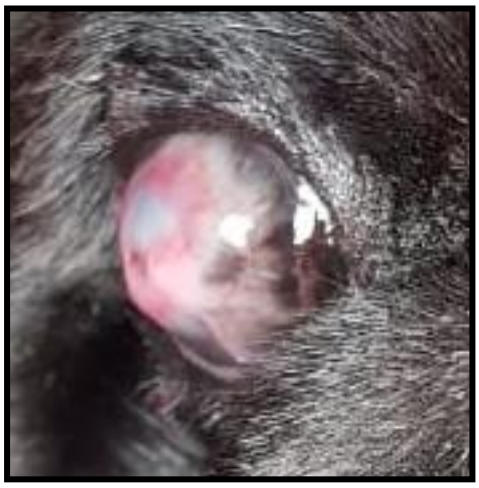


Fig.85 : Case (I-F) - 45th Day Post-operative

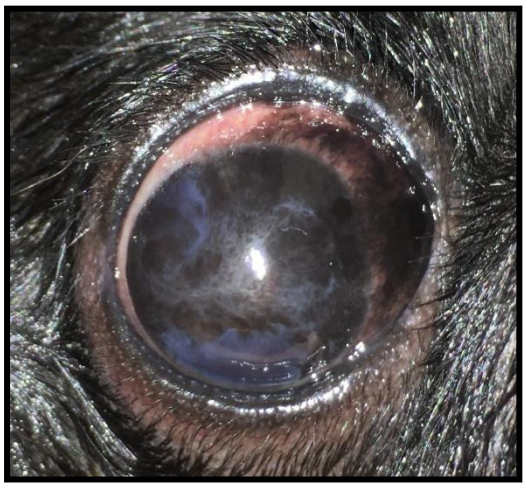


Fig.86 : Case (I-F) - 120th Day Post-operative

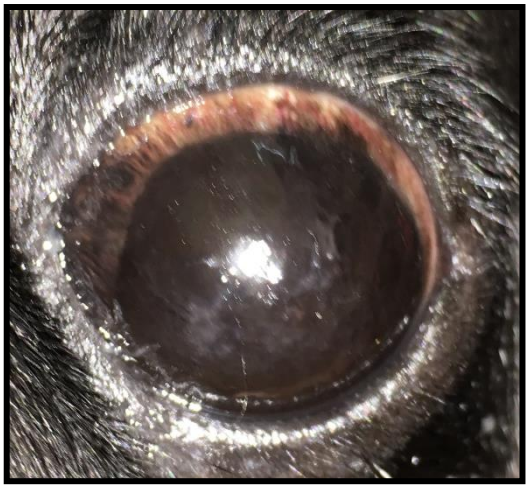


Fig.87 : Case (I-E) - Pre-operative

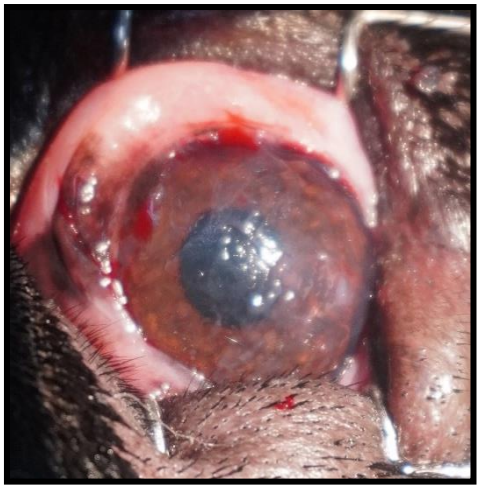


Fig.88 : Case (I-E) - Immediate Post-operative

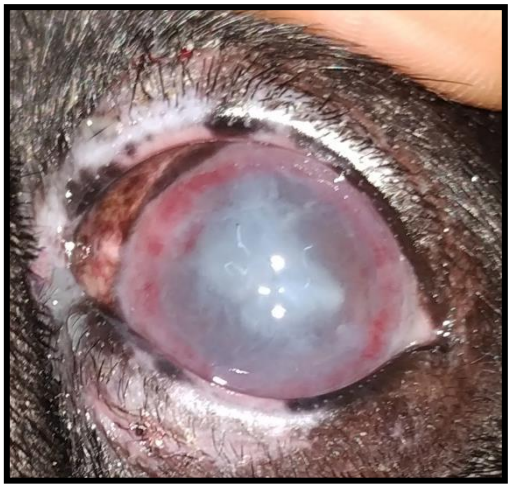


Fig.89 : Case (I-E) - 15th Day Post-operative

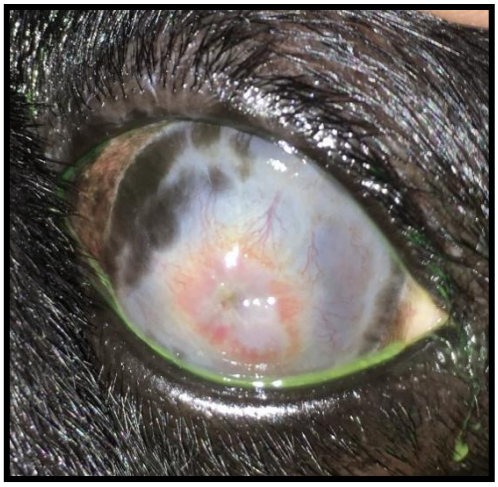


Fig.90 : Case (I-E) - 30th Day Post-operative

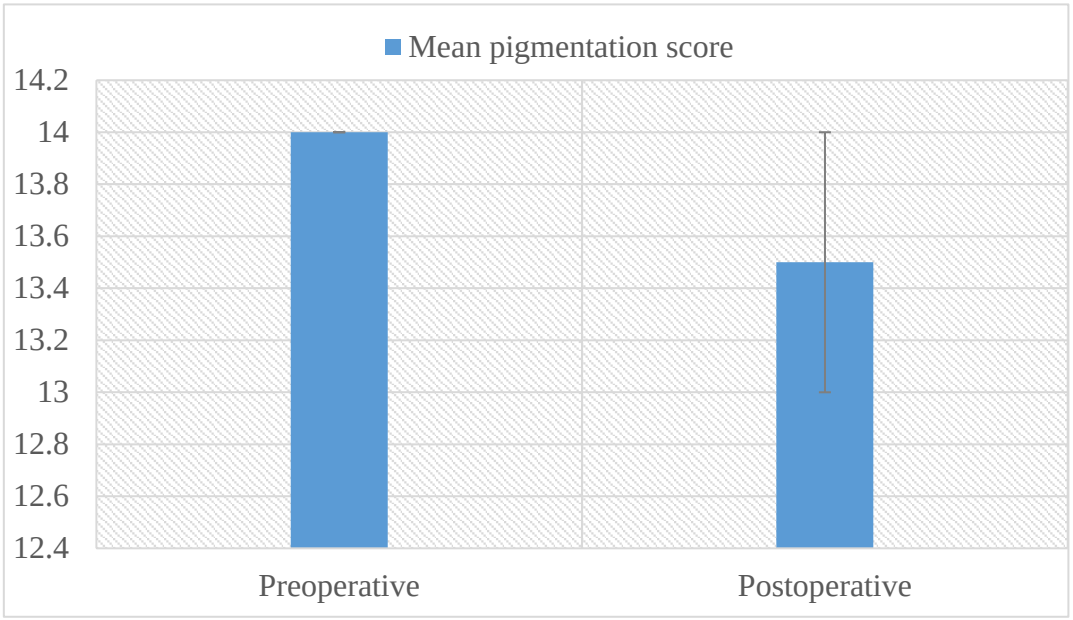


Fig.93 : Comparison of preoperative and postoperative Mean ± SE pigmentation score of Group I

Table 34 : Intra-operative & post-operative complications observed in Group I

Intra-operative findings	Post-operative findings
Less operating space at medial canthus	Initial corneal opacity and hyperaemic crests
Difficulty faced with cannulation of lower punctum	Corneal fibrosis, neovascularisation, corneal ulceration & re-pigmentation

Table 35 : Visual and tectonic outcome in Group I

VISUAL OUTCOME	TECTONIC OUTCOME
2/6 cases	6/6 cases

The post-operative complications reported by Peiffer et al. (1976) after superficial keratectomy were moderate to severe neovascularisation, corneal opacity or edema and corneal fibrosis with permanent scar formation which were similar to the observations recorded in the current study. Ledbetter & Gilger (2013) reported that the success rates of the surgical procedures involving the removal of pigmented cornea is limited by the post-operative complications such as frequent recurrence of the pigment and corneal fibrosis or scarring.

Table 36 : Summary of Preoperative observations of animals in Group I

Case no	Age (yr)	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
I-A	6	M	Right	MELE MCT KCS	Mucopurulent	Complete	Diffuse	Severe	14	2 years	- ve	NA	+ ve	NAD
I-B	5	M	Left	MELE MCT	Mucoid	Complete	Diffuse	Severe	14	3 months	- ve	NA	+ ve	NAD
I-C	5.5	M	Right	MCT	Mucoid	Complete	Diffuse	Severe	14	6 months	+ ve	NA	+ ve	NAD
I-D	6	F	Right	MELE MCT	Mucoid	Complete	Diffuse	Severe	14	2 years	Mild + ve	NA	+ ve	Cataract
I-E	5	M	Left	MELE MCT KCS	Mucopurulent	Complete	Diffuse	Severe	14	2 months	+ ve	NA	+ ve	NAD
I-F	4.5	M	Right	MCT	Mucopurulent	Medial	Patchy	Severe	14	3 months	- ve	NA	+ ve	NAD

Table 37 : Summary of Postoperative observations of animals in Group I

Case no	Postoperative (Day 15)	Postoperative (1 month)	Postoperative (Day 45)	Postoperative (2 months)	Postoperative (> 120 days)
I-A	Corneal opacity, hyperaemic crest	-	Corneal ulcer & fibrosis, hyperaemic crest, re-pigmenatation	Corneal ulcer healed, fluorescein staining (-ve), Progressive pigmentation, Neovascularisation, (Menace – ve)	-
I-B	Corneal opacity, hyperaemic crest	-	Re-pigmentation of entire cornea, (Menace – ve)	-	-
I-C	Corneal opacity, hyperaemic crest	-	Re-pigmentation of entire cornea, (Menace – ve)	-	-
I-D	Corneal opacity, hyperaemic crest	Corneal fibrosis, hyperaemic crests, re-pigmentation	-	Complete corneal healing, Progressive pigmentation, Neovascularisation, (Menace – ve)	-
I-E	Corneal opacity, hyperaemic crest	Corneal fibrosis, Neovascularisation, corneal ulcer, fluorescein staining (+ve) , re-pigmentation	-	Corneal ulcer healed, fluorescein staining (-ve), Progressive pigmentation, Neovascularisation,	Corneal transparency observed, mild opacity, neovascularisation, > 75% cornea pigmented, (Menace + ve)
I-F	Corneal opacity, hyperaemic crest	-	Corneal fibrosis, hyperaemic crest, re-pigmenatation	Complete corneal healing, Progressive pigmentation, Neovascularisation,	Corneal transparency observed, mild opacity, > 75% cornea pigmented, (Menace + ve)

4.4.2 Group II – Cryotherapy with Medial canthoplasty

Cryotherapy along with medial canthoplasty was performed in six eyes of six animals suffering from moderate to severe corneal pigmentation (Table 40) & (Fig.94, 100 & 104). Cryotherapy was a relatively easy procedure to perform and no such intraoperative complications were observed with cryotherapy as was observed with medial canthoplasty. The cryotherapy was performed in a two freeze-thaw cycles and in cases were the cornea was pigmented completely, the cornea was divided into two halves and two freeze-thaw cycles were performed in each half respectively (Fig.101).

Clinical examination was performed 5 days after the surgery and the pigmentation was seen disappeared in 3 eyes out of 6 which had moderate degree of pigmentation. However, in the 3 eyes which had severe degree of pigmentation, clinical examination revealed persistence of some amount of pigmentation on day 5 after surgery (Fig.95, 102 & 105). The other complications noticed on day 5 were corneal opacity and neovascularisation. As the superficial pigmented corneal epithelium eroded, stromal fibrosis was revealed in two eyes with a positive fluorescein stain uptake. One of the eyes exhibited ulcer on day 5 which healed completely by 45 days after surgery.

Ophthalmic examination 15 days after the surgery revealed positive menace response in 2 eyes which had negative menace response pre-operatively. The other 2 eyes which had negative menace response pre-operatively still exhibited negative menace response due to the prevailing cataract changes in the lens. However, mild recurrence of pigmentation was noticed in two eyes and the pigment which was seen persisting in the 2 eyes on day 5 after surgery started progressing further into the cornea. Neovascularisation and corneal opacity were present to a mild extent while the corneal fibrosis disappeared (Fig.96 & 106). Findings similar to that observed in Group I were recorded in this group with respect to the medial canthoplasty suture site healing and palpebral fissure length (Table 38).

Examination of the eye on day 30 after surgery revealed a lustrous cornea and tapetal reflex was visualised though a mild extent of neovascularisation and corneal opacity was still observed. However, the recurrence of pigmentation was noticed in all the eyes and the corneal pigmentation was seen progressing into the axial cornea in

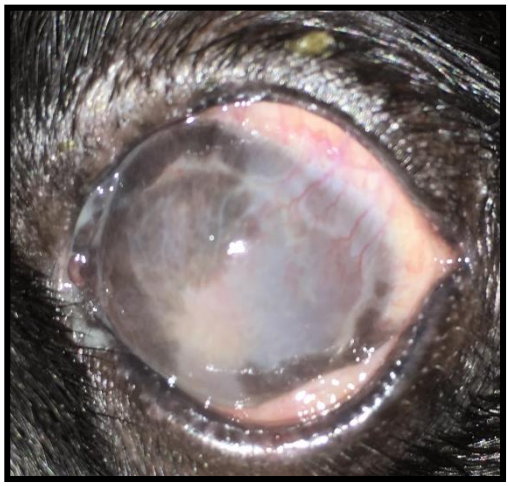


Fig.91 : Case (I-E) - 60th Day Post-operative

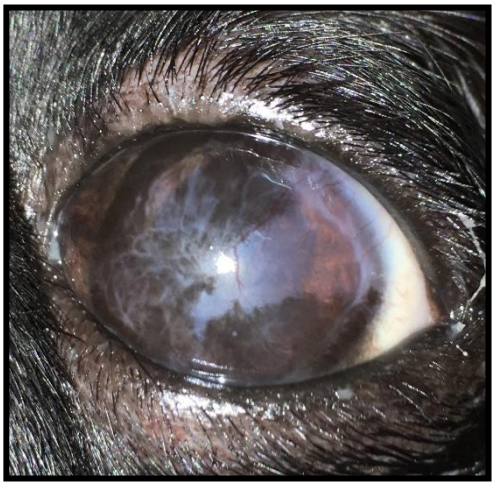


Fig.92 : Case (I-E) - 120th Day Post-operative

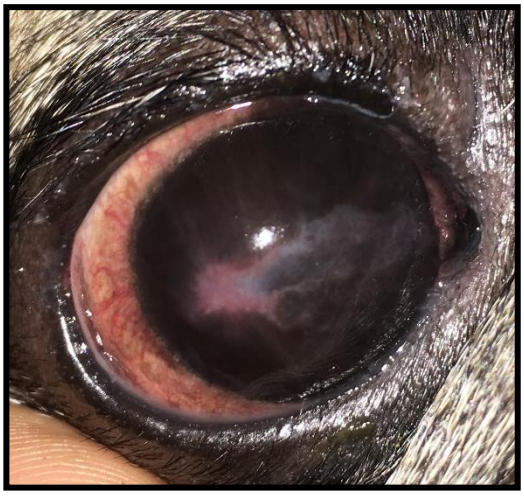


Fig.94 : Case (II-D) - Pre-operative

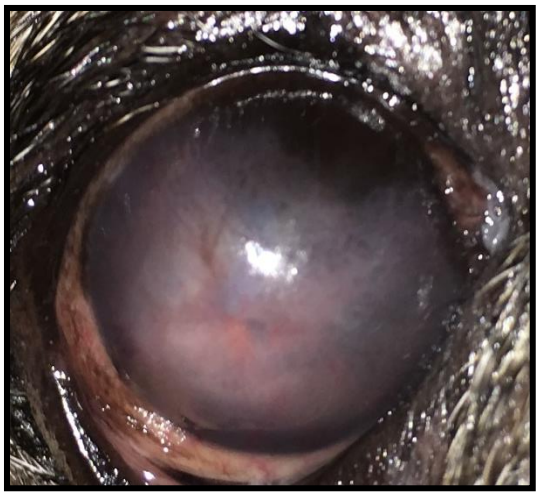


Fig.95 : Case (II-D) - 5th Day Post-operative

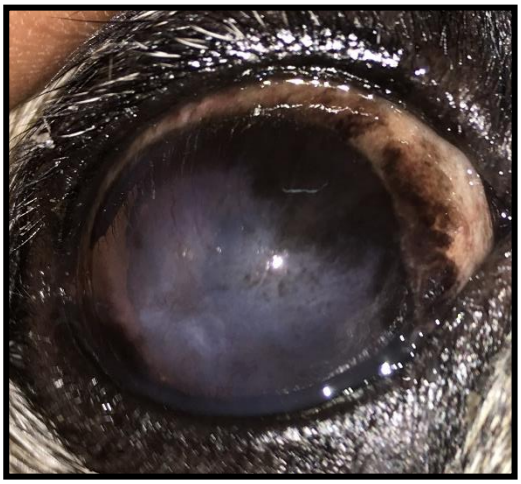


Fig.96 : Case (II-D) - 15th Day Post-operative

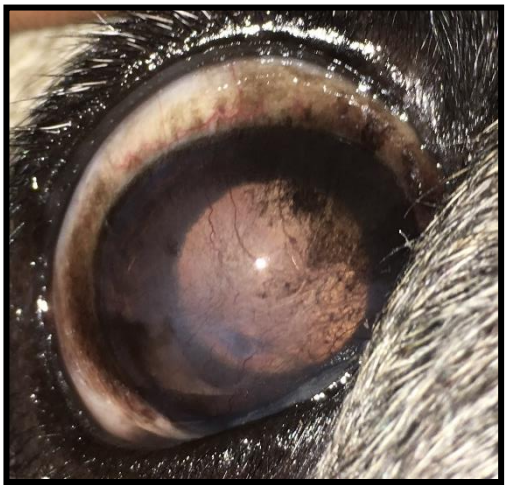


Fig.97 : Case (II-D) - 30th Day Post-operative

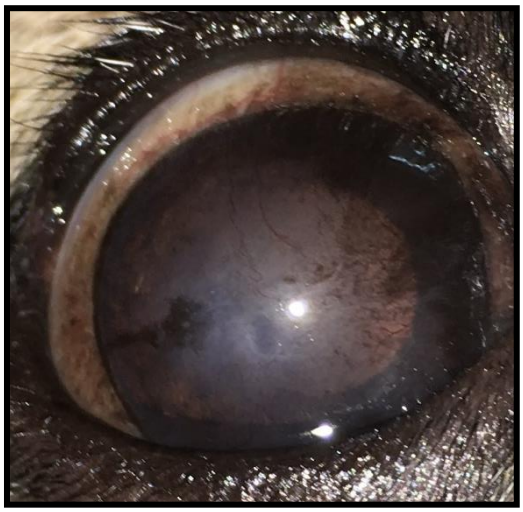


Fig.98 : Case (II-D) - 45th Day Post-operative

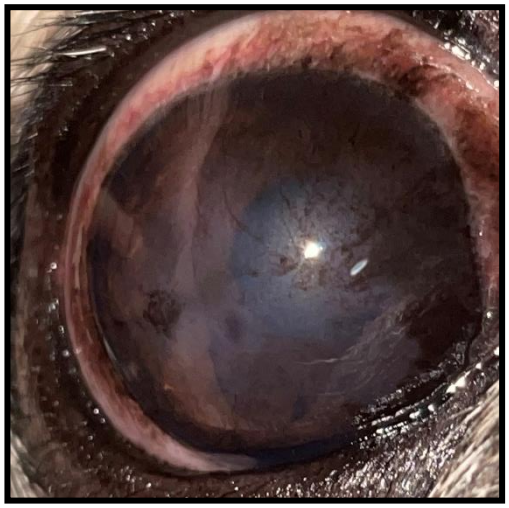


Fig.99 : Case (II-D) - 60th Day Post-operative

some eyes while in the other eyes, the intensity of pigmentation was seen to be severe compared to that observed on day 15 after surgery (Fig. 97, 103 & 107). The eyes which were followed up 2 months after the surgery revealed increased corneal transparency on examination and improved vision as reported by the clients based on the activity of the animals (Table 39 & 41) & (Fig.98 & 99). Though the pigment progression was noticed in these eyes, the rate of progression was relatively slower when compared to the eyes that underwent superficial keratectomy. The slower rate of pigment progression may be attributed to the less severe inflammatory changes incited to the corneal tissue with the cryotherapy compared to the superficial keratectomy.

The mean pigmentation score observed 30 days after the surgery was 6.58±1.44, which was relatively lower to that observed preoperatively in this group (Fig.108). The lower mean pigmentation score can be attributed to the disappearance of pigmentation as result of the cold sensitivity of the melanocytes and to the less traumatic nature of the cryotherapy procedure leading to less inflammation postoperatively.

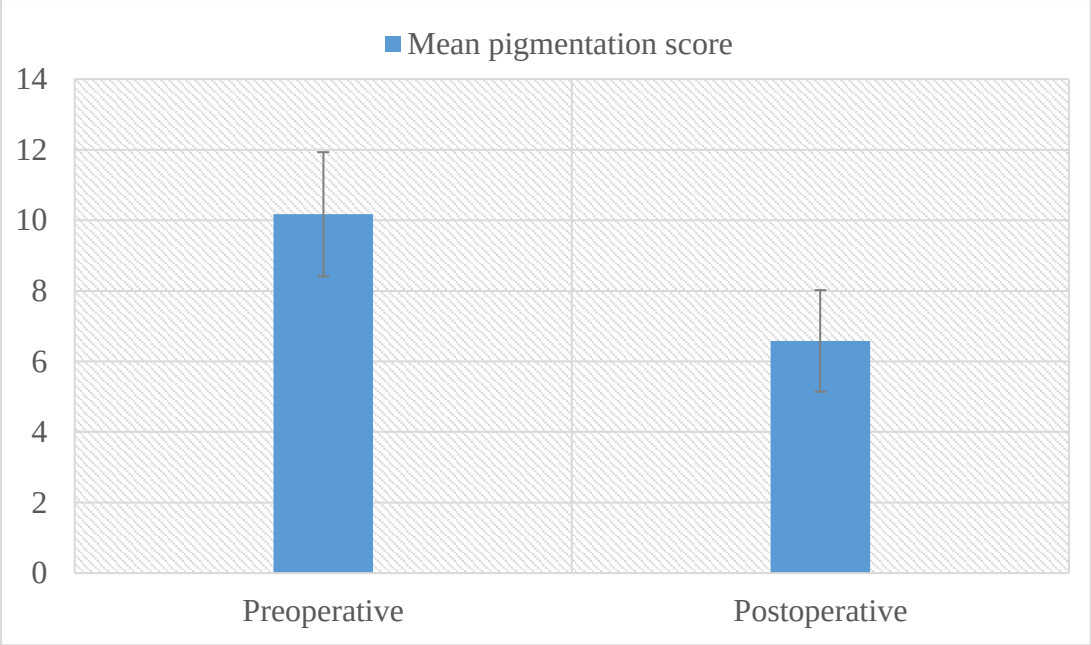


Fig.108 : Comparison of preoperative and postoperative Mean ± SE pigmentation score of Group II

Table 40 : Summary of Preoperative observations of animals in Group II

Case no	Age (yr)	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
II-A	4.5	M	Left	MELE MCT	Serous	Medial	Paintbrush	Moderate	6	3 months	+ ve	+ ve	+ ve	NAD
II-B	5.5	M	Left	MELE MCT	Serous	Medial	Patchy	Moderate	5	1 month	- ve	+ ve	+ ve	Cataract
II-C	10	M	Left	MELE MCT	Mucoid	Medial	Patchy	Severe	14	4 month	+ ve	NA	+ ve	NAD
II-D	6	M	Right	MELE MCT	Mucopurulent	Complete	Diffuse	Severe	14	4 month	- ve	NA	+ ve	NAD
II-E	8	M	Right	MELE MCT	Mucopurulent	Complete	Diffuse	Severe	14	5 month	- ve	NA	+ ve	NAD
II-F	4	M	Right	MELE MCT	Serous	Medial	Patchy	Moderate	8	1 month	- ve	+ ve	+ ve	Cataract

Table 41 : Summary of Postoperative observations of animals in Group II

Case no	Postoperative (Day 5)	Postoperative (Day 15)	Postoperative (Day 30)	Postoperative (2 months)
II-A	Disappearance of pigmentation, corneal opacity, corneal ulcer, fluorescein (+ve), Menace (+ve)	Corneal opacity, neovascularisation, corneal ulcer, fluorescein (+ve), faint re-pigmenatation, Menace (+ve)	Corneal opacity, neovascularisation, fluorescein (+ve), progressive pigmentation observed, Menace (+ve)	Progressive pigmentation observed, Menace (+ve)
II-B	Disappearance of pigmentation, corneal opacity, neovascularisation, fluorescein (-ve)	Mild opacity, neovascularisation,	Mild opacity, neovascularisation, re-pigmentation, Menace (-ve) due to cataract	-
II-C	Persistence of pigmentation, mild opacity and fibrosis, neovascularisation, fluorescein (faint +ve), Menace (+ve)	-	Mild opacity, neovascularisation, cornea lustrous, progressive pigmentation, fluorescein (-ve), Menace (+ve)	-
II-D	Persistence of pigmentation, fluorescein (+ve), mild corneal fibrosis, neovascularisation, Menace (Mild +ve)	Persistence & progression of the pigmentation observed, Menace (+ve)	Mild opacity, neovascularisation, progressive pigmentation, cornea lustrous and tapetal reflection observed, Menace (+ve)	Improved corneal transparency & progressive pigmentation observed, Menace (+ve)
II-E	Persistence of pigmentation, fluorescein (+ve), mild opacity and corneal fibrosis, neovascularisation, Menace (Mild +ve),	Persistence & progression of the pigmentation observed, fluorescein (faint +ve), Menace (+ve)	Mild opacity, neovascularisation, cornea lustrous, progressive pigmentation, fluorescein (-ve), Menace (+ve)	-
II-F	Disappearance of pigmentation, corneal opacity, neovascularisation, fluorescein (-ve)	Mild opacity, neovascularisation, re-pigmenatation	Mild opacity, neovascularisation, Progressive pigmentation, Menace (-ve) due to cataract	-

Azoulay (2014) performed cryotherapy for pigmentary keratitis and reported various post-operative complications similar to the current study such as corneal opacity, corneal inflammatory changes and superficial corneal ulcers which resolved after 1 month of surgery. The study reported recurrence of pigmentation in 8 eyes out of 16 eyes after 30 days of cryotherapy.

Table 38 : Intra-operative & post-operative complications observed in Group II

Intra-operative findings	Post-operative findings
Less operating space at medial canthus	Persistence or Incomplete disappearance of corneal pigmentation
Difficulty faced with cannulation of lower punctum	Corneal opacity, neovascularisation, corneal ulceration, stroma fibrosis & re-pigmentation

Table 39 : Visual and tectonic outcome in Group II

VISUAL OUTCOME	TECTONIC OUTCOME
4/6 cases	6/6 cases

The re-pigmentation rate reported by Azoulay (2014) was lower compared to the current study and this variation may be attributed to the difference in the study population of both studies. The current study was conducted only on pugs while the other study was conducted on a mixed population with only 1 brachycephalic breed of dog among the 9 dogs in the study population.

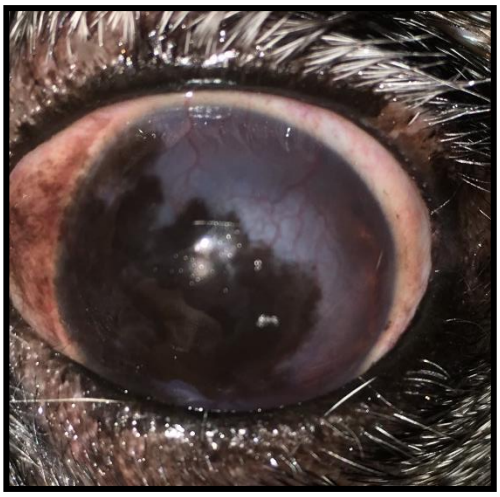


Fig.100 : Case (II-C) - Pre-operative

Fig.101 : Case (II-C) - Immediately Post-cryotherapy

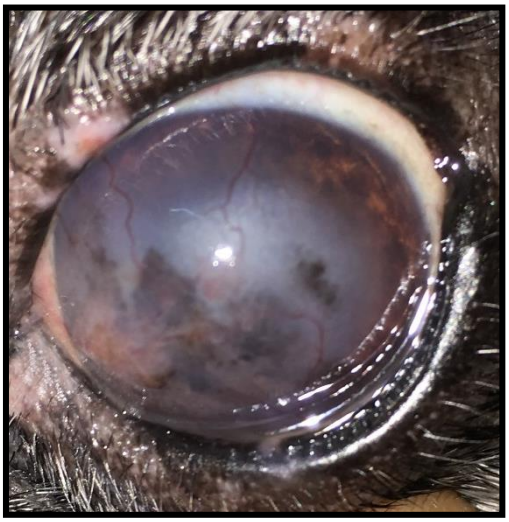


Fig.102 : Case (II-C) - 5th Day Post-operative

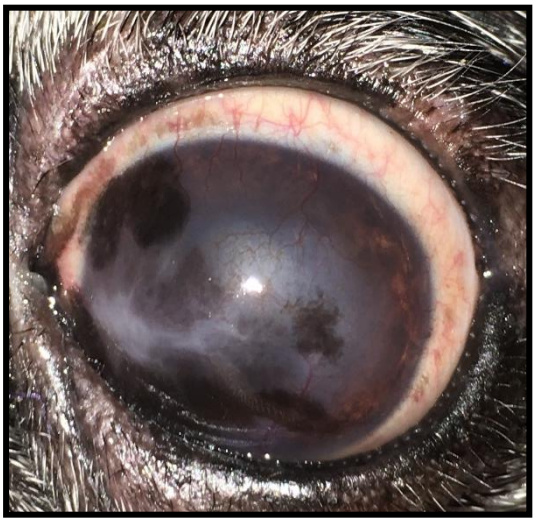


Fig.103 : Case (II-C) - 30th Day Post-operative

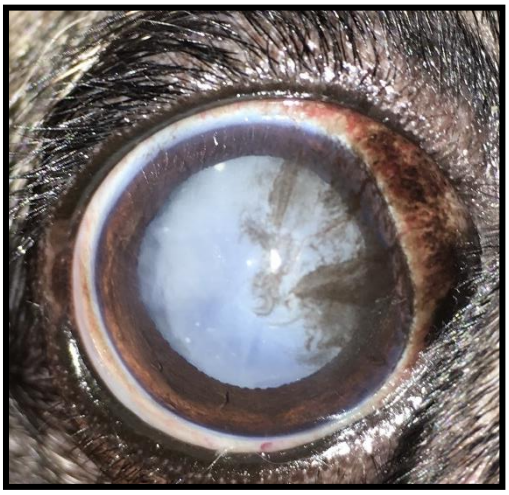


Fig.104 : Case (II-F) - Pre-operative

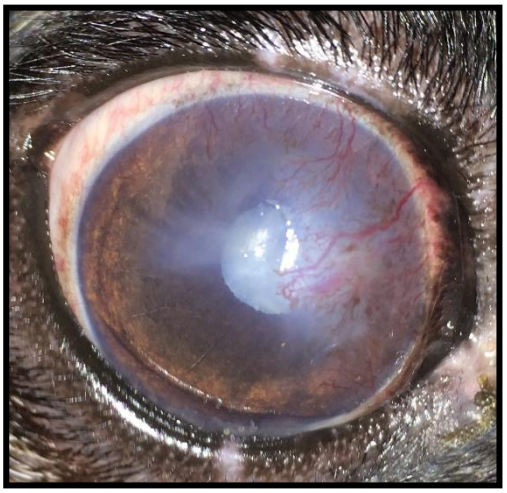


Fig.105 : Case (II-F) - 5th Day Post-operative

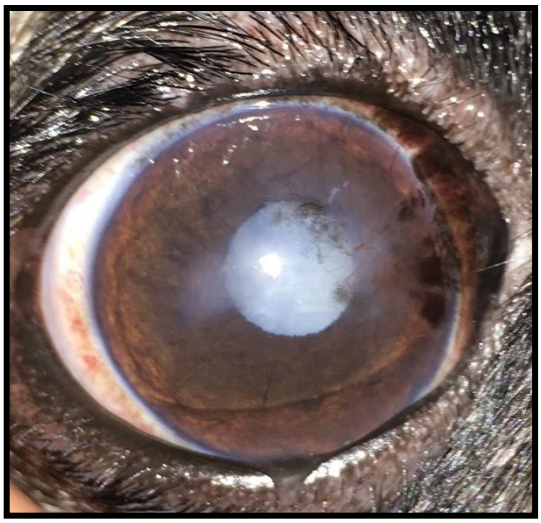


Fig.106 : Case (II-F) - 15th Day Post-operative

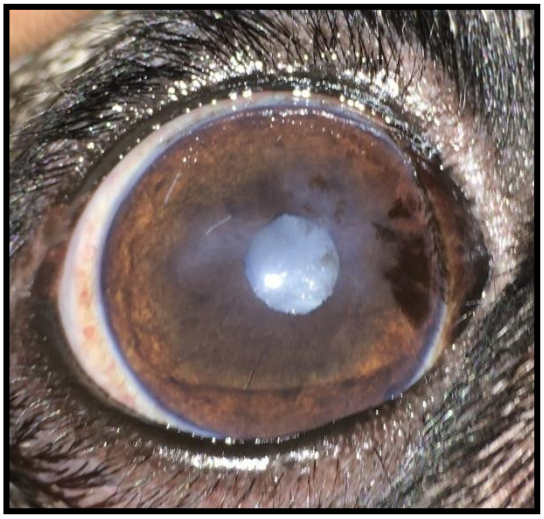


Fig.107 : Case (II-F) - 30th Day Post-operative

CHAPTER V

SUMMARY AND CONCLUSION

The study was conducted on clinical cases of pug breed of dogs presented to the Department of Veterinary Surgery and Radiology, GADVASU, Ludhiana with surgical affections. The study on the screening of pigmentary keratitis was conducted on 100 pugs with ailments other than ocular affections and the study on surgical management of pigmentary keratitis was conducted on 12 eyes of 12 pug breed of dogs presented primarily for ocular ailment.

All the 100 pug breed of dogs included in the screening study were studied for pigmentation of their eyes using photography of the eyes. The mean age of the animals in the study was 5.27±0.29 years (range, 0.42 to 10.0 years). Among the study population, 96 percent of the dogs had pigmentation of their eyes and only 4 percent of dogs had a clear and transparent cornea. All the animals that exhibited pigmentation of the cornea were affected bilaterally and (45.31%) had severe degree, (39.58%) had moderate degree and (15.10%) had mild degree of pigmentation.

The mean pigmentation score of the population was 8.84±0.37 while the mean score for the mild, moderate and severe group was 2.98±0.21, 6.89±0.18 and 13.35±0.16 respectively. The pigmentation was highly distributed among the adult age group followed by the senile and younger age group with the severity of pigmentation being mild to moderate in the younger group and moderate to severe in the adult and senile group respectively. Pigmentary keratopathy was highly distributed in the male population (54.17%) compared to the female population (45.83%) with the male and the female population exhibiting severe and moderate degree of pigmentation respectively.

The fawn coat colour (80.21%) was highly distributed among the study population with the black colour sharing only 19.79 percent of the study population. The incidence was more than 95 percent in both the coat colours with the fawn and black coat colour exhibiting severe and moderate degree of pigmentation respectively. No significant difference was recorded in the severity of pigmentation between the left and right eyes of the animals. The pattern of pigmentation was studied and categorised

With the diffuse pattern (34.38%) being highly distributed followed by the patchy (21.35%), medial pyramid (20.83%), streak or limbal brush border (12.50%) and paintbrush (10.94%) pattern of pigmentation.

The detection of corneal pigmentation was not significantly associated ($P>0.05$) with the sex, coat colour or the eye side (left vs right) but was significantly associated ($P<0.05$) with the age of the animal. The severity of pigmentation was not significantly associated ($P>0.05$) with the coat colour or the eye side (left vs right) but was significantly associated ($P<0.05$) with the age and sex of the animal.

During the study period, 141 clinical cases presented to the Department of Veterinary Surgery & Radiology, GADVASU, Ludhiana for primary ocular affections underwent complete ophthalmological examination. Among the study population, the younger age group shared the majority of ocular disorders followed by the adults and the senile group with the male population (71.63%) being over presented than the female animals (28.37%). Pugs (31.21%), Labrador Retriever (16.31%) and Spitz (7.80%) were the most commonly presented breeds for ocular affections representing more than 50 percent of the study population with various other breeds of dogs representing less than 5 percent of study population.

Bilateral affections (65.96%) were common among the population followed by unilateral affections of left (19.86%) and right eye (14.18%). Cornea (36.17%) was the most highly affected anatomical structure of the eye among the study population followed by the lens (17.73%), orbit & globe (16.31%), eyelids (15.60%), conjunctiva (7.09%), fundus (5.67%) and uvea (1.42%). Pigmentary keratitis (52.94%) was the most common corneal disorder recorded in the study population followed by corneal ulcers (31.37%), corneal opacity (11.76%) and descemetocele (3.92%).

All the 12 animals that underwent surgical treatment were selected randomly after complete ophthalmological examination and were randomly divided into two groups based on the surgical technique adopted. The animals of Group I (n=6) underwent superficial keratectomy along with medial canthoplasty and the animals of Group II (n=6) underwent cryotherapy along with medial canthoplasty.

The mean age of the animals in Group I was 5.33±0.25 years (4.5 to 6 years) while the mean age of the animals in Group II was 6.33±0.93 years (4.5 to 10 years).

Among the 12 animals, 10 were adults and 2 were senile animals while 11 out of 12 animals were male and 1 was a female. Multiple concurrent findings were recorded along with pigmentation in all the animals with MCT, MELE and KCS recorded in 12/12, 10/12 and 5/12 animals respectively.

Various clinical signs such as conjunctivitis, episcleritis, corneal and conjunctival melanosis, corneal opacity, corneal fibrosis and neovascularisation with partial to total vision were recorded in the animals with a mean pigmentation score of 14.0±0.00 and 10.17±1.76 in Group I and Group II respectively. All six animals in Group I had severe degree of pigmentation while 3 animals each had moderate and severe degree of pigmentation in Group II respectively.

Superficial keratectomy along with medial canthoplasty was performed in six eyes of six animals suffering from severe degree of pigmentary keratitis. The animals were studied postoperatively for complications and visual outcome. Clinical examination 15 day after the surgery revealed complications such as corneal opacity and hyperaemic crests while the examination on 30^{th} day revealed complications such as corneal ulceration, fibrosis, neovascularisation along with the complications recorded on day 15. Recurrence of pigmentation was from the medial canthus was noticed in 4 cases by 30 days while complete pigmentation of entire cornea was noticed in 2 cases by 45 days. Palpebral fissure length (unstretched) was recorded to be shortened by 0.4 – 0.5 cm compared to the contralateral eye. In cases studied 120 days after the surgery, corneal pigmentation was seen to cover more than 75 percent of the cornea although they had a positive visual outcome. The mean pigmentation score observed 60 days after surgery was 13.5±0.5 which was almost similar to the preoperative value of 14.0±0.0. The results stipulate that the major complication of superficial keratectomy was the re-pigmentation of cornea.

Cryotherapy along with medial canthoplasty was performed in six eyes of six animals suffering from moderate to severe corneal pigmentation. The postoperative study revealed that most of the corneal pigmentation had disappeared by day 5 with mild to moderate visual outcome. However, the cases which had severe pigmentation preoperatively had some amount of corneal pigmentation still persisting on the cornea by day 5. Corneal opacity, corneal ulcer and neovascularisation were the complications recorded on day 5. Clinical examination on day 15 revealed mild recurrence of

pigmentation in 2 cases while 2 eyes still had persisting pigmentation along with the observations seen on day 5. Cornea was relatively lustrous and transparent with visualisation of tapetal reflex on day 30 with progression of corneal pigmentation observed in some cases and the intensity of pigmentation increasing in other eyes. The eyes examined 2 months after the surgery revealed increased corneal transparency on examination and improved vision as reported by the clients based on the activity of the animals. The mean pigmentation score observed 30 days after the surgery was 6.58±1.44 which was relatively lower than the preoperative score of 10.17±1.76. The lower pigmentation score recorded postoperatively may be attributed to the less traumatic nature of the cryotherapy procedure leading to lesser inflammation compared with the superficial keratectomy.

Based on the above results, the following conclusions were made from the present study :

- The hospital occurrence of pigmentary keratitis in Pugs presented for affections other than ocular ailments was found to be 96 percent. The detection and severity of pigmentation was relatively higher in the adult and senile group compared to the younger age group.
- The detection of corneal pigmentation was significantly associated ($P<0.05$) with the age of the animal and the severity of pigmentation was significantly associated ($P<0.05$) with the age and sex of the animal.
- Superficial keratectomy performed along with medial canthoplasty, had a positive visual outcome in animals only for a short term with the recurrence of pigmentation observed as the major post-operative complication.
- Cryotherapy when combined with medial canthoplasty had a positive impact on the visual status of the animal with less severe complications and relatively long term maintenance of positive visual status.
- Cryotherapy performed along with medial canthoplasty was a better technique compared to superficial keratectomy performed along with medial canthoplasty in effective surgical management of pigmentary keratitis in pugs.

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