

Morpho-histological Study of the Nictitans Gland and Cartilage in Dogs and Cats in Sulaimani Province

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Abstract

This comparative study aims to assess the differences in the third eyelid gland and cartilage morphology and histology between dogs (*Canis lupus familiaris*) and cats (*Felis catus*). Twenty nictitans membranes were collected from 20 adult dogs and 20 adult cats. The nictitans membranes were dissected to examine the structure, shape, and positions of the nictitans gland and cartilage. Histologically, the samples were fixed in 10% neutral buffer formalin and then prepared for H&E stains. Generally, in both species, the cartilage consists of a long, slender body, and pigmented large dorsal and small ventral wings. The cartilage had a curving shape that resembled the eyeball's curvature and had two surfaces; palpebral and corneal or bulbar surface. At the basal end of the corneal surface, there are several lymph nodes aggregates. In the dogs, the nictitans cartilage was longer and slenderer than in the cats. The body of the cartilage was wrapped by a spherical and light pink nictitans gland in the dogs while the cats had oval nictitans gland. Microscopically, the nictitans gland in the dogs was composed of a compound tubuloacinar gland whereas; in cats, it was composed of a compound acinar gland. There was no difference histologically in the intralobular and the interlobar septa between species, the isogenous group of chondrocytes was higher in cats Vs. the dogs that showed few in number. In conclusion, the nictitans gland and cartilage were varied in their anatomical and histological structure. This should help researchers make preparations to prevent negative impacts and could help them investigate changes made more pronounced by disease conditions.

Keywords: Feral Cat, Nictitans Membrane, Eyelid, Gland, Cartilage.

دراسة شكلية و نسيجية للغدة النكتية والغضاريف في الكلاب والقطط في محافظة السليمانية

الخلاصة

هدفت هذه الدراسة المقارنة إلى تقييم الفروق الشكلية بين غدد الجفن الثالث وغضاريف في كلاب (*Canis lupus familiaris*) والقطط (*Felis catus*). تم جمع عشرين غشاء من الجفن الثالث من 20 كلاب بالغة و 20 قطط بالغة. تم تشريح أغشية النكتيتان لفحص بنية وشكل ومواضع غدة النكتيتان والغضاريف. من الناحية النسيجية، تم تثبيت العينات في محلول فورمالين بنسبة 10% وتم معاملتها بالطرق الروتينية وصبغها بصبغة هيماتوكساليين يوزين. في كلا النوعين، يتكون الغضروف من جسم ضيق طويل، يليه أجنحة ظهرية كبيرة وأجنحة بطنية صغيرة. كان للغضروف شكل منحنٍ يشبه انحناء مقلة العين وله سطحان؛ الجفني والقرنية أو سطح بصلي. في النهاية القاعدية لسطح القرنية، توجد عدة تجمعات من العقد الليمفاوية. في الكلاب، كان غضروف النكتيتان أطول وأكثر رشاقة من القطط. تم لف جسم الغضروف بغدة الجفن الثالث الكروية الوردية الفاتحة في الكلاب بينما كان لدى القطط غدة الجفن الثالث بيضاوية. مجهرياً، كانت غدة الجفن الثالث في الكلاب تتكون من غدة نيببية عنبية مركبة؛ في القطط، كانت يتألف من مركب غدة عنبية. لم يكن هناك فرق تشريحي بين الفصيصات والحاجز البيئي بين الأنواع، كانت المجموعة المتجانسة من الخلايا الغضروفية أعلى في القطط مقابل الكلب الذي أظهر عددًا قليلاً. في الختام، اختلفت غدة الجفن الثالث وغضاريفها في بنيتها التشريحية والنسيجية. يمكن أن يساعد هذا الباحثين في التحقيق في التغييرات التي تحدثها حالة المرض ويجب أن تساعد في الاستراتيجيات لمنع النتائج غير المرغوب فيها.

Introduction

Animals have a noticeable semilunar flap of the conjunctiva at the medial angle of the eye known as the third eyelid (1). It is necessary for the production and movement of tears, for the removal of ocular waste, for the health of the immune system, and the protection of the eye globe (2). The chronic inflammatory disease known as keratoconjunctivitis sicca (KCS) is a common problem in dogs, which is caused by abnormalities in the quantity or quality of the tear film (3). In dogs, the third eyelid's leading edge is typically visible and pigmented. The bulk of the third eyelid is hidden within the orbit when the eye is in its normal position in a living dog; only the free edge is visible in the ventromedial aspect of the palpebral fissure, which is partially covered by the lacrimal caruncle (4). Consequently, by manually moving the globe into the orbit, the third eyelid can be seen for inspection (5). In dogs, a flat T-shaped hyaline cartilage consisting of a crossbar, upper and lower branches, and supports the third eyelid anatomically. The oval-shaped superficial gland known as the nictitans gland surrounds these crossbars. The ventral oblique muscles partially cover the nictitans gland, which is situated between the ventral and medial straight muscles (6). To match the shape of the underlying globe, the cartilage is curved. beneath the surface of the bulbar conjunctiva, several diffuse lymphoid follicles could cause a "cobblestone" appearance (7). Due to the absence of a particular muscle to draw the third eyelid over the globe, as it is present in cats, the

movement of the third eyelid in dogs is primarily passive (5).

Histologically, In dogs, hyaline cartilage composes the majority of the cartilage (2). Elastic fibers in cats extend into the perichondrium from the nearby connective tissue. An elastic network is regularly constructed around the chondrocytes and dispersed in isogenous groups of two or four cells or individually (8). The third eyelid's superficial gland in dogs has a multilobar tubuloacinar structure and is serous (9). It is encircled by an enormous intraperiorbital fat body that is covered by a dense connective tissue capsule that has adipocyte infiltration in various places. The connective tissue capsule, which is likewise heavily populated with adipocytes, produced interlobar septae that were both thick and thin. This gland structure had broad and small lobes, which were divided by interlobar septae. Each lobe was composed of tubules and acini. Tall conical cells with eosinophilic cytoplasm made up the acini, which had a restricted lumen. Tubules with large lumens are made up of a single layer of cubic cells. Both the interlobar septae and the center sections of the lobes, which are composed of simple columnar epithelium, include some major ducts. There are no goblet cells in the main ducts (6).

In the cats, A T-shaped segment of elastic cartilage that is attached to nearby connective tissue and extends into the perichondrium reinforces the middle third of the nictitating membrane (10). The lymphoid follicles are more

widely distributed on the bulbar surface, and the nictitans gland, which secretes a serous fluid, is situated vertically near the cartilage's base (11).

The presence of nictitans membrane with their associated structures are very important for the maintenance of healthy eyes, and for this reason, we aimed to explore the anatomical and histological configurations of the nictitans cartilage and gland in dogs (*Canis lupus familiaris*) and cats (*Felis catus*).

Materials and methods

Animals

Twenty dogs (10 males and 10 females) and twenty feral cats (10 males and 10 females) of different ages and weights, were used in this study. The study was conducted from September 2021 to August 2022 on those animals were euthanized by intramuscular injection of an overdose of xylazine and ketamine mixture (10 mg/ml Ketamine, 2 mg/ml Xylazine) (12), after the animal reached the Veterinary Teaching Hospital for reasons of car or other accidents rather than diseases, that were critically injured by different traumatic injuries and their lives were thresholded. The animals were admitted to the Veterinary Teaching Hospital, belonging to the College of Veterinary Medicine/ University of Sulaimani for clinical inspection and post-mortem findings.

Ethical Approval

This study was approved by the Veterinary College Research Ethics Committee (VREC,

030510), University of Sulaimani, based on CONCEA (National Animal Experiment Control Council) ethical norms for animal experimentation.

Anatomical dissection

The samples were collected immediately after post-mortem by careful anatomical dissection using a small incision from the skin on the lateral angle of the eyelid (temporal angle). Then by a blunt dissection between the sclera and the eyeball, the eyeball, and eye adnexa such as; extraocular muscles, connective tissue, and periorbital fats were detached. Finally, the optic nerves were severed and the eyeballs were removed from the orbit. The cartilage and gland were carefully dissected from the nictitans membrane using a scalpel and sharp-sharp pointed scissors (13).

Histological preparation

Samples of the nictitans gland and cartilage were prepared according to (14) and stained with H&E stain. The tissue samples were examined and photographed using a light microscope and an AmscopeTM, Japan camera, respectively.

Results and Discussion

Anatomical study

An adapted conjunctival fold called the nictitating membrane (NM) was normally located within the nasal angle of the eyes and it partially covered the eyeball in our study's dogs and cats, resembling that of domestic (1) and wild animals

including red kangaroo (15), koala (16), bear (17), roe deer (18), and wild ruminant (19), as well as in many other vertebrates including sharks (20), amphibians (21), reptiles and birds (22), as well as, it was previously described in the literature in the other canines' species (23). The marginal edge of the nictating membranes was thick and strongly pigmented with black pigment (Fig. 1) similar to canine (7).

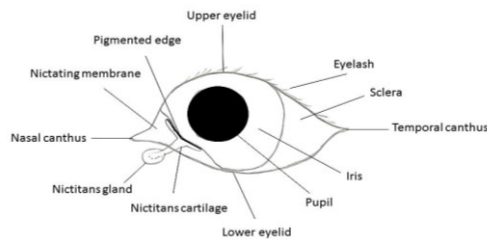


Figure 1: Schematic illustration of the eye globe and structures of the nictating membrane in the dogs and cats after concluded from dissection of nictitans membrane samples.

The third eyelid was a thin membrane that connected the upper eyelid to the lower eyelid. In both species, (NM) was structurally supported by irregular T-shaped cartilage named nictitans cartilage (NC) (Fig.2 A). The NC was curved to conform to the cornea's convexity and consisted of two surfaces and two portions; palpebral convex surface and bulbar or corneal concaved surface. At the base of the corneal surface, there were several clustered lymphatic nodules of the nictitans cartilage (Fig.2 B) as Cooper's literature (7), other breeds such as Beagles, the Pointer mixed-breed, and Pit Bull Terriers dogs (24), and other cat breeds (11). The NC in the cats was

smaller and broader than in the dogs. The lateral portion of the NC consisted of the wing and body (Fig. 3 A and B). The body extends to the medial canthus of the eye, and the wings were parallel to the leading edge of the lid and consist of a dorsal wing and ventral wing that were strongly pigmented and thick. The dorsal wings were directed dorsally and longer than the ventral wings that were directed ventrally. These features of the T-shaped cartilage that was composed of a long straight crossbar, holding a long curved dorsal wing and the ventral wing are similarly reported in the other species of dogs (6), crab-eating foxes, and the maned wolf (23, 25), and cats (8, 10, 23, 26). The body of the NC at the base of the palpebral surface was wrapped by uniform, undivided, and light pink nictitans gland (NG). The NG in the dogs was spherical vs. cats were oval-shaped. The ventral oblique muscles partially covered the NG situated at the base of the body NC between the medial and ventral straight muscles, which were varied from the other reported breeds of dogs in the literature such as captive South African painted females and the domestic dogs, which they have light pinked oval shaped nictitans gland (6, 26). Both species' ocular globes were the same size and inside the orbit, it is visible as a spherical structure (Fig. 2 B), as the captive female dog (6), and other cat breeds such as Burmese, domestic short hair, Persian, domestic long hair, and Devon Rex, they had a spherical to ovoid shapes (27). In addition, in the Feral cat, it was noticed that the color of the eyes was light brown, which is dissimilar to that

of the Persian cat which varied from light blue, and dark blue to light orange and dark orange (28). Concerning sexual dimorphism, no differences were found between males and females, as well as, between the right and the left side of the NC, NG, and eyes in both species.

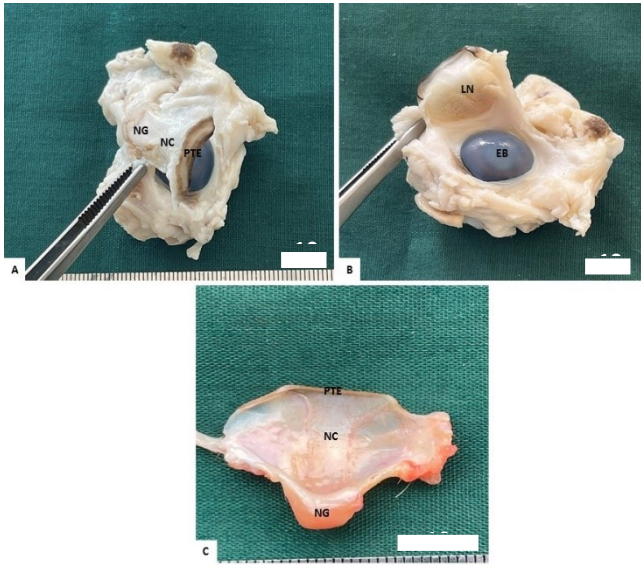


Figure 2: Anatomical structures of the nictating membrane in the male dogs and cats revealed A; Pigmented cartilage and nictitans gland related to the shape of the eyeball. B; Lymphoid nodules at the corneal surface of nictating cartilage. C; Pigmented nictitans cartilage and gland of the third eyelid in the male cat at the corneal side. NG; nictitans gland. NC; nictitans cartilage. PTE; pigmented third eyelid. LN; lymph node. EB; eyeball (scale bar = 10mm).

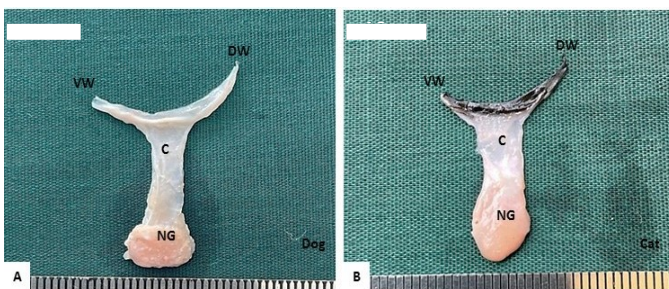


Figure 3: Anatomic structure of the nictitans cartilage in both species. A; nictitans cartilage and its gland in the right third eyelid of the adult male dogs. B; nictitans cartilage and nictitans gland in the right third eyelid of the adult male cats. VW; ventral wing. DW; dorsal wing. C; cartilage. NG; nictitans gland (scale bar = 10mm).

Histological study

Histological features of the nictitans gland in dogs revealed compound tubuloacinar units that surrounded the third eyelid's cartilage which were noticed by routine stain (Fig. 4A) while in the cats, composed of the compound acinar gland that was covered by a thin layer of connective tissue capsule (Fig. 4B). This finding is similar to the other breeds of dog (29), Mongrel dog (9), Captive female dog (6), and Domestic dogs (26, 30), and the other breed of cats (11).

The microscopic structure of the chondrocyte in the dog's third eyelid with huge numbers of single chondrocytes had an oval-spherical shape, and few of an isogenic group composed mostly of 2 cells and more amount of extracellular matrix (Fig.4C) vs. cat in which the third eyelid was surrounded by the cartilage with oval-shaped chondrocytes mostly seen as an isogenous group vs. to those found in a dog (Fig. 4D). This study demonstrated that the third eyelid's cartilage in the dogs of our study was similar to that of the captive female dog (6), other dogs (8), and Mongrel dogs (9). In Feral cats, the cartilage

tissue was similar to the other species of the cats such as (8, 10, 11), Equidae, lion, and Suidae in which the elastic fibers are particularly numerous, but non-uniformly distributed (31).

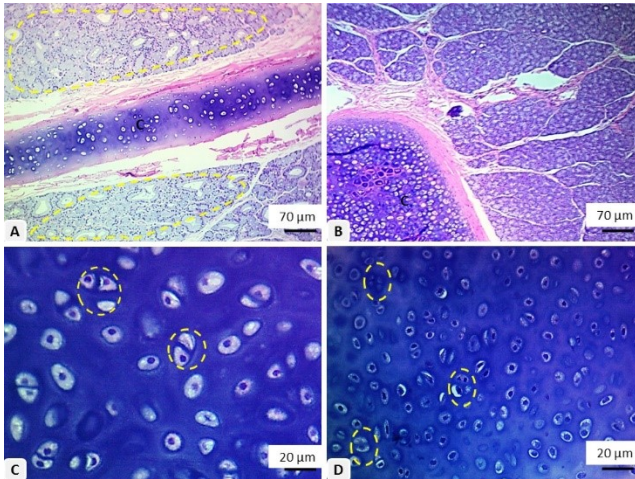


Figure 4: Microscopic section of the nictitans membrane revealed; A; Compound tubulo acinar nictitans gland (yellow dash line) and cartilage (C) in the dogs. B; Compound acinar nictitans gland and cartilage (C) in the cats. C and D; An isogenous group (yellow dash line) in dog and cat respectively, (H&E stain). C: cartilage.

In both species, the gland is separated into multiple thick and thin interlobular septa by the connective tissue from the capsule, which also transmits trabeculae into the glandular tissue (Fig. 5 A and B). Many interlobular and intralobular ducts within the trabeculae between the lobes and lobules were found in the nictitans gland in both species, which are made up of a simple cuboidal epithelium that resemble the dogs of Alexandre literature (29).

The glandular parenchyma in the dogs contains both alveoli and tubules (mixed secretory cells) (Fig. 5A), whereas the tubular gland less predominates over than acinar gland vs. cat species in which glandular parenchyma that was composed entirely of alveoli (Fig. 5B). In both species, the acini showed small lumen with a spherical shape of one-row pyramidal cells and an oval nucleus near the basement membrane with eosinophilic cytoplasm (Fig. 5D). While the dogs' tubules had wide, irregular lumen and a single layer of tall cells with oval nuclei embedded in the basement membrane and eosinophilic cytoplasm (Fig. 5C) (6, 9, 11, 26, 29, 30). According to the literature of Paszta that recorded; there were many adipocytes in between the secretory units, which were not observed in our investigation (6).

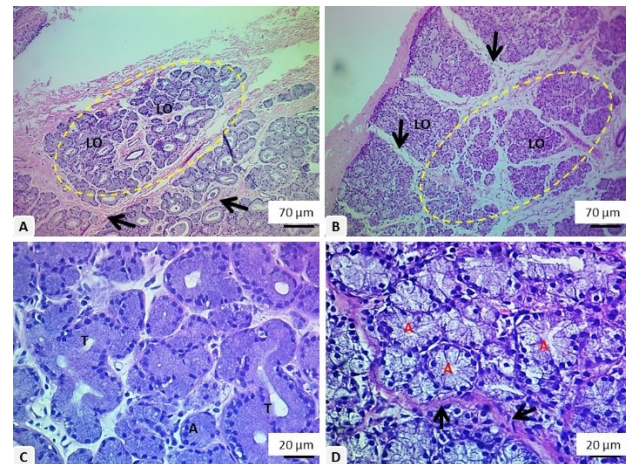


Figure 5: Microscopic section of the nictitans membrane shown; A: Compound tubulo acinar nictitans gland parenchyma that was divided into lobe (yellow ring) and lobule and trabeculae (black arrows) in the dog. B: Compound acinar

nictitans gland parenchyma that was divided into lobe (yellow ring) and lobule and trabeculae (black arrows) in the cat. C and D: Tubulo-acini and acini structures in dog and cat respectively, (H&E stain). LO; lobules, A; acini, T; tubule.

Conclusion

This study concluded the normal anatomical and histological structures of the nictitans gland and cartilage in the local breeds dogs and cats. They were approximately similar in morphology but varied in their anatomical and histological structures in both species such as the nictitans cartilage in dogs was longer and narrower than the cats, and their nictitans gland was spherical rather than being oval-shaped in cat. The nictitans gland in dogs was made up of compound tubuloacinar serous units, whereas the nictitans gland in cats was made up entirely of compound acinar units.

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