The structural characterization of the lacrimal gland in the adult dog (Canis familiaris)

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ABSTRACT

To elucidate the macroscopical and microscopical structure of dog's lacrimal gland, 15 adult dogs (Canis familiaris) were utilized in this study. Macroscopically, the lacrimal gland was located on the dorsolateral aspect of the eyeball and bounded dorsolaterally by the orbital ligament, zygomatic process of the frontal bone and frontal process of malar bone. Its shape was nearly rectangular with the average width (1.38 ± 0.13 cm) and length (1.52 ±0.12 cm). In between the dorsal and lateral rectus muscles, the lacrimal artery, vein and nerve were observed. Microscopically, the lacrimal gland was enveloped by the fibromuscular capsule and lobulated. Each lobule was formed of secretory endpieces and ducts. The secretory endpieces were mucous and serous. However, the mucous type was predominant. The lining cells of the secretory endpieces expressed a positive reaction to alcian blue and Periodic acid Shiff. A series of duct system within the lobules and septa was seen. The scanning electron microscop ic examination revealed that the secretory endpieces covered with granular secretory substance. Several crystals with sharp taper ends were seen around the secretory endpieces.

Keywords: lacrimal gland; dog; orbital ligament; secretory endpieces.

1. INTRODUCTION

In recent years, dogs become an important laboratory animal because certain occurring diseases in dogs simulate human disorders (Kijas et al., 2003). Dogs have multiple uses for the human such as guarding property, hunting, protection assisting police and military (Udell and Wynne, 2008). Although the accessory structures of the eye (the eyelids, conjunctiva, lacrimal apparatus, third eyelid and its associated glands) are not participating directly in the vision process, they are necessary for the eye protection in all animals (Gelatt et al., 2013). The lacrimal gland is a part of the lacrimal apparatus that consists of both secretory and drainage portions in domestic mammals (Konig and Liebich, 2007). Macroscopically, the canine LG was situated inside the orbit on the dorsolateral aspect of the eyeball (Cabral et al., 2005; Murphy et al., 2013; Park et al., 2016). Also, in man Edward and Bedrossian (2005) and Records (2006) explained that the main lacrimal gland occupies the lateral third of the orbital roof and it lies in the lacrimal fossa of the frontal bone. On the other hand, Dursum (2000) and Aslan et al. (2005) in cattle and Simoens and Budras (2003) and (Dyce et al., 2010) in bovine reported that, the broad dorsal part of the lacrimal gland lies under the root of the zygomatic process. While a long thin tail of the lacrimal gland has extended around the lateral margin of the orbit and located in the glandular lacrimal fossa. The lacrimal gland was lobulated, flat and irregular in outlines with morphological variations between different breeds of dogs (Park et al., 2016). Microscopically, the lacrimal gland is a multilobar tubuloacinar gland in most of the species. It is classified according to its secretion as serous gland in European bison (Kleckowska-Nawrot et al., 2015a) and human (Hamid et al., 2015) or mucoserous (mixed) gland as in rat (Draper et al., 1998), sheep (Gargiulo et al., 2000), Roe Deer (Kleckowska-Nawrot et al., 2013), Alpaca (Kleckowska-Nawrot et al., 2015b) and dog (Shaker and AL-Obeady, 2016). The preocular tear film is a trilaminar structure which consists mainly of a watery aqueous layer, overlying a thin mucous layer, with a superficial
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The aqueous component of the tear film is secreted mainly via the lacrimal gland. In addition, mucosubstances and lacrimal proteins include lysozyme, lactoferrin and growth factors are secreted from the lacrimal gland. Therefore, it plays an essential role in maintaining a healthy environment for the ocular surface (Fullard and Snyder, 1990). The tear film disorders are the most common cause of the dry eye disease that impairs the vision or even causes blindness of dogs (Giuliano and Moore, 2007; Ribeiro et al., 2008).

Numerous investigations were concerned with studying the lacrimal gland in the different animals. However, few reports were documented in dogs. So this work was designed to elucidate the structure of the lacrimal gland in adult dogs (Canis familiaris).

2. MATERIAL AND METHODS

2.1. Animals

This study was conducted on 15 healthy adult dogs (Canis familiaris) of both sex (nine males and six females), their ages aged between two and four years. They were collected from different localities all over Sharkia Governorate, Egypt. They were humanely euthanized. Handling of these animals was according to the guideline of Zagazig University.

2.2. Macroscopical examination

Six fresh heads were carefully dissected for studying position, relations and the shape of lacrimal glands. The length and width of ten lacrimal glands were measured by using stainless Caliper. The obtained measurements were statistically analyzed for the mean ± standard error (SE) by using the SPSS software program (version 16.0; Chicago, USA). Four fresh heads were infused with normal saline through the right and left common carotid arteries. The heads were divided into two equal groups, then injected by gum milk (Latex 60%) colored red and blue with Scib paints through the right and left common carotid arteries and the lingofacial vein for the demonstration of the arteries and veins, respectively. The injected heads were preserved in 10%formalin, 3% glycerine 1% thymol fixative solution to be carefully dissected latter. Two fresh heads were fixed in the previous fixative solution, then they were soaked in glacial acetic acid 5-10% for 24hrs. After that, they were dissected for studying the nerve supply of the lacrimal gland. For Computed Tomography (CT), one dog was sedated by intramuscular injection of 0.5 mg/kg xylazine.

Then, the dog was anesthetized by intravenous injection of 20-25 mg/kg thiopental. The dose of thiopental given at incremental doses for maintenance of prolonged anesthesia (Hall et al., 2001). Computed Tomography (CT) images were performed by CT Optima 680, 16 Multislices apparatus in the AL-Rhma center at Mahal ElKobra, Al Gharbiyah Governorate, Egypt.

2.3. Microscopical examination

2.3.1. Light Microscopy

The lacrimal glands' specimens from one head were immediately dissected and fixed in 10% neutral buffered formalin for 24 h. They were processed to get 5 μm thick paraffin sections and stained with hematoxylin and eosin (H&E) stain to demonstrate general structure, Crossman's trichrome stain to detect collagen and muscle fibers, Combination of Van Gieson’s and Weighert’s elastic stains to demonstrate the collagen and elastic fibers. Alcian blue (pH:2.5) to demonstrate acidic mucopolysaccharides and Periodic acid Shiff (PAS) technique to demonstrate neutral mucopolysaccharides (Bancroft and Gamble, 2002). The stained sections were examined under the light microscope and photographed by a digital Dsc-W 130 super steady cyber shot camera connected to an Olympus BX 21 light microscope in the Histology and Cytology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

2.3.2. Scanning Electron Microscopy

Fresh specimens of the lacrimal gland about 1 mm³ of one head were immediately fixed in a buffered GA/FA fixative (1% glutaraldehyde and 10 % formaldehyde in 0.1 M Phosphate buffer at pH. 7.4 and 4ºC) for two hours, followed by washing with distilled water 3 times for twenty min each at 4ºC and post- fixed in 1 % osmium tetroxide for 2 h at 4ºC. After rinsing with Phosphate buffer solution for 20 min, the specimens were dehydrated in ascending grades of ethyl alcohol and drying in a critical point dryer. Specimens were mounted on a metal base and coated with gold in a vacuum evaporator. Specimens were examined by a JSM-35 (JEOL, CO, USA) scanning electron microscope with an accelerating voltage of 25KV, at the Faculty of Medicine, Tanta University, Egypt.

3. RESULTS

3.1. Macroscopic examination

The lacrimal gland situated on the orbital face of the frontal bone on the dorsolateral aspect of the
eyeball (Fig. 1A), enveloped by a thin sheet of periorbital fascia (Fig. 1B). It was bounded dorsolaterally by the orbital ligament, zygomatic process of the frontal bone and frontal process of the malar bone (Fig. 1C). It was related ventrally to dorsal and lateral rectus muscles (Fig. 1D) and caudally to temporal muscle (Fig. 1C). It was flattened with thin regular rostral border and the thick caudal border with a small notch (Fig. 1E). It was lobulated, irregular in outline, nearly rectangular in shape and pink in color (Fig. 1E & 1F). The average width and length of the lacrimal glands measured about 1.38 ± 0.13 cm and 1.52 ±0.12 cm, respectively (Table 1).

Table 1: Elucidate the different measurements of dog's LG.

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<tr>
<th>Length of LG (cm)</th>
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3.2. Blood and nerve supply:

The lacrimal gland was supplied by the lacrimal artery, which was originated from the ventral branch of external ethmoidal of the external ophthalmic artery (Fig. 2A & 2B). The latter arose from maxillary artery after passing through foramen rotundum (Fig. 2A). The lacrimal artery reached to the caudal border of the lacrimal gland as a single branch and split on its ventral surface into right and left fine branches (Fig. 2C). The lacrimal gland was drained by the lacrimal vein, which poured in the external ethmoidal vein. The latter was received by the ophthalmic Plexus which continued with the pterygoid plexus of the maxillary vein (Fig. 2D & 2E). The lacrimal nerve arose from the dorsal aspect of the ophthalmic nerve and divided into two branches to supply the lacrimal gland (Fig. 2F). The main blood and nerve supply of the lacrimal gland reached it accompanying each other between the dorsal and lateral rectus muscles (Fig. 2E).

3.3. Microscopic examination

3.3.1. Light microscopical observations

The lacrimal gland consisted of stroma and parenchyma. The stroma was composed of a capsule of moderately dense connective tissue that was housing bundles of smooth muscle fibers. The capsule sent septa that divided the parenchyma into variable-shaped lobes and lobules (Fig. 3A). The adipose cells were observed in the capsule and within the glandular lobule. These adipose cells were clustered or singly distributed (Fig. 3A & 3B). The stromal connective tissue of the capsule and septa consisted primarily of collagen fibers (Fig. 3B) and few elastic fibers (Fig. 3C). The capsular connective tissue was stained positively with Alcian blue while smooth muscle bundles were stained positively with Periodic Acid-Schiff (Fig. 3D). The parenchyma was composed of the secretory endpieces and the duct system. Both mucous and serous secretory endpieces were detected, but the mucous secretory endpieces were predominant. The mucous secretory endpieces were mainly tubular in shape and had a wide lumen. It lined by tall columnar cells with pale acidophilic vacuolized cytoplasm and basal oval nuclei. The boundaries of these cells were distinct (Fig. 4A). The serous secretory endpieces were mainly acinar in shape and had a narrow lumen. It lined by pyramidal-shaped cells with homogenous acidophilic cytoplasm and nearly central rounded nuclei. The boundaries of these cells were hardly discernible (Fig. 4B). The lining cells of some secretory endpieces were positively stained with Alcian blue and Periodic Acid Schiff (Fig. 4C & 4D).

The secretory endpieces open directly into intercalated ducts. Some of the intercalated ducts were lined with simple squamous epithelium (Fig. 5A) and other by simple cuboidal epithelium (Fig. 5B). All the interlobular ducts were lined with simple cuboidal epithelium and surrounded by thin layer of connective tissue (Fig. 5C). The interlobar ducts were lined with simple columnar epithelium and surrounded by a thick layer of connective tissue (Fig. 5D).

3.3.2. B-Scanning Electron Microscopical observations

The surface morphology of the dog's LG had the capsular smooth muscle bundles and the variable shaped lobules (Fig. 6A). Numerous crystals of variable sizes and shapes with sharp taper ends were seen around secretory endpieces (Fig. 6B). Several rounded secretory endpieces covered with granular secretory substance and the secretion were also noticed. The circular opening of the intralobular duct, large and small blood vessels
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Fig. 1. Photo macrographs clarifying the position, relation, shape and color of dogs' lacrimal gland. A: A computed tomography image of dog's head axial section. B, C, D: Dorsolateral view of dog’s head after reflection of the lacrimal gland. E: Formalinized dog's lacrimal gland. F: Fresh dog's lacrimal gland in situ. Lacrimal gland (1), orbital ligament (reflected) (2), periorbital fascia (3), medial canthus of the eye (4), lateral canthus of the eye (5), zygomatic process of the frontal bone (6), frontal process of the malar bone (7), temporal muscle (8), dorsal rectus muscle (9), lateral rectus muscle (10), thin rostral border (black arrow), thick caudal border (red arrow) of dog's lacrimal gland.
Fig. 2. Photo macrographs of dogs' heads after removal of the vertical ramus of the mandible clarifying the blood and nerve supply of lacrimal gland. A, B, C, D, E: Dorsolateral view, F: lateral view. Lacrimal gland (LG), dorsal rectus muscle (a), lateral rectus muscle(b), alar canal (c), ophthalmic nerve (on), maxillary artery (1), external ophthalmic artery (2), external ethmoidal artery (3), dorsal branch (4) of external ethmoidal artery, lacrimal artery (5), infraorbital artery (6), lacrimal vein (7), external ethmoidal vein (8), ophthalmic plexus (8*), pterygoid plexus (9), maxillary vein (10), lacrimal nerve (11), the two branches (11*) of lacrimal nerve.
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Fig. 3. Photomicrographs of the dog's lacrimal gland showing A: A thick capsule (line) houses smooth muscle bundles (SM) and groups of adipose cells (AC). The septa (arrowhead) divide the gland into variable-shaped lobules (L). H&E stain; scale bar: 300 μm. B: The capsular connective tissue is mainly formed of collagen fibers (arrowhead), bundles of smooth muscle fibers (SM) and an abundant number of adipose cells (AC). Some adipose cells (ac) are noticed within the glandular lobule. Crossman's trichrome stain; scale bar: 200 μm. C: Few elastic fibers are distributed in the capsule (arrows). Van Gieson’s and Weigert’s elastic stains; scale bar: 15 μm. D: The capsular connective tissue is stained positively with Alcian blue (arrow) while smooth muscle bundles are stained positively with Periodic Acid-Schiff (arrowhead). Combination of Alcian blue stain and Periodic Acid-Schiff technique; scale bar: 15 μm.

Fig. 4. Photomicrographs of the dog's lacrimal gland showing types of the secretory endpieces. A: The mucous secretory endpiece is tubular in shape and has a wide lumen (L). It is lined by tall columnar cells with pale acidophilic vacuolized cytoplasm (arrowhead) and basal oval nuclei (arrow). The boundaries (zigzag arrow) of these cells are distinct. H&E stain; scale bar: 15 μm. B: The serous secretory endpiece is acinar in shape. It is lined by pyramidal-shaped cells with homogenous acidophilic cytoplasm (arrowhead) and nearly central rounded nuclei (arrow). H&E stain; scale bar: 15 μm. C: Some secretory cells stained positively with Alcian blue (arrows), Alcian blue stain; scale bar: 20 μm. D: Some secretory cells are stained positively with Periodic Acid-Schiff (arrows), Periodic Acid-Schiff technique; scale bar: 30 μm.
Fig. 5. Photomicrographs of the dog's lacrimal gland showing the lining epithelium of the progressive ductal segments. A: The intercalated duct is lined with simple squamous epithelium (arrow); B: Other intercalated duct is lined with simple cuboidal epithelium (arrow). C: The interlobular duct is lined with simple cuboidal epithelium (arrow) and surrounded by thin layer of connective tissue (zigzag arrow). D: The interlobar duct is lined with simple columnar epithelium (arrow) and surrounded by a thick layer of dense irregular connective tissue (zigzag arrow), H&E stain; scale bar: 15μm.

Fig. (6): Scanning electron micrographs of the dog's lacrimal gland showing its surface morphology. A: The variable shaped lobules (arrowheads) and the capsular smooth muscle bundles "arrow" are observed. scale bar: 500 μm; B: Several crystals with sharp taper ends (arrows) were arranged around secretory endpieces. scale bar: 10 μm; C: Part of the single lobule showing several rounded acini covered with granular secretory substance (closed arrows). The circular opening of the intralobular duct, large "arrow" and small (zigzag arrow) blood vessels are observed; scale bar: 100 μm; D: Higher magnification of the boxed area in C. Notice rounded secretory endpiece (arrowhead) with its discharged secretion (arrow). scale bar: 10 μm.
4. DISCUSSION

The examined dog's lacrimal gland was located on the dorsolateral aspect of the eyeball. It was bounded dorsolaterally by the orbital ligament, zygomatic process of the frontal bone and frontal process of the malar bone. The shape of lacrimal gland was lobulated, flattened and irregular in outlines. Morphometrically, the average width and length of the lacrimal gland were 1.38 ± 0.13 cm and 1.52 ±0.12 cm, respectively (Cabral et al., 2005; Murphy et al., 2013; Park et al., 2016).

In this study, the dog's lacrimal gland was enveloped by a thin sheet of periorbital fascia and rectangular in shape which disagreed with the results of Murphy et al. (2013) and Park et al. (2016), who recorded that, lacrimal gland was non encapsulated and ranged in shape from round, triangular, or oval to heart- or dumbbell-shaped. Also, this rectangular lacrimal gland differed from the shapes that have been investigated in other animals; bilobed in cattle (Pinard et al., 2003) and human (Lorber, 2007), elongated in camel (Alsafy, 2010; Ibrahim et al., 2006; Mohammadpour, 2008; Mohammadpour, 2011), ovoid in donkey (Alsafy, 2010; El-Bhery, 2015), triangular in Roe Deer (Kleckowska-Nawrot et al., 2013), oval to spherical in Lori sheep (Abbasi et al., 2014) and elongated oval in sheep (El-Bhery, 2015).

The lacrimal artery was the main blood supply of the dog's lacrimal gland. It was the ventral branch of the external ethmoidal artery of the external ophthalmic artery. While, (Park et al., 2016), revealed that, the lacrimal artery was detached from the muscular branch of the external ophthalmic artery near the apex of the extraocular muscle cone.

The results concerning the venous drainage of the dog's lacrimal gland were similar to the earlier literature and differed from that observed in bovine (Buda and Budras, 2003). Who clarified that the venous blood of the orbit was drained through the dorsal external ophthalmic vein, which poured in the superficial temporal vein.

The investigated lacrimal gland was innervated by lacrimal nerve, which reached to the gland accompanied with the lacrimal artery between the dorsal and lateral dorsal muscles which came parallel to Park et al. (2016).

The light microscopic examination of the dog's lacrimal gland structure showed a mixed tubuloacinar gland with predominant mucous secretory endpieces. The same results were obtained in the most studied mammalian species as in senile rat (Draper et al., 1998), Philippine water buffaloes (Maala et al., 2007; Shadkhast and Bigham, 2010), swine (Menaka and Puri, 2015) and dog (Shaker and AL-Obeady, 2016). On the contrary, the predominant serous type is reported in the young rat (Draper et al., 1998), sheep (Gargiulo et al., 1999), Roe deer (Kleckowska-Nawrot et al., 2013) and Alpaca (Kleckowska-Nawrot et al., 2015b). While the pure serous type is documented in human (Hamid et al., 2015).

The lining cells of secretory endpieces contained both PAS and Alcian blue positive cytoplasmic granules indicates the presence of acidic and neutral mucopolysaccharides. Similar data have been reported previously for the Philippine water buffalo (Maala et al., 2007), American bison and cattle (Pinard et al., 2003). While, in European bison, the mucopolysaccharides are acidic (Kleckowska-Nawrot et al., 2015a). Consequently, lacrimal gland appeared a primary source to the mucous layer of the tear film, in addition to the conjunctival epithelia (Arango et al., 2001; Gasser et al., 2011). The function of the mucus is to protect corneal and conjunctival epithelia (Mantelli and Argueso, 2008).

In the canine Cabral et al. (2005), European bison Kleckowska-Nawrot et al. (2015a) and alpaca Kleckowska-Nawrot et al. (2015b) indicated the intralobular adipose cells have been found, which was also characteristic of the investigated dog. According to Payne (1994) reported such cells are lipid-laden cells in the harderian gland that act as carriers and storage for the porphyrin pigment. The importance of porphyrins, is photoprotective for the eye and conjunctiva.

There were several classes of ducts which varied in location and epithelial lining. The intercalated, interlobular and interlobar ductal segments. The present study revealed that intercalated ducts were lined with simple squamous epithelium and simple cuboidal epithelium in other. This investigation is partially goes in hand with Seamon et al. (2008) in rabbit and dog, Abbasi et al. (2014) in Lori sheep and El-Fadaly et al. (2014) in adult albino rat who reported that the intercalated ducts have only simple cuboidal epithelium lining cells. On the contrary, Shaker and AL-Obeady (2016) revealed that there are no intercalated ducts in the dog.

The epithelial cells lining interlobular ducts were simple cuboidal epithelium in the examined dog's lacrimal gland. This result was in accordance to that mentioned in rabbit (Seamon et al., 2008).
and dog (Shaker and AL-Obeady, 2016). Results differ from that reported in Iranian river buffalo (Shadkhast and Bigham, 2010), bison and cattle (Pinard et al., 2003) where pseudostratified lining epithelium are reported. The stratified cuboidal epithelium is detected in Lori sheep (Abbasi et al., 2014) while a single layered or a double-layered columnar epithelium are recorded in the guinea pig (Gasser et al., 2011).

The present study revealed that interlobar ducts were lined with simple columnar epithelium. These results were in partially coinciding with Seamon et al. (2008) who reported that interlobar ducts are lined with simple to pseudostratified and stratified columnar in the rabbit.

Numerous crystals with sharp taper ends were loosely arranged around secretory endpieces of the examined lacrimal gland. According to, Breustedt et al. (2005) in the human, Seamon et al. (2008) in the rabbit and Madhurantakam et al. (2010) in the dog the presence of crystals in the tear film are tear lipocalin (TLC). Tear lipocalin has a variety of extracellular functions in tears, including binding and release of lipids, regulation of tear viscosity and serving as a biomarker for dry eye disease in human (Dartt, 2011).

It could be concluded that the lacrimal gland of the dog was dorsolaterally situated to the eyeball with special covering by a thin sheet of periorbital fascia. The orbital ligament, zygomatic process of the frontal bone and frontal process of the malar bone were the dorsolateral boundary of the gland. The microscopical investigation revealed a tubuloacinar gland with seromucoid secretory endpieces, but the mucous secretory endpieces were predominant. Therefore, the lacrimal gland acts as a source of tear film mucin (neutral and acidic mucopolysaccharide). Several crystals with sharp taper ends were loosely arranged around the secretory endpieces.

5. REFERENCES


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