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A comparative histological study of trachea between mature and immature local breed dog (*canis familiaris*

Mohammed Dhyaa Abdulkareem[1], Mohammed Sulaiman Dawood[2]

[1]Department of Anatomy and histology, College of Veterinary Medicine, Baghdad University, Iraq. [2]Department of Anatomy and histology, College of Veterinary Medicine, Baghdad University, Iraq

Abstract. Aim: The present study aimed to investigate the histological features of trachea of mature and immature local breed. Materials and Methods: six adult dogs were used in the present study. The animals were injected by0.04 mg/kg atropine Sulphate, subcutaneously as a premedication drug after 20-30 minutes injected by Xylazine 2% and Ketamine 10%, the dose was used 1 mg/kg, then the animals were opened up, and the trachea were removed, The specimens fixed in formalin 10% for 48hr. then processed according to paraffin embedding technique protocols and the tissue sections are stained with hematoxylin and eosin, and masons trichrome combined with Alcian blue (pH2.5)-PAS. Results: The results showed that, in immature dog the trachea was a ridge tubular part of upper respiratory tract build up by tunica mucosa, tunica submucosa and tunica adventia the epithelialis mucosa was composed of ciliated pseudo stratified columnar epithelium with goblet cells that measured 25.526±1.34µm. In mature dog, the tunica mucosa of trachea was build up by epithelialis mucosa and lamina propria and thin interrupted muscularis mucosa. The epithelialis mucosa was composed of ciliated pseudo stratified columnar epithelium with goblet cells that measured (20.91±0.9µm). The histochemical study showed that, with combine AB (2.5pH) - PAS stains the ciliated epithelial cells were secreted acidic mucopolysaccharids while the goblet cells secreted weak neutral mucopolysaccharids.

Keywords: dog, trachea, histology.

Introduction

The respiratory system is a vital organ that plays an important role in respiration called the aeration system. This system limited the gas exchange via the respiratory construction and outside atmosphere (Maton *et al.*, 2010). The olfaction, vocalization and body temperature regulation deem another respiratory activation beside the ventilation which consisted of gases exchange and conduction (Sellnow, 2006; Baba and Choudhary, 2008). The mammalian respiratory system consisted of numerous anatomical structures classified as conducting and respiratory part, the first one nasal cavity, nasopharynx, larynx, trachea and bronchi; however, the second bronchioles, alveolar ducts, and alveoli which were responsible for gas exchange. The distribution of the air and blood in a wide space via large exact network of airway and blood vessels will assist the gas exchanging (Glenny and Robertson, 2011). Kuehne and Junqueira**,**(2000)and Hamid *et al***.,** (2005) mentioned that the wall of the trachea has consisted of four layers in most domestic animals, which were pseudostratified columnar epithelial mucosal layer**,** submucosa**,** muscularis, and adventitia, the incomplete hyaline cartilaginous ring supports these layers.

Materials and Methods

Experimental animals

Six adult healthy dogs (*canis familiaris*) aged (2-5) years, were used. The animals were injected by0.04 mg/kg atropine Sulphate, subcutaneously as a premedication drug(Dawood,2016; Salman and Dawood,2022) after 20-30 minutes injected by Xylazine 2% and Ketamine 10% (AL-Falahi et al 2016), After euthanized the animals, then the animals were opened up, and the trachea were removed, The specimens are fixed in 10% formalin for 48 hr.(Dawood,2018) then processed according to paraffin embedding technique protocols(-Dawood,etal.,2022; Rasheg,etal.,2016 ;Mohammed, 2017) and the tissue sections are stained with hematoxylin and eosin, and masons trichrome combined with Alcian blue (pH2.5)-PAS(Batah and Mirhish, 2019)

Results

The results showed that the trachea of immature dog consisted of tunica mucosa, tunica submucosa and tunica adventia (fig. 1). The tunica mucosa was build up by epithelialis mucosa and lamina propria without muscularis mucosa (fig, 2).The epithelialis mucosa was composed of ciliated pseudo stratified columnar epithelium with goblet cells that measured 25.526±1.34µm. The epithelialis mucosa composed of three types of cells; Ciliated cells, non-ciliated (Goblet cells) and basal cells. The ciliated

columnar cells were the predominated type had eosinophilic cytoplasm; the goblet cells were a few in numbers 1-2/HPF (table4) had bright cytoplasm, and the basal cells composed a line of cells which arranged at the basement membrane and had vertically oriented darkly stained nuclei that revealed active mitotic figures (fig. 2). The lamina propria was thick layer which continuous with tunica submucosa due to lack of muscularis mucosa, it composed of well vascular fibrous connective that revealed little of tracheal glands, the thickness of lamina propria-submucosa measured 128.87 ±2.03µm (fig. 3 & 4). The lamina propria-submucosa supported by overlap hyaline cartilage that followed by circular bindles of smooth muscle (fig. 1). Tunica adventia was thin layer of fibrous connective tissue (fig. 1). The tracheal glands were solitary patches of mixed compound tubular glands that build up by large mucous alveoli which surrounded by serous demiulons, these glands were dispersed around the trachea (fig. 4& 5). The histochemical study showed that, with combine AB (2.5pH) - PAS stains the ciliated epithelial cells were secreted acidic mucopolysaccharids while the goblet cells secreted weak neutral mucopolysaccharids. On the other hand, the secretory units of the tracheal glands were both types of secretory activities (Acidic and neutral mucopolysaccharids (fig. 6&7). In mature dog, the tunica mucosa of trachea was build up by epithelialis mucosa and lamina propria and thin interrupted muscularis mucosa (fig, 8 & 9). The epithelialis mucosa was composed of ciliated pseudo stratified columnar epithelium with goblet cells that measured (20.91±0.9µm). The epithelialis mucosa composed of three types of cells; Ciliated cells, non-ciliated (Goblet cells) and basal cells. The ratio of the ciliated columnar cells was as well as that of the goblet cells 5-7/HPF, the basal cells composed a line of cells which arranged at the basement membrane showed resting status (fig.10). The lamina propria was thin layer of fibrous connective tissue that separated from submucosa by thin layer of interrupted smooth muscle fibers of muscularis mucosa (fig. 9 & 10). The tunica submucosa was very thick layer that measured 57.04±1.3 µm, it composed of loose connective tissue occupied by furthermore tracheal glands and supported by vascular fibrous connective that revealed little of tracheal glands (fig. 9 & 11). The submucosa supported by overlap C-shape hyaline cartilage that followed by bindles of smooth muscle (fig. 8). Tunica adventia was very thin layer of fibrous connective tissue (fig. 8). The tracheal glands were furthermore specially at the dorsal aspect of the trachea and dispersed around the tracheal submucosa. The histochemical study showed that, with combine AB (2.5pH) - PAS stains the ciliated epithelial cells were secreted acidic mucopolysaccharids while the goblet cells secreted weak neutral mucopolysaccharids. On the other hand, the secretory units of the tracheal glands were both types of secretory activities (Acidic and neutral mucopolysaccharids (fig.12).

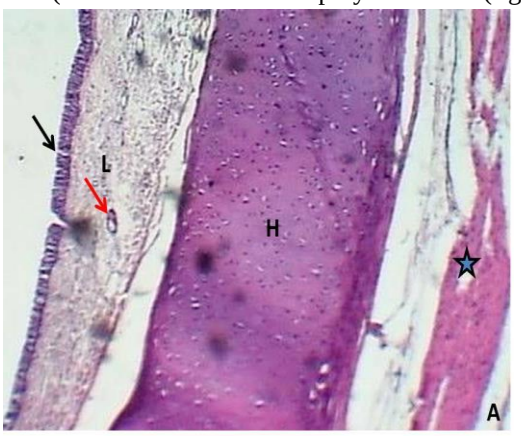

Figure 1. Transverses section of trachea (immature dog) shows: epithelalis mucosa (Black arrow), lamina propria submucosa (L) tunica adventia (A), hyaline cartilage (H) & smooth muscle (Asterisk) & tracheal gland (Red arrow) .H&E stain 40X

Figure 2. Section of trachea (immature dog) shows ciliated epithelial cells (Black arrow), goblet cells (G), basal cells (Red arrows), duct (D), opening of duct (O), fibrous tissue (Asterisk) & blood vessels (Blue arrow).H&E stain 400x.

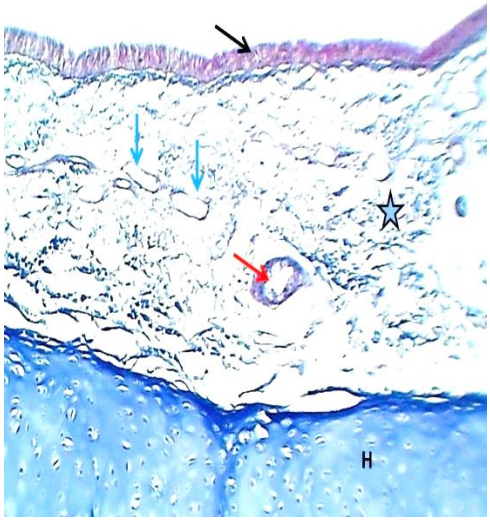

Figure 3. Section of trachea (immature dog) shows epithelial mucosa (Black arrow), tracheal gland (Red arrow), collagenous fibrous tissue (Asterisk), blood vessels (Blue arrow) & hyaline cartilage (H). Masson trichrom stain 200x.

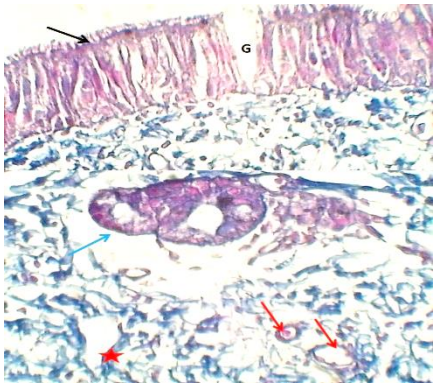

Figure 4. Section of trachea (immature dog) shows epithelial mucosa (Black arrow), tracheal gland (Blue arrow), collagenous fibrous tissue (Asterisk), & blood vessels (Red arrow). Masson trichrom stain 400x.

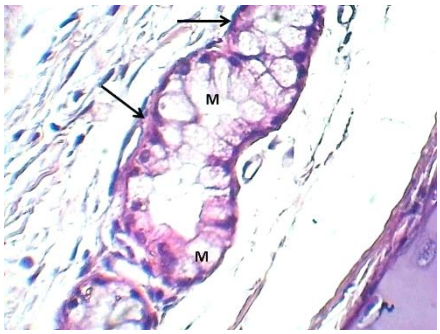

Figure 5. Section of trachea glands shows: mucous secretory alveoli (M) & serous demiulons (Black arrows). H&E stain 400x.

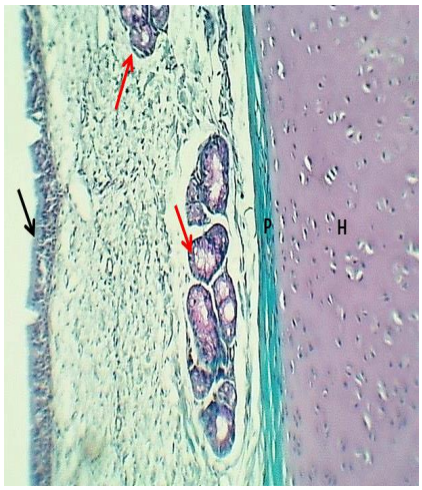

Figure 6. Section of trachea shows epithelial mucosa (Black arrow), tracheal glands (Red arrows), perichondrium (P) & hyaline cartilage (H). Combine AB & PAS stains 100x.

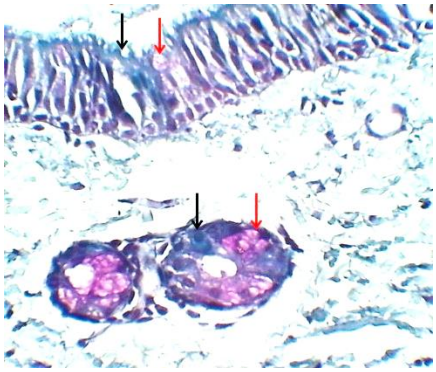

Figure 7. Section of trachea glands shows: acidic mucoploysaccharid (Black arrows), and neutral mucoploysaccharid (Red arrows). Combine AB & PAS

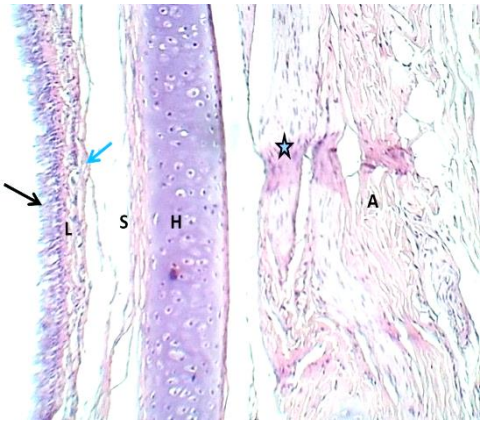

Figure 8. Transverses section of trachea (Mature dog) shows: epithelalis mucosa (Black arrow), lamina propria (L), muscularis mucosa (Blue arrows), hyaline cartilage (H) & trachealis muscle (Asterisk) & adventitia (A) .H&E stain 40x.

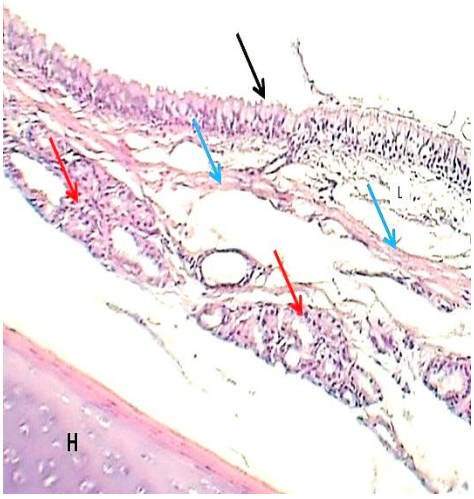

Figure 9. Transverses section of trachea (Mature dog) shows: epithelalis mucosa (Black arrow), lamina propria submucosa (L), muscularis mucosa (Blue arrows), hyaline cartilage (H) & submucosal tracheal glands (Red arrows) .H&E stain 100x.

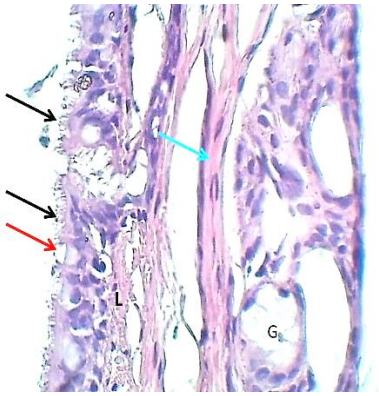

Figure 10. Section of mucosa & submucosa (Mature dog) shows ciliated epithelial cells (Black arrow), goblet cells (Red arrow), fibrous tissue lamina propria (L) & muscularis mucosa (Blue arrow) & tracheal glands (G).H&E stain 400x.

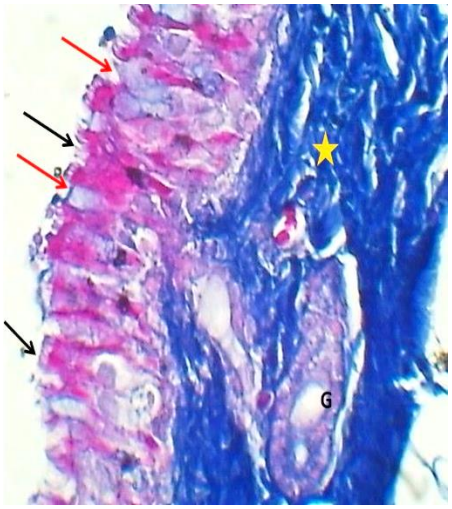

Figure 11. Section of mucosa of trachea (Mature dog) shows ciliated cells (Black arrow), goblet cells (Red arrows), tracheal gland (G), and collagenous connective tissue (Asterisk). Masson trichrom stain 400x.

Figure 12. Section of mucosa of trachea (Mature dog) shows: acidic mucoploysaccharid (Black arrows), and neutral mucoploysaccharid (Red arrows). Combine AB & PAS stains 400x

Discussion

The results of the present study of both animals coincided with Moussa and Hassan (2015) in red fox, showed that the tracheal and bronchial wall formed of mucosa, submucosa and adventitia. But the mucosa consisted of pseudostratified columnar epithelium without cilia, as well as, mentioned four type of respiratory cells instead of three type which mention in the current study, (Al-Abasi, 2001) in camel and Al-Umeri, (2015) in sheep also mention four type of cells, Hussein, and Abdul Zahra, (2016) in camel, demonstrated that the wall of the trachea consist of mucosa, submucosa, hyaline cartilage and adventitia. but, Mariassy and Plopper (1983**)** and Hamid *et al.***,** (2005) reported that the tracheal wall of the most domestic animals consisted of four layers; mucosa**,** submucosa**,** muscularis and adventitia, oppositely, the current study recorded three layers. Generally**,** (Kuehne and Junqueira**,** 2000) stated that the mucosal layer was lined by typical respiratory epithelia and there were three type of cells, while additionally recored longitudinal folds in dorsal membranous section of mucosa**.** Yang *et al.***,** 2010) recorded a dissimilar result associated with goblet cells who demonstrated that these cells has Microvillus projections and crescent shaped nucleus. Meanwhile (Das *et al.***,** 1978) declared that the trachea lined with typical respiratory epithelia, while the proximal part of the tracheal wall was line by tall columnar ciliated with goblets cells and lesser triangular basal cells, and mentioned dissimilar result who showed that the ciliated cell number was more five times than the goblet cells. Dias and Celis, (2011) in human recorded that the trachea lined by typical respiratory epithelia, as well as, great numbers of serous mucous cells of basal membrane situation like the dog, but the Submucosa layer composed of cartilaginous ring of hyaline cartilage as same the present study. Meanwhile Yousif (2019) in small ruminants recorded the same tunicae as mentioned in the current study but lamina propria and muscularis mucosa was missed as in the immature dog, but the other structures and features were similar to the present study, also stated tracheal glands were simple tubuloalveolar mucous glands instead of mixed compound tubular glands as in the dog. Hamid *et al.* (2005) observed that the external submucosal layer limited with the adventitial layer and consisted of dense connective tissue with a C-shaped ring of hyaline cartilage which were opened dorsally to form free ends that connect by a smooth muscle fibers named trachealis muscle this result was coincided with the present study On the other hand Abdul-Raheem and AL-Haaik (2006**)** in small ruminants demonstrated that the submucosal glands were compound mixed tubuloacinar**,** therefore the chief portion of the secretion were mucous and the rest of glands were serous**,** while the other details were similar to the present study except the presences of intermediate cells

which were not found in the current study, as well as Evans *et al.***,** (1999) and Jumaah (2017) has the same results of the current study. Al-Abasi (2001) mentioned that the tracheal glands in camels were serous and few number of mucous as the current results, whereas Choi *et al.***,** (2000) mentioned that the Submucosal glands are absent in laboratory animals (rabbits**,** guinea pigs and mice. Hussein, and Abdul Zahra, (2016) in camel showed that the Goblet cells of trachea produce limited quantities of acidic and neutral mucosubstances which were appeared positive reaction toward PAS stain and revealed purple color due to mucopolysaccharide, similar finding was observed by Raji and Naserpour, (2007). The mucous produced by goblet cell act as a protective barrier for the epithelium by lubricating, insulating and providing an appropriate condition for mucociliary clearance (Buchner and Maxwell, 1993).

The results of the all histological measurements of the present study refer to that the respiratory system has continues development until reach to the maturation age approximately about 37 weeks (8.5 month**)**, (The rate of lung development can vary greatly, and the lungs are among the last organs to fully develop.

Conclusion: The results referred that the lung of dog has different blood supply from other domestic animals and the

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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