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Morphological Investigations of the Thyroid Gland in Dogs

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ABSTRACT

The present study aimed to comprehensively examine the thyroid glands of young and mature male dogs through anatomical, ultrasonographic, computed tomography (CT) imaging techniques, histological, and immunohistochemically. Five male dogs were used. Grossly, the thyroid glands of dogs consist of two separated lobes that are localized on the lateral aspect of the cranial part of the trachea. Both lobes appeared as dark red flattened ellipsoid bodies. The right lobe was localized more cranially than the left lobe. Ultrasonography revealed the thyroid glands as a hypoechoic organ, but it was also more echogenic or isoechoic than the surrounding muscles. CT imaging provided high-resolution images for evaluating the gland's morphology and any potential pathological changes. Histologically, the dog's thyroid gland was covered with a thick capsule, while the trabeculae of the gland are poorly expressed. Thousands of follicles represent the Parenchyma of the gland within the collagenous capsule, The shape of the thyroid follicle ranged from spherical to elliptical. Immunohistochemicals showed a strong positive reaction for TTF1 in follicular cells at a young age and moderate reactions in adult animals and this result might be associated with the physiological role of TTF1 in the differentiation of endodermal origin follicular cells into active endocrine cells. Similarly, immunostaining showed a stronger reaction for SYN in the parafollicular cells of young age than that of adult animals and this result might be associated with the physiological roles of SYN in the differentiation of neuroectodermal origin parafollicular cells into endocrine cells.

1. INTRODUCTION

In dogs, the thyroid gland is an essential endocrine organ that controls body metabolism, it generates hormones that regulate the body's growth, development, and energy utilization[1] The thyroid gland has anatomical features that vary among vertebrates; the thyroid gland varies in shape, color, and location, and it varies within a species even during its development[2] The shape of the thyroid gland has been extensively researched across several animal species. Several investigations have identified interspecific and intraspecific variations, A significant amount of research focuses on the anatomy of the thyroid gland in both fetuses and adult animals, encompassing dogs, cats, cattle, small ruminants, and wild species[3]. The normal thyroid gland lobes in dogs are flattened ellipsoids two separated bodies, located dorsolateral at the cranial section of the cervical part of the trachea. These lobes contain four ovoid glands, known as parathyroid glands[4]

The thyroid gland is vascularized by two major arteries, which are at least as much as the blood supply to a parenchymatous organ of the same size in one body. The caudal thyroid artery usually originates from the brachiocephalic trunk or, in certain cases, from the caudal section of the common carotid artery, whereas the cranial thyroid artery is derived from the common carotid artery [5]

Ultrasonography is the most effective technique for visualizing visceral organs in animals, whether outside or inside the body cavities Because of the unique position of the thyroid gland as it is located superficially in the body, 1.5–2 cm below the skin's surface, it can be examined with a high-frequency transducer (7–12 MHz) or 10 MHz on average. Each lobe was located using the trachea medially, the sternothyroid muscle ventrally, and the common carotid arteries laterally. The dorsally situated esophagus can serve as an extra landmark for the left lobe [6]

Nowadays, CT is crucial for the diagnosis and management of numerous illnesses. It is also an important research tool in the field of veterinary anatomy[7]

You need to know how the thyroid gland and other organs normally look to make an accurate diagnosis. Due to its ability to provide an axial slice of the area of interest and precisely measure the tissue absorption of X-ray beams as they pass through, CT is one of the most crucial diagnostic imaging tools in veterinary medicine. This allows for greater differentiation between individual soft tissue structures than radiography[1,8]

Histologically, the thyroid gland contains two types of cells: follicular cells also called thyrocytes, and C cells, or para-follicular cells. The follicular cells form what is known as a thyroid follicle, which is a sphere of cells surrounding a lumen. The shape of the cells varies depending on their synthetic activity; if the follicle's secretory activity is high, the thyrocytes will be columnar, or cuboidal while the squamous form indicates the follicles in the resting inactive stage [9,10,11]

The thyroid follicle is formed of follicular cells and intra-follicular colloids. The thyroid's functional activity affects the size, shape, and height of the follicular epithelium and the follicles. Furthermore, the intra-follicular colloid exhibits morphological variability contingent on the thyroid's functional state, The thyroid gland is responsible for secreting two main hormones critical for the normal life of animals, as thyrocytes secrete thyroxine (T4) and triiodothyronine (T3) that control basal metabolic rate and protein synthesis, while calcitonin hormone which acts to decrease the level of calcium in the blood in contrast to parathyroid hormone function, produced by parafollicular cells[9,10,11,12] Although there are some studies on age-related morphological changes of the thyroid gland, there are few on the relationship between Structural changes and functional activity of dog thyroid gland

One of the most important nuclear proteins found in thyroid gland follicular cells is thyroid transcription factor 1(TTF1), which is first described as a mediator of thyroid-specific gene transcription, that is why it was named thyroid transcription factor 1, the role of TTF1 in the thyroid gland is very critical as it regulates a particular gene group those directly contribute to the manufacture of thyroid hormones, such as sodium iodide symporter, thyroid peroxidase (TPO), thyroglobulin, and TSH receptor[13] TTF1 is mainly expressed in differentiated cells derived from the foregut endoderm and neuroectoderm, including thyroid follicular cells and type II alveolar epithelial cells[14]

TTF1 is not present only in the thyroid gland but also in many other regions, as it is identified in the lung epithelium of both humans and rats, during the embryonic period and after birth, and in the embryonic forebrain. [15] Recently, TTF1 has been widely used as a molecular tumor marker for lung and thyroid gland cancer diagnosis [16]

Synaptophysin is a membranous glycoprotein, and it was initially isolated from bovine presynaptic vesicles and frequently used as immunohistochemical markers for neuroendocrine tumors like malignant tumors of the C cells. (MTCs) is a rare thyroid cancer developing from a deviation in C cells that secretes calcitonin and chromogranin A into the bloodstream [17]. On the other hand, synaptophysin can occur in normal and neoplastic neuroendocrine cells of neural type [18]. And play an essential role in the differentiation of the neuroendocrine cells [19]

The increase of dogs as pets in Egypt provides them with a high level of health care and the observation of a high incidence of diseases concerning this species' thyroid gland. Thus, this study aims to examine the thyroid gland in normally apparent healthy male dogs from all perspectives by using many morphological tools to provide enough information to enrich our knowledge about the thyroid gland and to provide basic diagnostic knowledge about the thyroid gland in dogs.

2. MATERIALS AND METHODS

2.1 Animals:

In this study, five healthy-looking male dogs were included for the examinations of the anatomy, ultrasonography, CT imaging, histology, and immunohistochemistry of the thyroid glands. The age of used animals was ascertained by dentition according to [20]. Anatomical, ultrasonographic, and CT studies were carried out on three mature male dogs whose ages ranged from (2 to 3.5) years old. The histological and immunohistochemical study was carried out on the previously mentioned group and two young male dogs whose ages ranged from (2 to 3) months, The animals were collected from Sheibin El-Kom city; Menoufia governorate some were hopeless cases admitted to the pet clinic of the Faculty of Veterinary Medicine Menoufia University, the use of animals was permitted by their owners to be included in the study. The samples were collected and investigated at the Department of Anatomy and Embryology Faculty of Veterinary Medicine, Menoufia University, Egypt. During this study restricted animals from this area to avoid environmental conditions that could affect thyroid gland activity.

2.2. Gross Anatomical examination:

The animals that were used for the gross anatomy of the thyroid gland were euthanized by Ketamine (100 mg/Kg) and xylazine (10mg/Kg) IM injection [21]. This step was followed by canalization and infusion of 10% formalin through the common carotid artery and usually 2 - 3 liters were used based on the animal size. and if there was resistance to the injection of the embalming solution, it meant that enough solution had been injected [22]. For complete fixation and preservation, the animals were subsequently submerged in 10% formalin. After complete fixation the neck region of the examined cadaver was dissected through a ventral midline incision then the neck region was dissected, the size, shape, location, and relation of the thyroid gland were examined.

2.3. Ultrasound examination of thyroid gland:

The thyroid gland ultrasound examination was performed at the Department of Surgery, Anesthesia and Radiology, Faculty of Veterinary Medicine, Menoufia University using an ultrasound machine (ESAOTE S.p.A–Italy). The animals were manually secured on the dorsal recumbency, and the neck was extended to provide adequate access to the cranial region of the neck. The hair was then clipped from the ventral aspect of the neck, and the two thyroid gland lobes were scanned using a micro-curved probe with frequency (5 MHz). Slowly moving the probe caudally in the midline, the scanning started just caudal to the larynx.

2.4. CT examination of the thyroid gland:

To perform a CT examination the animal was sent to AL Mawaddah Radiology Center where we could perform a thyroid gland CT scan using a 16-row scanner (Siemens somatom go now- Germany) with parameters 120 Kv, 150 Ma, slice thickness 2mm. Scanning started from the larynx to the thoracic inlet as soon as euthanasia was performed using a 20% pentobarbitone solution[23].

2.5. Histological Examination:

Following the removal of glands from surrounding tissues, the glands were fixed in 10% neutral buffer formalin, cut into tiny pieces measuring 3–4 mm, and refixed again in 10% neutral buffer formalin. Followed by washing and dehydration in increasing alcohol grades (70, 90, and 100%). The next step was xylene cleaning. [24] The samples were then dehydrated in ascending grades of ethanol, cleared in xylene, and impregnated with melted paraffin wax. Finally, paraffin blocks of the processed samples were prepared. Thin sections (5-7 µm thick) were cut and mounted on egg albumin-glycerin coated glass slides, dried in an electrical incubator for 30-60 minutes, and stained with the following stains hematoxylin and eosin (H and E), Masson's trichrome technique, Van Gisson stain and Periodic Acid Schiff reaction (PAS), The slides were examined by a light microscope and photographed by digital camera.

2.6. Immunohistochemistry:

For immunohistochemistry, two groups of animals were selected, the first group included mature animals ranging from (2 to 3.5 Years) and the second one included young animal (2 to 3 months) for detection of Thyroid transcription factor-1 (TTF-1) and Synaptophysin (SYN) This investigation was completed in the department of pathology - faculty of medicine Tanta University Egypt. The immunostaining was performed on the formalin-fixed paraffin section. Antigen retrieval was carried out by submerging the slides in citrate-buffered (pH 6) distilled water and then microwaving for 15 minutes at 850 W and 15 minutes at 300 W in a microwave. After that, the slides were left for 20 minutes to cool. The endogenous peroxidase was inhibited by applying 0.03% hydrogen peroxide for five minutes and then washing with water and phosphate-buffered saline (pH 7.4). [25] The antibody used for synaptophysin was rabbit anti-SPY at a 1:100 dilution, meanwhile, the mouse MoAb (M3675, diluted at 0.54 microgram immunoglobulin G/ml, DAKO Corporation) used for TTF1 detection, then sections were incubated for an entire night at 4°C in a humidity chamber. The slides were then incubated at room temperature for 1h, rinsed with PBS three times, and incubated with appropriate biotinylated secondary antibodies for 30 min. For visualization, a commercial ABC kit was used and incubated with DAB for 5 min. Sections were counterstained with hematoxylin. The sections were examined by light microscope and photographed by digital camera

RESULTS

3.1. Gross anatomy of the thyroid gland in the dog

The canine thyroid gland was made of two separate lobes and was localized on the lateral aspect of the cranial part of the trachea (Fig 1A) Each lobe appeared to be enclosed by thick per-tracheal fasciae (Fig 1C). The right and left lobes were not connected by an isthmus. In most examined animals, the right lobe was more cranial than the left lobe as the right lobe extends from the cricoid cartilage of the larynx to the fourth tracheal ring. The left lobe occupies the space from the second tracheal ring to the sixth ring (Fig1B). In one animal the two lobes have the same extension from the first to the fifth tracheal ring (Fig1D). The two lobes were dark red, flattened, elongated, and had two pointed ends; however, the caudal end was typically smaller than the cranial one.

Each thyroid lobe has two surfaces, the lateral surface is related to the sternocephalic muscles, while the medial surface is typically associated with the lateral surface of the trachea (Fig1D)

The ventral border of both lobes was related to the dorsal aspect of the sternothyroid muscle. On the other hand, the topographical relation of the dorsal border has differed between the left and right lobes based on the surrounding structures.(Fig1D) On the left side, the esophagus was in contact with the dorsal border of the left lobe while the dorsal border of the right lobe was in contact with the carotid sheath with its content of right common carotid right vagosympathatic trunk and right caudal laryngeal nerve)

The arterial blood supply of the thyroid gland is achieved by two main arteries, the cranial and caudal thyroid arteries on both sides (Fig1A). The cranial thyroid artery was detached from the common carotid artery at the level of the larynx and passes through the sternomastoid muscle the artery entered the right and left lobes of the gland from its cranial pole. The caudal thyroid artery originated from the brachiocephalic trunk directed cranially and entered the right and left lobes of the gland from its cranial pole caudal pole.

3.2. Ultrasound examination

The ultrasonography of the dog's normal thyroid gland lobes revealed that both lobs of the glands were located between the trachea medially and the common carotid artery laterally. They had bilateral symmetry in shape as a thin, homogenous fusiform structure that is easily distinguished from the surrounding organs. The thyroid appeared to be a hypoechoic organ, but it was also more echogenic or isoechoic than the surrounding muscles. The thyroid gland is encased in a smooth, fibrous capsule that provides a thin, well-defined, hyperechoic layer around the gland. (Fig 2)

3.3. Ct examination

The thyroid gland is easily identified by CT because of its high attenuation in comparison to the surrounding soft tissues. The thyroid glands lobes appear homogenous on both sides of the trachea in a dorsolateral location, and on the transverse section, they appear as an ovoid or triangular structure that is related medially to the lateral surface of the trachea and the common carotid artery laterally. On long-axis CT, however, the thyroid gland has a distinctive appearance as an elongated ovoid shape. On the other hand, we were unable to identify the parathyroid glands using CT (Fig3)

3.4. Histological examination

The thyroid gland is enclosed by a connective tissue capsule that sends trabeculae into the parenchyma, the trabeculae divide the thyroid lobe into many lobules (Fig 4A). The thyroid gland consists of the outer stroma of connective tissue and internally located parenchyma. The internal parenchyma contains two types of cells: thyrocytes are deeply stained, and numerous forming a glycoprotein-containing structure called thyroid follicles that vary in size. At young ages, the follicles are small and centrally located while the large-size follicles are present at the periphery (Fig 5). In old age, the large follicles become dominant and present not only in the periphery but also at the center of the gland (Fig 4C) The thyrocytes display variation in the shape between small and old aged dogs as in the young age follicular cells tend to be cuboidal or low columnar which indicates that the thyroid gland is in a state of activity (Fig 5B) while the cells tend to be more squamous which told us that the gland tends to be more inactive than that of the young age, the other endocrine cells called Para-follicular (C cells), are large and have lightly stained cytoplasm with deeply stained nuclei. These cells are fewer in number and are present as single cells or as an aggregation between follicles. (Fig 4C)

The immunohistochemistry for the SYN showed strong immunostaining within parafollicular cells of young animals and a moderate reaction in adult animals. The reaction mainly localized in the cytoplasm of the parafollicular cells (Fig 9A) (Fig 8A). as well as immunostaining showed a stronger reaction for TTF1 in the follicular cells of young animals than that of adult animals (Fig 9B) (Fig 8B).

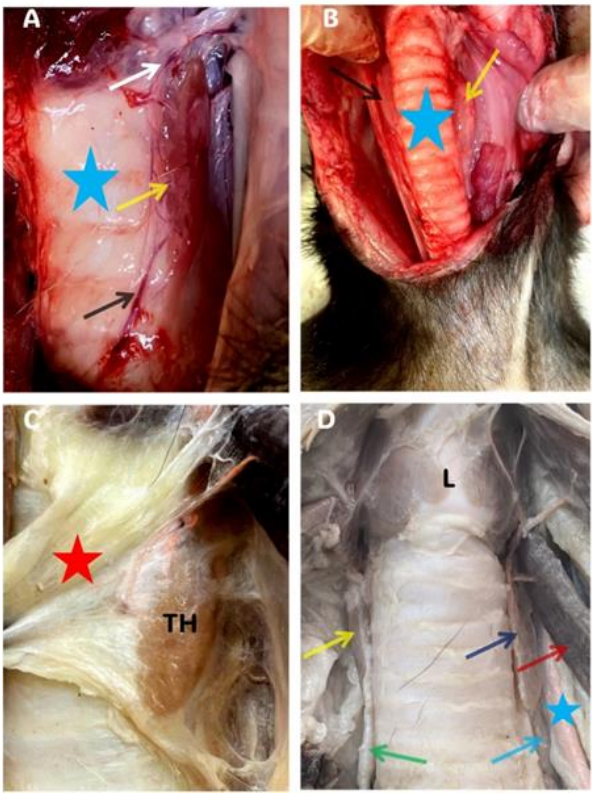


Fig. 1.In situ ventral views of the thyroid glands in male dogs. Panel A: - illustrates the gross anatomy of a normal canine thyroid gland in adult animals and shows the trachea (blue star), cranial thyroid artery (white arrow), caudal thyroid artery (black arrow), left lobe of the thyroid gland (yellow arrow). Panel B: - is a normal canine thyroid gland in adult animals and shows asymmetrical location of the two lobes, the right lobe (black arrow), left lobe (yellow arrow), and trachea (blue star). Panel C: - showed pre-tracheal fasciae (red star) that enclose the left lobe of the thyroid gland (TH). Panel D: - shows the anatomical relationships of thyroid lobes. Sternothyroideus muscle (red arrow), the right lobe (yellow arrow), the right common carotid artery (green arrow), the left thyroid lobe (dark blue arrow), the right common carotid artery (blue star), the esophagus (light blue), and larynx (L).

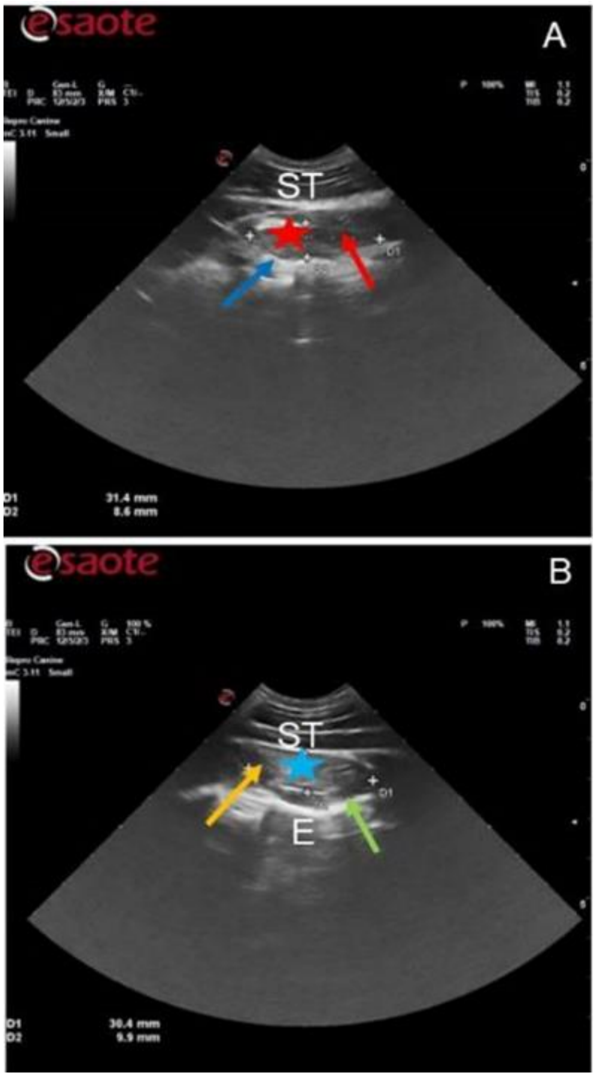


Fig.2. Ultrasonography showed the ultrasound examination of the two lobes of the canine thyroid gland. Panel A: - shows the right lobe of the thyroid gland (red star), capsule (blue arrow), caudal parathyroid gland (red arrow), and sternothyroid muscle (ST). Panel B: - shows the left lobe of the thyroid gland (blue star), capsule (green arrow), cranial parathyroid gland (orange arrow), sternothyroid muscle (ST), and esophagus (E).

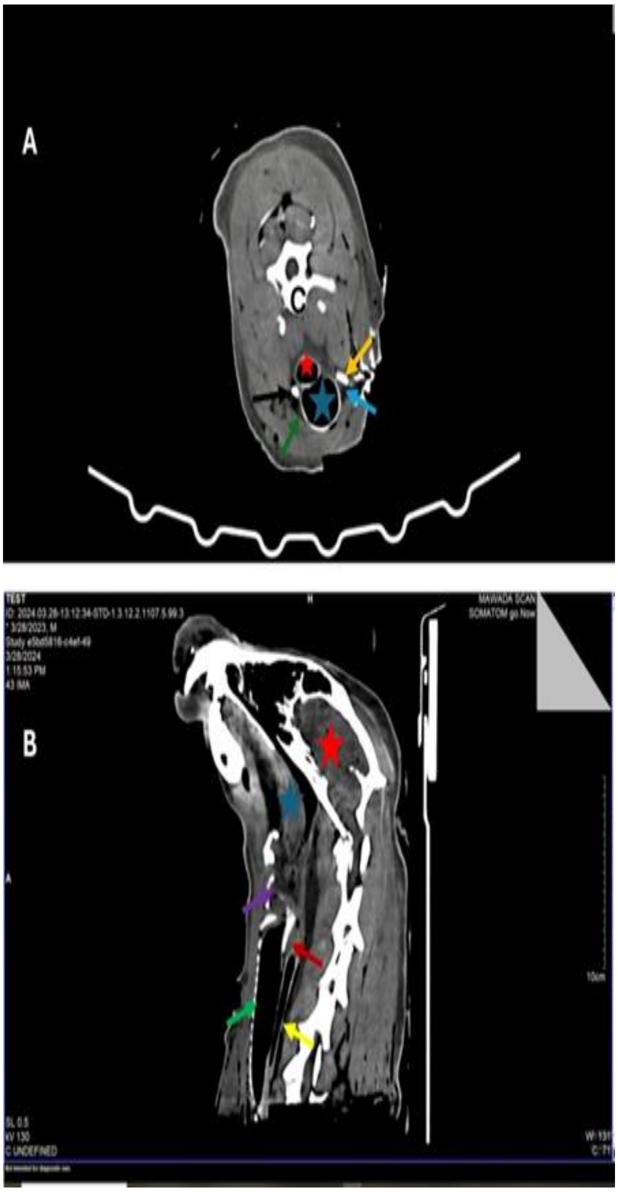


Fig.3. Displays the CT examination of the canine thyroid gland in two axes. Panel A: - shows the thyroid gland in transverse axis the two lobes of the thyroid gland right lobe (blue arrow) and left lobe (green arrow) around the trachea (blue star), Esophagus (red star), right common carotid artery (orange arrow), left common carotid artery (black arrow). Cervical vertebrae (C). Panel B: - Illustrates the examination of the thyroid gland at the longitudinal axis as the thyroid gland (red arrow), trachea (green arrow), esophagus (yellow arrow), larynx (purple arrow), brain (red star), tongue (blue star).

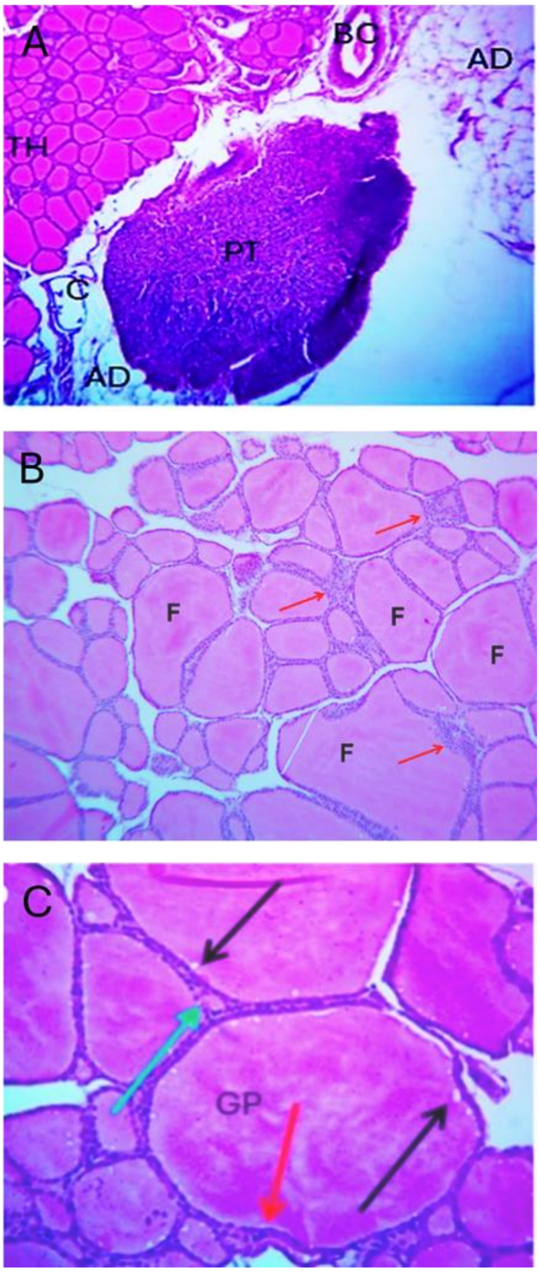


Fig.4. Photomicrograph, thyroid gland of mature male dogs (H&E-stained sections) showing: Panel A: - thyroid gland (TH), parathyroid gland (PH), blood capillary (BC), capsule (C), and adipose tissue (AD) (X10). Panel B: -showing Para-follicular cells (red arrow), as an aggregation between the thyroid follicles (F) (X 20). Panel C: - shows a large thyroid gland follicle lined by simple squamous epithelium. (red arrow) distended by glycoprotein (GP), Para-follicular cell (green arrow), and vacuoles (black arrow) (X40).

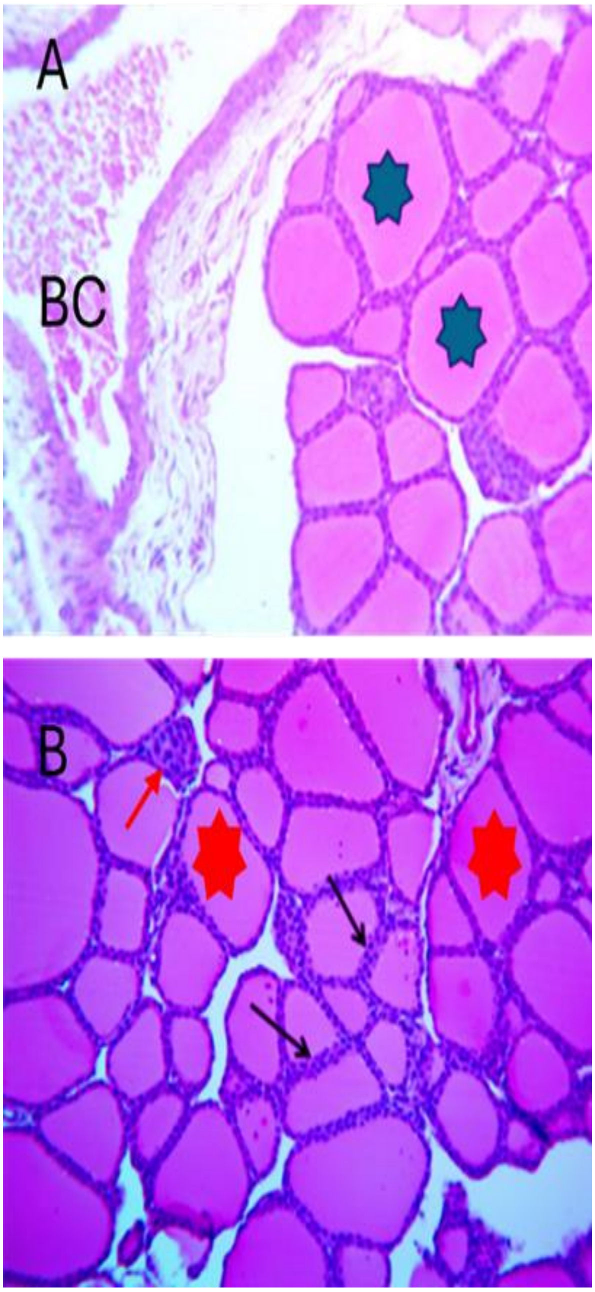


Fig.5. photomicrograph thyroid gland of young male dogs (H&E) stained section Showing Panel A: - small sized follicles (green stars) at the periphery, blood capillary (BC) (X40). Panel B: - thyroid follicle(red stars) lined by cuboidal hyper-proliferation follicular cells (black arrows), aggregation of para-follicular cells (red arrow) (X40).

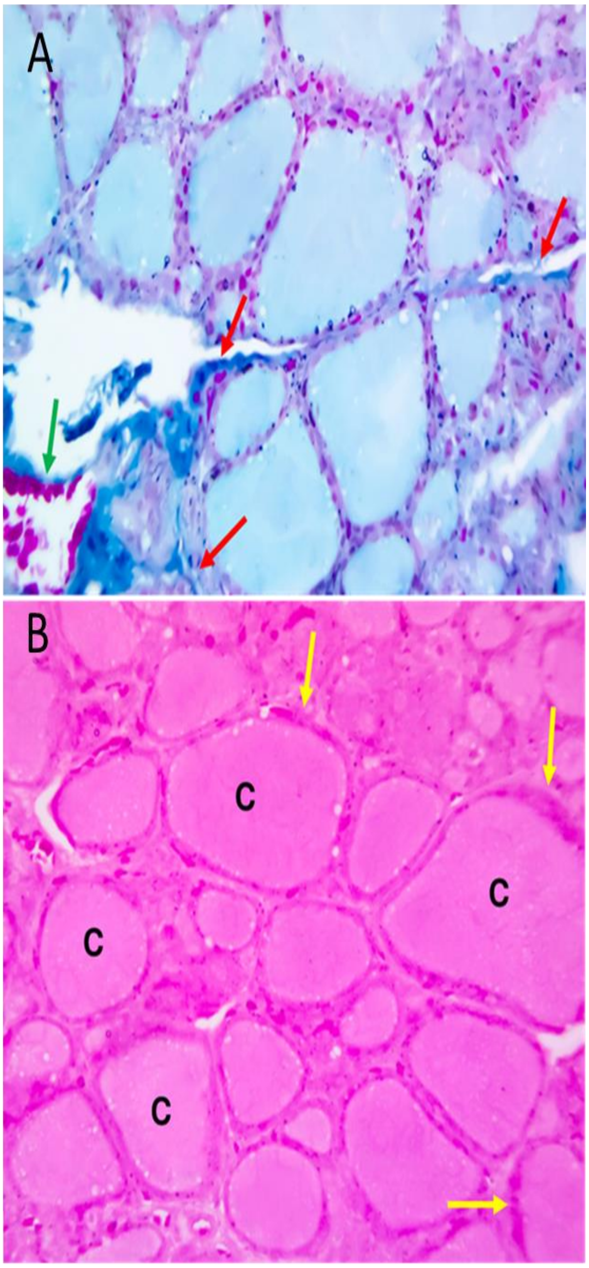


Fig.6. Photomicrograph the thyroid gland of mature male dogs with (Masson's trichrome stain & periodic acid Schiff stain) stained section Showing Panel A: - shows moderate staining of collagen fibers (red arrows) between thyroid follicles, blood capillary with its connective capsule (green arrow) (X40). Panel B: - shows strong PAS staining in the colloid (C), moderate staining of basement membrane (yellow arrows) (X40).

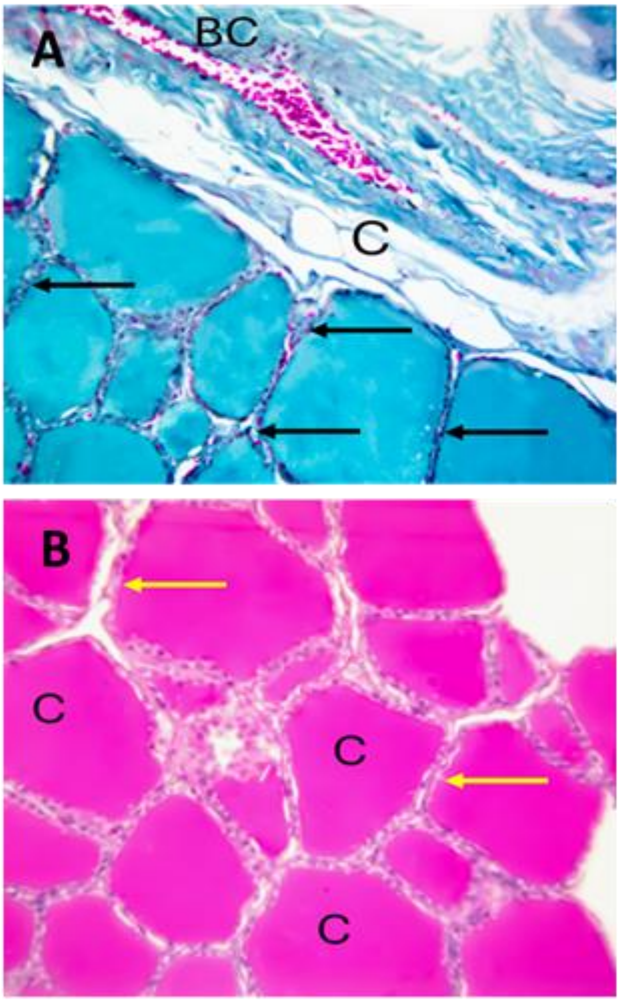


Fig.7. Photomicrograph of the thyroid gland of young male dogs with (Masson's trichrome stain & periodic acid Schiff stain) stained section Panel A: - shows mild staining of collagen fibers (black arrows) between thyroid follicles, blood capillary with its connective capsule (BC), thyroid gland capsule (C) (X40). Panel B: - shows strong PAS staining in the colloid (C), moderate staining of basement membrane (X40).

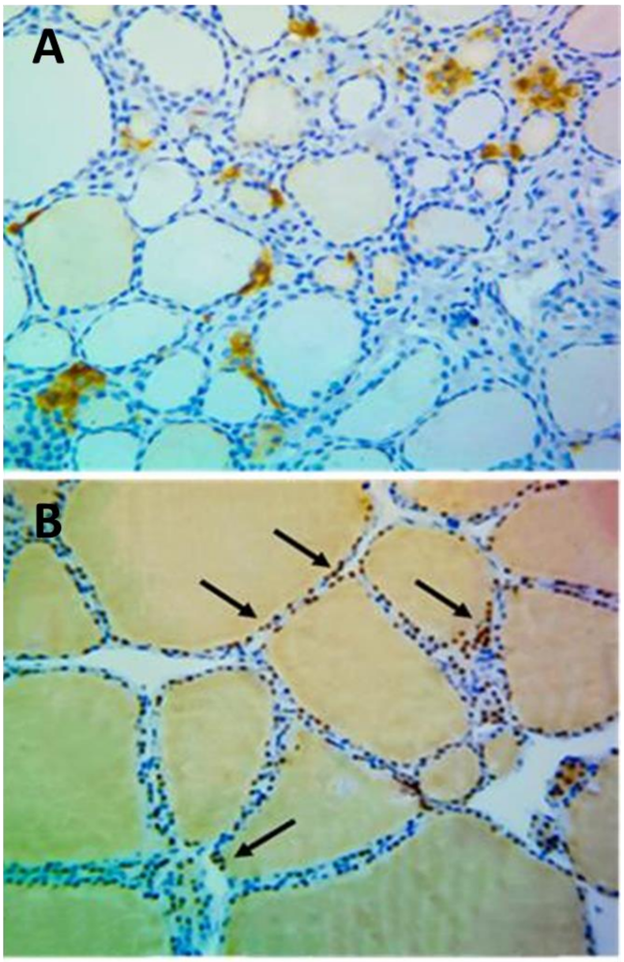
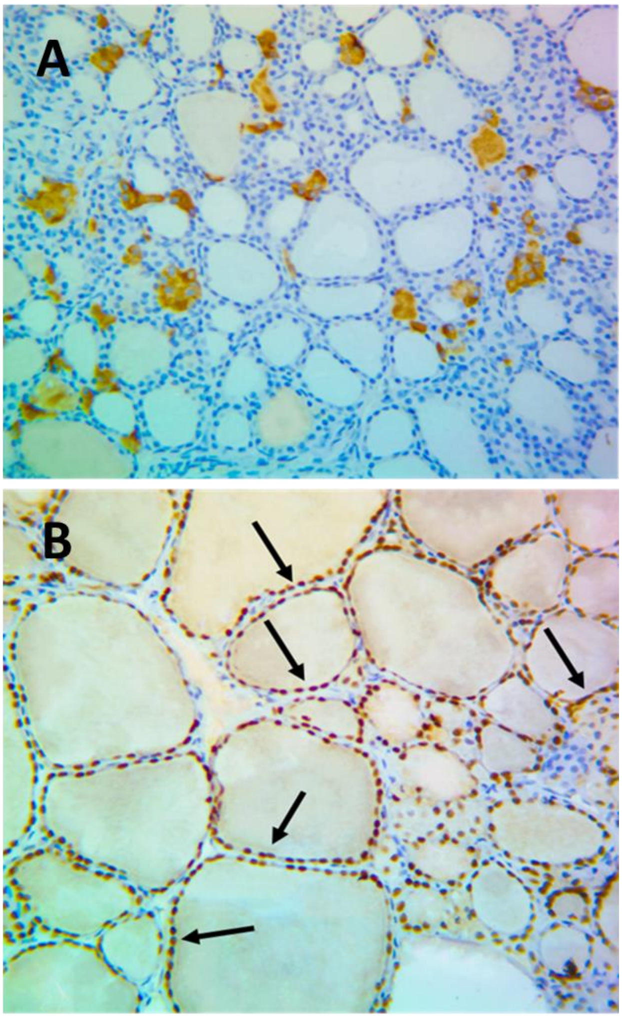


Fig.8. photomicrograph of thyroid gland with IHC (TTF1& SYN) of mature male dogs. Panel A: - moderate immunohistochemical reaction of SYN in Para-follicular cells the reaction product is cytoplasmic and stained brown (X40). Panel B: - moderate immunohistochemical reaction to TTF1 in follicular cells the reaction product is nuclear and stained brown (black arrow) (X40).



1. Fig.9. photomicrograph of thyroid gland with IHC (TTF1& SYN) of young male dogs. Panel A: - strong immunohistochemical reaction to SYN in Para-follicular cells the reaction product is cytoplasmic and stained brown (X40). Panel B: - strong immunohistochemical reaction to TTF1 in follicular cells the reaction product is nuclear and stained brown (black arrow) (X40).

2. DISCUSSION

In this study, we focused on studying the features of the normal thyroid gland in male dogs anatomically, ultrasonography, by using CT imaging technique histologically and immunohistochemistry. The present results showed that the thyroid gland of dogs consists of two separated flattened ellipsoid lobes, on both sides of the trachea caudal to the cricoid cartilage of the larynx, and this result is in agreement with the previous literature [1,2,5,6,10,27]. Each lobe is supplied with two main arteries: the cranial and caudal thyroid arteries and this observation matched with[5,26,27] The two lobes appeared to have an asymmetrical location around the trachea as the left lobe was located more caudally as previously mentioned by [5]. In one examined case the two lobes had the same location and a similar case in goat mentioned by [2]. The thyroid gland can easily be located and viewed by ultrasound examination due to its unique superficial location just under the skin, as in longitudinal images the lobes were fusiform or elliptical with a smooth capsule and this diagnostic imaging matched with [6]. Knowledge about normal thyroid gland ultrasonography is important as it can be used as a guide for the diagnosis of canine thyroid gland abnormality or cancer masses. Large, nonhomogeneous masses with variable delineation and occasionally several cysts are the hallmarks of thyroid carcinomas. Invasion of neighboring structures such as the esophagus, fascial sheaths, and cervical vasculature can be identified; This information is useful in deciding whether surgical surgery is a realistic therapeutic choice [28].

Recently, there has been a wide application of CT imaging, especially in pets to study the thyroid gland due to its high attenuation value relative to the surrounding soft tissues. The present CT imaging of the thyroid lobes of dogs showed the lobes as ovoid structures in the transverse sections and elongated ovoid structures on long-axis imaging and this observation coincides with [1] and with

[7] in goat CT of the thyroid in dogs could help identify a cervical tumor of uncertain origin from thyroid malignancy, The primary indication for thyroid CT in humans is neoplasia; this technique is useful for determining the tumor's local invasiveness, detecting distant metastases to the lungs and lymph nodes, and evaluating ectopic thyroid tissue that is present in the mediastinum, lateral neck, and oral cavity [28].

In the present study, the thyroid gland of dogs is enclosed by a connective tissue capsule that sends trabeculae into the parenchyma in agreement with [10] who describe that the canine thyroid lobes is surrounded by a double-layered connective tissue capsule derived from the deep pre-tracheal fascia, the deep one is considered the true capsule that sends trabeculae to carry blood and nerve supply to the internal gland. The parenchyma is formed of two types of cells that vary in number, size, function, and arrangement inside the gland, the more numerous small-sized cells that present in a sphere structure called follicles are named thyrocytes and are the primary cells, the other type is less in number, large sized present as groups or as single cells between the primary cells, that cells named para-follicular cells, this structure agreed with the observation found by[9,27] The thyroid gland displays changes in follicles and their follicular cells that changes related to the age of the animals as the young animals have small well-regulated follicles surrounded by simple cuboidal thyrocytes, that change into simple squamous surrounding large irregular follicles in adult dogs, the same results were recorded in calves [9], in pigs [31], in mice [32].

In the present study, TTF1 has been detected in the follicular cells of the canine thyroid gland in mature and young dogs with different degrees of reactions where there are strong positive reactions in follicular cells at young ages and moderate reactions in adult animals. This observation might be related to the physiological roles of TTF1, as it takes part in the formation of thyroid hormones by regulating a particular gene group that directly contributes to the formation of T3 &T4 hormones and also plays a crucial role in maintaining the architecture, function and differentiation of thyroid gland[13] In young animals the thyroid gland is more active than in adult animals therefore a direct relationship between TTF1 and the activity of the thyroid gland meanwhile it has an indirect relation with the age of the animals [13,16] Thyroid, lung, and other tissue development, as well as the occurrence of numerous clinical conditions such cancers, thyroid disorders, and nervous system disorders, are all strongly correlated with TTF1 expression and deletion, so TFF-1 is a potential target for cancer treatment since its knockdown prevents cancer cells from proliferating [16].

SYN shows a similar reaction to that of TTF1 with the two groups of dogs, as it has a stronger cytoplasmic reaction in young animals than in mature animals, this finding may be related to SYN's physiological functions in the development of Para-follicular cells into endocrine cells. At present, it is recommended to stain synaptophysin to demonstrate neuroendocrine differentiation in thoracic malignancies. as one crucial indicator of neuroendocrine neoplasms is the expression of neuroendocrine characteristics [29,33].

3. CONCLUSIONS

Finally, we can conclude that ultrasonography and CT are applicable methodologies to evaluate thyroid gland morphology and abnormality in dogs. TTF1 immunostaining has a stronger reaction within the follicular cells at an early age and a moderate reaction in adult animals, and this result might be associated with the physiological role of TTF1 in the differentiation of endodermal origin follicular cells into active endocrine cells. Similarly, the immunostaining showed a stronger reaction for SYN in the parafollicular cells of an early age than that of adult animals and this result might be associated with the physiological roles of SYN in the differentiation of neuroectodermal origin parafollicular cells into endocrine cells.

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