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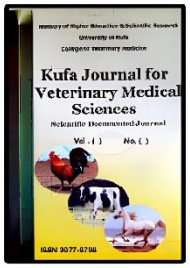

Histomorphometric Study of the Thyroid Gland of the Adult Male Squirrel *(Sciurus Anomalus)*

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Abstract:

The aim of the current study is to determine the histological and histochemical characteristics of the thyroid glands in local male squirrels. So, thyroid gland tissue was freshly collected from an adult healthy local male squirrel for histological preparation and examination. All histological sections were prepared and stained with routine stains H & E, in addition to specific stains including Masson Trichrome and PAS stains.

The histological results showed that thyroid glands consisted of glandular parenchyma enclosed by a capsule which mainly consisted of collagen fibers and sent septa into glandular parenchyma to form lobules that contained follicles of thyroid glands. These follicles of glandular were lined by simple cuboidal epithelium and some epithelium displayed a simple squamous epithelium, and were filled with colloid material which was positive with PAS stain. Accordingly, capsule thickness, follicle diameters, and heights of the epithelium of glandular parenchyma did not show any significant differences between the right and left lobes at ($p< 0.05$). Also, parafollicular cells (C-cells), are detected as single cells interspersed between the follicular cells or as a small group of (1-2) cells between the follicles. These findings showed that the main structures of the thyroid gland are similar to most species of mammalian glands.

Keywords: Follicles, Glandular Parenchyma, Parafollicular cells, Thyroid glands.

Introduction:

Previous studies have mentioned that Caucasian squirrels, or as called Persian squirrels (*Sciurus anomalus*) consider a mammal species and belong to order; Rodentia; genus: *Sciurus*; species: *Sciurus anomalus* in the genus of Sciurus [1]. Thyroid gland is produced different hormones including thyroxine (T4) and triiodothyronine (T3), and considered an unique gland among the endocrine glands due to its stores much amounts of hormone in an intact form within extracellular compartments in the center of follicles and a small amounts of hormones in intracellular location [2]. These glands have a similar follicular structure in the most animal species in despite of there some gross, histological and ultrastructural variations have been recorded among the species. Also,

thyroid gland actions are affected in response to environmental like seasons and nutritional influences which might be varied among the animals' species [3].

Anatomically, the thyroid gland is a bi-lobed organ, situated in the neck region of the animal, anterolateral to larynx and upper trachea. It is embedded between tissue of trachea and has two lobes are linked by a thin band of thyroid tissue called the isthmus that passes anterior to cranial part of trachea [4]. Histologically, thyroid gland structure is differing according to \ or related to many factors between different animals. Hence, in most species, thyroid gland is surrounded by a capsule which send trabeculae into the parenchyma of the gland, dividing it into many lobules, and these lobules are consisted of an aggregation of different sizes and shapes of follicles which lined by a single layer of epithelium and contains a variable amount of colloid substance [5]; [4]), and based on inter follicular connective tissue rich in capillaries [6]**.**

Previous research found that the height of follicular cells varies depending on the follicle's activity state of glands [7]. Therefore, the epithelium of follicular cells appears in the resting state as squamous or low cuboidal and cuboidal or columnar in active state [8].

Moreover, the follicular cells produce thyroid hormones T3 and T4 [9] and in addition to calcitonin [8]. Parafollicular cells are secreted mainly calcitonin hormone which regulate calcium level in the blood and few other regulatory thyroid peptide as somatostatin, chromogranin A [10]. These hormones play an important role in the regulation of the cellular metabolic activities of all parts of the body and involving in the development of mammalian foetus [11] and [12]. The colloid periphery adjacent to apical surface of the follicular cells is vacuolated in an active follicle. The colloid has a smoother peripheral surface in inactive follicles and there are no vacuoles [4].

Up to date, there are no much available studies or data in the previous literature research to identify the thyroid gland of the local adult male squirrel, therefore this study focused on the thyroid gland tissue of the squirrel to avail more data about thyroid glands of the squirrel for other applications and research studies.

Materials and Methods:

Animal ethical approval

This study has conducted according to instructions and regulations of animal ethics of College of Veterinary Medicine/ University of Al-Qadisiyah. In the current study (4) adult male Squirrel *(Sciurus anomalus*) were used for the histological and histochemical study. All healthy adult squirrels were euthanised and killed, then the thyroid gland tissue are immediately collected from the neck region of squirrels and washed by saline solution. Specimens were collected in March 2023, so small piece (1 cm^3) of tissue were taken from right and left lobe of thyroid gland, then proceed with routine histological technique as following**;** specimens were fixed in (10%) neutral buffered formalin for (48) hours. Then tissue was dehydrated in a graded series of ethanol (70%, 80%, 90% and 100%) two hours for each concentration. After that, tissue was cleared by xylene for twice one hour of each step. Then, specimens are embedded in paraffin twice; two hours for each using oven set at (58) C°. Next, specimens embedded in paraffin wax to be prepared for sectioning by rotary microtome. The sections thickness was sectioned at (5-6) µm thickness. Finally, all sections slides were stained with the following stains: Harris Hematoxylin and Eosin stain**:** for general histological structures; Periodic Acid Schiff Reagent (PAS) **f**or detecting the carbohydrate muco-polysaccharides, muco-protien, glycoprotein and basement membrane; Masson Trichrome Stain for determining collagen fibers and smooth muscle fibers [13]; [14].

Histomorphometry measurements

The thickness of the capsule of the right and left lobs was measured at 10× magnification. Furthermore, the epithelial height was found on the epithelial basement membrane to the apical surface of the epithelial cells under 400 × magnifications [15]. The, follicular diameter was conducted by measuring horizontal and vertical distances of the follicles to identify the size of follicles under 10× magnification [16]. All histological

sections examined using light microscope (Olympus/ Japan), and sections were photographed by Olympus (Japan) microscope, at different magnifications (×4; ×10; ×20; ×40; ×100).

Statistical Analysis

The histological measurements data was analyzed using SAS (2012) [17], so this program software was used to explore the significant differences studied between histological measurements applying for T test and possibility at ($p< 0.05$).

Results:

Our result showed that histological structure of thyroid gland in adult male squirrel revealed similar common pattern to mammalian histological structure. So, capsule showed that the thyroid gland in adult male squirrel was covered by capsule which were consisted two layers: externally thin outer layer of adipose tissue which display a clear cellular limit interpose with the collagenous fibers and a few elastic fibers (Fig. 1).

While, internally a thin capsule constructed of an inner dense irregular connective tissue and constituted from collagen and elastic fibers, contained spindle shape fibroblasts and attached to glandular parenchyma of the gland and send different septa and divided glands into lobules and based the follicles (Fig. 1, and 2). Also, thin trabeculae accompanied capsular small and medium size blood vessels into the glandular parenchyma and separating it into clear and different size lobules (Fig.3). The mean capsule thickness of right and left lobes were132.2 ± 0.01 µm and 132.6± 0.04 µm respectively (Table 1). Statistical analysis found there were no significant differences between the right and left lobes at $p< 0.05$ (Table1, and Fig.4).

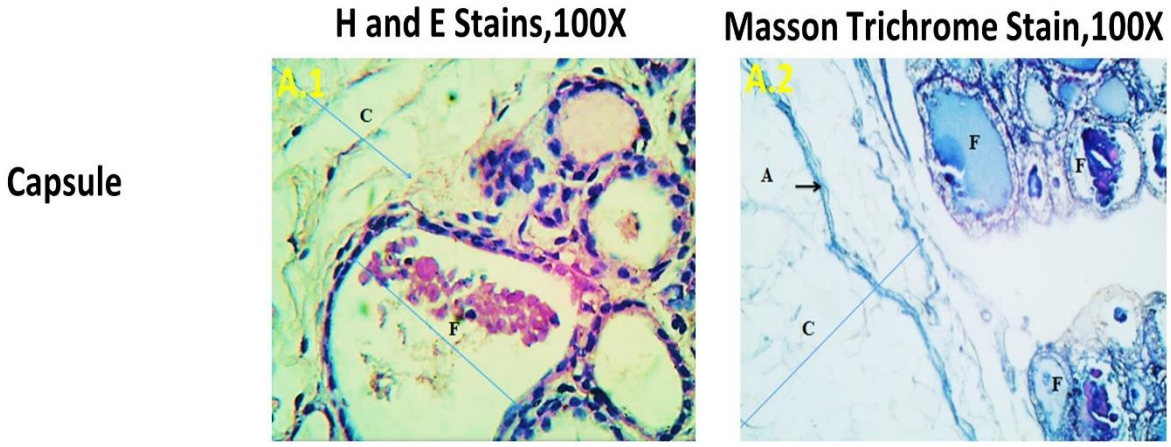

Figure1: Histological Section of the lobe of thyroid gland in local male squirrel Shows capsule. A1: lobe of thyroid gland stained with H&E Stain, capsule (C), Follicles (F), A2: Capsule (C), Collagen fibers (black arrow), Adipose tissue (A), capsule (C), and Follicles (F), stained with Masson Trichrome Stain.

Follicles

Our study found that lobules in both right and left lobes consisted of an aggregation of different shapes and sizes of follicles which surrounded by a basement membrane comprised of thin connective tissue, collagen fibers, fibroblasts, fibrocytes and many capillaries (Fig. 2). Also, different shapes of follicles were observed as rounded**,** oval**,** elongated, polygonal, and irregular shape follicles**.** However, rounded and oval shape follicles were the predominant follicles in the thyroid glands tissue (Fig.2). Furthermore, follicles showed that there were three sized follicles were detected according to size: small**,** medium and large -sized follicles**.** Moreover, the thyroid gland was consisted of a thin network of inter follicular connective tissue rich in blood capillaries which surround all follicles**.** These follicles were spread

through right and left lobes and contained colloid substance (Fig. 2, A1). In the right lobe the average diameters of small, medium and large size follicles accounted for 18.1± 3.56 µm, 49.6± 3.20 µm and 64.3±2.1 µm respectively (Table 1), As, left lobe was accounted for 18.3± 3.49 µm, 49.2±3.29 µm and 64.10± 2.3 µm, respectively (Table 1). However, there were no significant differences between right and left lobes at p< 0.05 (Table 1, and Fig.4). The lining epithelium of the follicles was revealed as a low simple cuboidal epithelium with spherical nuclei and some follicles were lined by simple squamous epithelium (Fig. 3). The average height of follicular epithelium was accounted for 4.3 ± 0.2 µm and 4.5± 0.5 µm, in the right and left lobes respectively (Table 1). There was no significant difference between right and left lobes at (p < 0.05), (Fig.4).

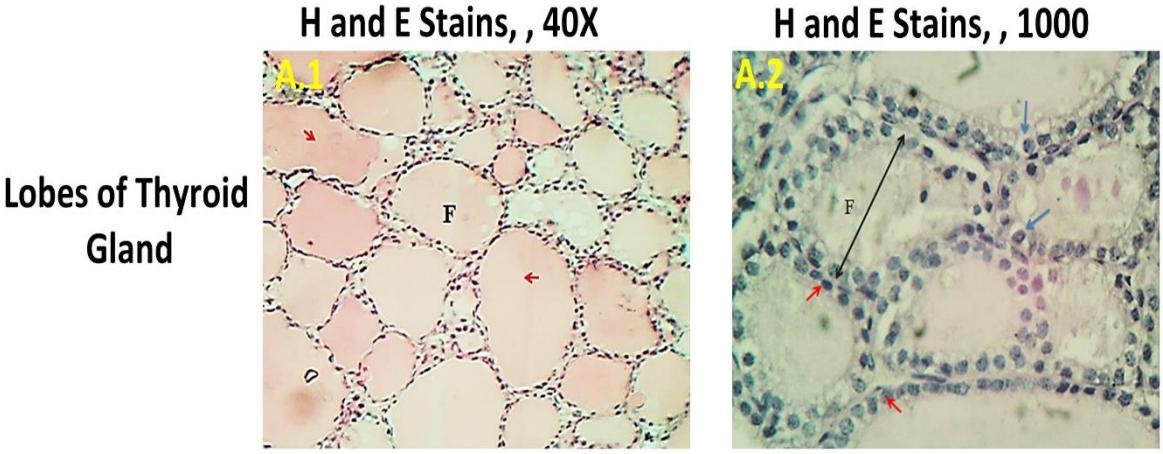

Figure 2: Histological section of the lobe of thyroid gland in male local male squirrel shows lobules (Red arrow); A1: Follicles (F), colloid (red arrows) stained with H&E Stain; A2: Follicles (F), simple cuboidal epithelium (red arrows), Parafollicular cells (blue arrows) stained with PAS Stain.

Parafollicular cells (C-cells)

The parafollicular cells appeared as large oval to rounded cells in shape, and more lightly stained cytoplasm than follicular cells and densely stained nuclei. These cells were observed among the follicular cells as aggregations groups. The base of follicular and the parafollicular cells were rested on the basement membrane, while few other cells found among the follicles and distributed as groups between follicles (Fig. 2,and 3).

Colloid Substance

Thyroid follicles were contained a variable amount of homogenous eosinophilic colloid substance in the intra follicular spaces (Fig. 2,3), and no uniformly stained colloid in which many or few peripheral visible empty vacuoles present in the stored colloid. Some follicles were observed without colloid (Fig.3,A2). The colloid substance showed a different degree of density; some follicles were contains densely stained eosinophilic colloid substance which reacted strongly positively with PAS and showed a magenta color which confirmed that colloid is very rich in glycoproteins and carbohydrates. Few follicles contain faintly stained colloid which showed weak positive reaction towards the PAS stain ((Fig.3, A2).

Table1: Histological Parameters of the Right and Left Lobes of the Thyroid glands in male Local Squirrel.

Histological parameters(µm)	Right Lobe	Left Lobe
Thickness of capsule	132.2 ± 0.01	132.6 ± 0.04
Diameter of small Follicles	18.1 ± 3.56	18.3 ± 3.49
Diameter of Medium Follicles	49.6 ± 3.20	49.2 ± 3.29
Diameter of Large Follicles	64.3 ± 2.1	64.10 ± 2.3
Height of Follicular Cells (epithelium)	4.3 ± 0.2	4.5 ± 0.5

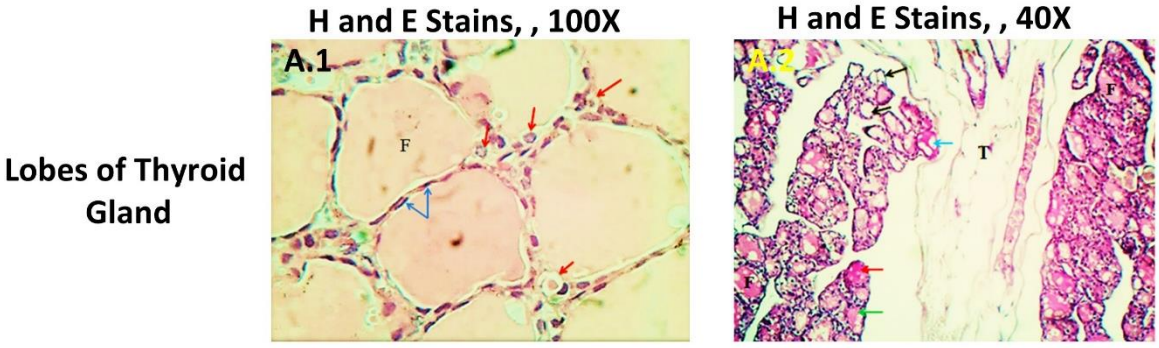

Figure 3: Histological section of lobe of thyroid gland of the male local squirrel, A1: shows: Follicles (F), simple squamous epithelium (blue arrows) Parafollicular cells (red arrows) stained with H&E Stain.A2: Follicles (F), vacuoles (blue arrow), empty follicles (black arrows), strong positive reaction (red arrow), light positive reaction (green arrow), Trabeculae (T), stained with PAS Stain.

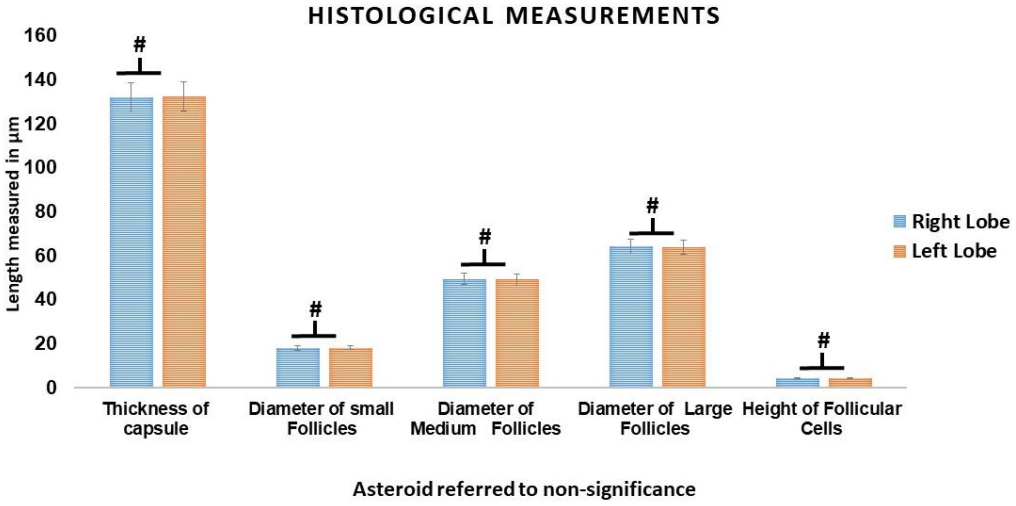

Figure 4: This histogram illustrated thick of the capsule, the different diameters of the follicles. Asteroid (#) is meaning not significance at ($p < 0.05$).

Discussion:

Our study has reported that thyroid glands of squirrels are surrounded by a thin capsule of dense irregular connective tissue and formed of two layer structure of collagenous fibers and small blood vessels internally and adipose tissue externally, similar result was reported by [18]in goats and [19] in the dog. So results confirmed that the main histological structure of thyroid gland in squirrels has high similarity to other domestic animals.

Also, the current result revealed that thickness of capsule of both right and left lobs was quite similar to each other and no significant differences between right and left lobs., however, the thickness of capsule in both right and left lobes in local squirrels was thinner than that reported by [20] in sheep, this is related to different species and sheep has large size in comparison with a squirrel.

Generally, thickness of capsule of different species has a high variation because there are different responses between animals to environmental conditions, foods and other habits. Therefore. These findings disagree with study [21] that observed in rabbits in which the thickness of the capsule varies greatly even within the same gland, due to the uneven location of the fatty layer [21].

Importantly, vascularized trabecular strands extend from capsule toward the glandular parenchyma of glands and divide it into distinct lobules to reach all aggregation the follicles' basement membrane. Inter follicular connective tissue contains a network of capillaries which provides a numerous blood supply for follicular cells. This finding was parallel with [22] in sheep, but in contrast in buffalo with [16] in which the trabeculae were divided into parenchyma into poorly lobules in comparison with thyroid gland of squirrels. Accordingly, these outcomes that squirrels has very active thyroid glands in comparison with other species which need to organize the metabolism of squirrel, in addition to different sources of food might be related to activating thyroid glands in the squirrels.

Many studies assured that the thyroid gland an endocrine gland secretes (T3, T4 and calcitonin) hormones which play an important role in the metabolism and maintenance of calcium, constant body temperature in mammals [23] respond quickly to changes in environmental condition. This action of thyroid allows the animals to adapt to the variation in environmental temperatures and the thyroid hormones are involved in thermoregulation of the body [24] These findings are consistent with our study for histological structures of thyroid glands of squirrel in particular distributions of capillaries and many lobules which are developed and this allowed to squirrels to be adapted with different conditions and seasons.

The thyroid parenchymal tissue of squirrels was characterized by a various follicle, thin inter follicular connective tissue containing collagen fibers and fibroblast, extensive peri-follicular capillaries and sinusoids, as observed by [6] in pigs. So, the present findings displayed that thyroid gland has a large similarity to these species but it has some specific different patterns, for enhancing each lobule in the right and left lobes was made of an aggregation of different shapes and sizes of follicles. As well, three main sizes of follicles were found and randomly distributed, this result was agreed with [25] in cats, but disagreed that observed in camels which the follicles were two sizes only; small and large size follicles [26] These variations are normally considered between different species according to weight, foods and environmental conditions. Even though, statistical analysis showed that follicular diameters have non-significant differences between the right and left lobes of local squirrels, this result may be due to that the two right and left lobes are the same considered the main functional structures of the thyroid gland in squirrels.

In the current study, the histological examination of thyroid in squirrels showed uniformly distributed variable sized follicles in other species and recorded by [22], but it was a different pattern in wild African grasscutter *(Thryonomys swinderianus)* in which the small follicles were remarkably located centrally and the large follicles were located peripherally [27]. However, the follicular epithelium was similar to goat and lined with simple cuboidal epithelium, [20].

Also, the height of follicular cells displayed that there were no significant differences between right, and left lobes. This result may indicate that the squirrel was in the same stage of activity, and confirmed in goat by [28]. At this time, the follicles were lined mostly by low simple cuboidal to flattened epithelium, and epithelial height is an indicator to follicular activity according to [29]. Previous studies found that active follicles are lined by simple cuboidal epithelium or simple columnar epithelial cells, while in inactive state, it lined by simple cuboidal to squamous epithelial cells [30], and this assured high activity of thyroid glands of squirrels compared to other species.

Correspondingly, parafollicular cells of the thyroid structure of squirrel observed oval–rounded in shape larger and lighter than the follicular cells with dense stain nuclei and they were occupied two locations; the inter follicular cells and parafollicular positions inside the gland. These cells were recorded in other species [31] in rats and [32] in cat. Then again, it was differed from those found in buffalo as a single cells or groups of two - three cells in same place of tissue [33].

The C- cells contain small cytoplasmic granules representing the stored hormone calcitonin which regulates calcium concentration in blood [34]. Moreover, thyroid follicles of squirrel contained different amounts of homogenous colloid substance which reacted positively for PAS stain, due to its thyroglobulin an iodinated glycoprotein substance [35], and similar to Iraqi goats (2017). Besides, some of follicles contain peripheral colloid vacuoles between the epithelial cells and follicular colloid, which indicate their metabolic activity. These findings highly agreed with [36] in cattle. For this reason, that functional activity of the follicles can be evaluated based on the color of colloid inside them [37] which established that high activity of thyroid glands of squirrels in relation to our results. In conclusion, this study reported that thyroid glands of squirrels have a specific pattern in histological structures in comparison with different species. Also, it was very active the glands might be related to foods and environmental circumstances of squirrels' life. However, squirrel has large similarities to goats and cats, and more investigations are needed.

Conflict of interest: None.

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