Original Article

Anatomical and Histological Study of Thyroid and Parathyroid Glands in the Persian Squirrel (*Sciurus anomalus*)

Ghasem Akbari[1*], Gholamreza Hamidian[1], Mahdi Yaghmori[1], Mohammad Babaei[2]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamadan, Iran.

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How to Cite This Article Akbari, Gh., Hamidian, Gh., Yaghmori, M., & Babaei, M. (2026). Anatomical and Histological Study of Thyroid and Parathyroid Glands in the Persian Squirrel (*Sciurus anomalus*). *Iranian Journal of Veterinary Medicine, 20*(1), 143-152. http://dx.doi.org/10.32598/ijvm.20.1.1005509

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ABSTRACT

Background: The thyroid gland is one of the largest endocrine glands among vertebrate species

Objectives: This study aims to examine the thyroid and parathyroid glands of the Persian squirrel duo to limited anatomical knowledge.

Methods: Five adult male Persian squirrels were included in this study. After euthanasia, the thyroid and parathyroid glands were dissected and macroscopically evaluated. Subsequently, tissue samples from these glands were fixed, processed, and subjected to hematoxylin and eosin (H&E) and specific staining methods for microscopic analysis.

Results: The thyroid gland of the Persian squirrel was composed of two unequal pale brown lobes without an isthmus. The caudal part of the gland was pointed, while the cranial edge was convex. The left parathyroid gland was located at the cranial edge of the thyroid gland, and the right parathyroid gland was located within the parenchyma of the thyroid gland. The cranial thyroid artery facilitates the blood supply to the glands. The colloidal substances were periodic acid schiff (PAS) positive. They appeared pale to deep orange in Masson's trichrome staining, indicating the presence of glycoprotein with a high protein content in the more intensely colored colloids. Parafollicular cells were mainly clustered between follicles. The parathyroid gland consists of small secretory cells in a rope-like arrangement. These cells exhibited acidophilic cytoplasm, euchromatin round to oval nuclei, and a weak reaction to PAS staining.

Conclusion: Information regarding the thyroid and parathyroid glands can be highly beneficial for veterinary practitioners dealing with diseases related to the endocrine system.

Keywords: Anatomy, Histology, Parathyroid gland, Persian squirrel, Thyroid gland

Article info:
Received: 25 Jan 2024
Accepted: 10 Mar 2024
Publish: 01 Apr 2025

*** Corresponding Author:**
Ghasem Akbari, Associate Professor.
Address: *Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.*
Phone: *+98 (41) 33307101*
E-mail: *g.akbari@tabrizu.ac.ir*

Introduction

The thyroid gland has received significant attention from researchers due to its development in various disorders, including hypothyroidism and hyperthyroidism. Phylogenetically is one of the largest endocrine glands among vertebrate species (Onwuaso & Nwagbo, 2014). It is crucial in regulating body growth, metabolism, and tissue development and differentiation (Choksi et al., 2003). These hormones include thyroglobulin, triiodothyronine, and thyroxine (Gartner, 2020). Evaluating thyroid histology is crucial for assessing thyroid function. The gland consists of numerous follicles of varying sizes that form its functional and histological units. These units have three main components: follicular epithelial cells, parafollicular cells, and colloidal cavities (Reece & Rowe, 2017). Thyroid hormones are synthesized by follicular cells, constituting a major portion of the gland parenchyma. These hormones are first stored in follicular fluid, broken down, and converted into end products. Ultimately, they are released into the bloodstream (Baljit, 2017).

The parathyroid glands produce parathyroid hormone, which plays a crucial role in regulating serum calcium and phosphorus concentrations by regulating bone metabolism, absorption from the digestive tract, and excretion in the urine. The parathyroid glands originate from the epithelium of the third and fourth pharyngeal sacs, which also give rise to the internal parathyroid glands and the parathyroid glands IV, indicating their embryonic origin (König et al., 2007). While present in all vertebrates, the number and position of parathyroid glands can vary significantly.

The morphology of parathyroid glands has been extensively studied in various mammals, including camels (Kausar & Shabid, 2006), sheep, dogs, cattle (Greco & Davidson, 2017), goats (Joshi & Mathur, 2015), Bakerwali goats (Dar et al., 2018), African giant rats (Enemali et al., 2016), rats and mice (Ali, 2020; Ingbar, 1985).

Squirrels belong to the order Rodentia, and Persian squirrels (*Sciurus anomalus*) are found in Armenia, Azerbaijan, Turkey, Syria, Greece, and Georgia. Despite being wild animals, the trend of keeping Persian squirrels as pets has been increasing, leading to increased visits to veterinary clinics (Akbari & Kianifard, 2017).

Information on naturally occurring thyroid and parathyroid diseases in small mammals is extremely limited. Although there is an abundance of research on experimentally induced thyroid and parathyroid diseases in laboratory rodents, this does not follow the pattern of naturally occurring diseases in these species and often has limited value when evaluating pet rodents. Naturally occurring thyroid and parathyroid gland dysfunction is poorly documented in rodents, and further research is needed to explore these diseases (Thorson, 2014; Barber, 2004; Soltani & Dalimi, 2018; Mohammed Ibrahim et al., 2022; Badi et al., 2022; Sheikholeslami et al., 2019). Therefore, morphological data can be useful to veterinary practitioners in thyroid disorder management. Due to limited information about the anatomy and histology of the parathyroid glands in Persian squirrels, as well as the growing popularity of keeping them as pets, this study was conducted. The results of this study may be useful to diagnose the pathobiology and clinical treatment of endocrine diseases in Persian squirrels.

Materials and Methods

Five adult male Persian squirrels (*S. anomalus*) were included in this study. These animals were euthanized by a 10% ketamine overdose for reasons unrelated to endocrine disease, either due to various causes in veterinary clinics in Tabriz City, Iran, or as a result of car accidents. This research focused on the anatomical, histological, histochemical, and stereological aspects of the thyroid and parathyroid glands.

Anatomical study

First, to determine the blood supply to the thyroid and parathyroid glands, the common carotid trunk was carefully examined, and the position, shape, and relationship of these glands with other structures were observed. The size and weight of the glands were measured using a digital caliper (Guanglu, China) and a three-zero scale (ELECTRONIC BALANCE, HL-323A). Following these observations, samples were transferred to 10% formalin for further analysis.

Histological and histochemical study

After tissue processing, tissue samples were embedded in paraffin blocks and sliced into sections measuring 7 µm thickness by microtome (LEICA, RM2145). These sections were stained with hematoxylin and eosin (H&E) for routine histological examination. Periodic acid-Schiff (PAS) staining was performed to evaluate the follicular fluid, while Masson's trichrome staining was used to evaluate the connective tissue between follicles.

Results

Anatomical result

The thyroid gland in Persian squirrels (*S. anomalus*) is paired and situated on both sides of the caudal larynx. It begins at the junction of the thyroid cartilage with the cricoid, at the end of the sternothyroid muscle, and at the second and third cervical vertebrae levels. The gland extends below these muscles to the third tracheal cartilage on the right and second tracheal cartilage on the left. It appears as a thin, elongated brown structure. The oval base of the gland was located in the rostral direction, and the pointed tip was located in the caudal direction. The isthmus of the gland is not present in the Persian squirrel. The right thyroid gland was slightly larger than the left thyroid gland (Figure 1). The mean measurements for the right thyroid were 10.48±0.39 mm in length, 2.53±0.23 mm in width, and 1.9±0.17 mm in thickness, while for the left thyroid, the values were 10.04±0.44 mm, 1.84±0.31 mm, and 1.68±0.15 mm, respectively. The mean weight of the glands was 0.0082±0.00083 g on the right side and 0.0058±0.00083 g on the left side. The parathyroid gland was observed in the rostral and lateral parts of the left thyroid gland. It exhibited an oval shape and light brown color (Figure 1b). However, the specific location of the left parathyroid gland was not determined during anatomical studies. The cranial thyroid artery provides blood supply to these glands. The correlation between body weight and thyroid gland weight was 0.97, which was significant (P<0.01). However, no significant correlation was observed between body length and thyroid gland length, with a correlation coefficient of 0.6 (P>0.05) (Table 1).

Histological and histochemical study

The thyroid gland is surrounded by connective tissue that penetrates the gland, dividing it into unknown parts. The main parenchyma of the gland consists of thyroid follicles of irregular and polyhedral shapes and different sizes. These follicles contained follicular fluid (Figures 2a, and 2b) and were surrounded by follicular cells. Follicular cells were short to long cuboids, rarely cylindrical, with round to oval nuclei that appeared as relatively heterochromatin (dark and pigmented) (Figures 2b, and 2c). The colloidal material reacted positively to PAS staining (Figure 3a). It appeared pale orange to deep orange in Masson's trichrome staining (Figure 3b), which indicated the presence of glycoproteins with a high protein content in the colorful colloids. The connective tissue contained collagen fibers between the follicles (Figure 4b).

Parafollicular cells, which are typically located between follicles, were mostly observed in the form of cell accumulations or clusters. Individual parafollicular cells were rarely observed within follicle walls. The cytoplasm of parafollicular cells appeared bright, with large euchromatin and round to oval nuclei. These cells exhibited weak PAS staining. (Figure 4a).

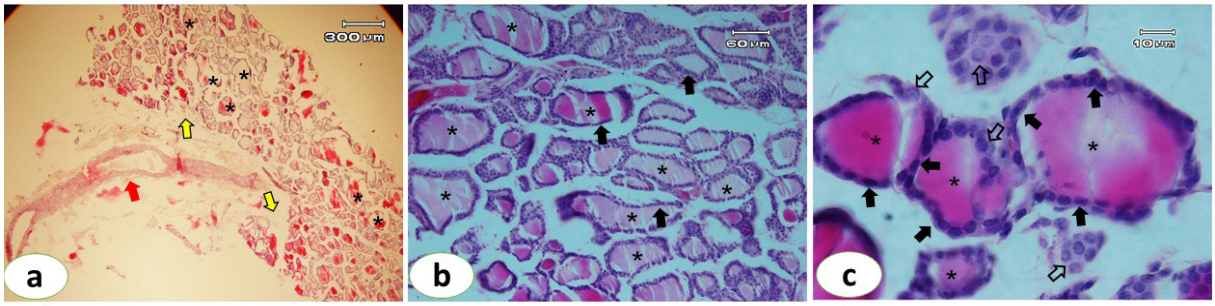

Figure 2. The micrograph of the thyroid gland from the Persian squirrel, H&E staining

a) The gland is surrounded by connective tissue, which penetrates the gland and divides it into unknown parts (yellow arrow), the cranial thyroid artery (red arrow) is visible. The main parenchyma of the gland consists of thyroid follicles (*) with irregular shapes and different sizes containing follicular material.

b) Thyroid gland consists of follicles with irregular and polyhedral shapes, and in different sizes that contain colloid material (*) and are surrounded by short to long cuboidal and rarely cylindrical follicular cells (black arrow).

c) The wall of thyroid follicles is surrounded by short to long and rarely cylindrical follicular epithelial cells with round to oval and relatively heterochromatin nuclei (black arrow). The interior of the follicles is filled with colloids (*). Parafollicular cells are mainly observed as cellular aggregates between follicles and are rarely found within the walls of follicles, which have bright cytoplasm with a large and round to oval euchromatin nucleus (hollow arrow).

Table 1. Macroscopic measurements of the thyroid gland in Persian squirrel

Variables	Mean±SD
Body weight (kg)	0.356±0.041
Left thyroid weight (g)	0.0058±0.00083
Right thyroid weight (g)	0.0082±0.00083
Thyroid weight (g)	0.014±0.0016
Body length (cm)	32±1.695
Right thyroid length (mm)	10.482±0.395
Left thyroid length (mm)	10.042±0.439
Thyroid weight (g)/body weight (kg)	0.039±0.001
Thyroid length (mm)/ body length (cm)	0.3276±0.0152

Tissue sections of the left parathyroid gland revealed that this gland was located at the base of the thyroid gland. It consisted of small secretory cells arranged in a rope (Figure 3a). These cells had acidophilic cytoplasm, and their nuclei were euchromatin and round-to-oval in shape (Figure 3b). The reaction of the parathyroid gland to PAS staining was weak (Figure 4c). In the histological sections of the right thyroid gland, it was found that the right parathyroid gland was located within the parenchyma of the thyroid gland (Figure 3c). The cell structure of the right parathyroid gland is similar to that of the left parathyroid gland. Both glands showed weak PAS and Masson's trichrome staining.

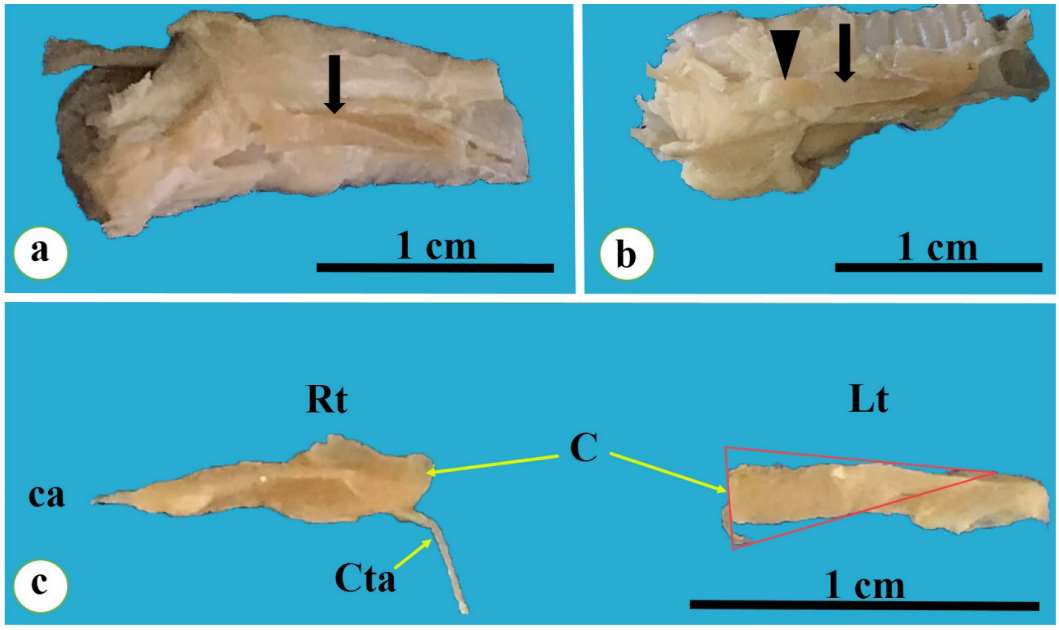

Figure 1. The gross photographs of the thyroid and parathyroid glands

a) The position of the right thyroid gland on the larynx and trachea (lateral view), b) The position of the thyroid and parathyroid glands on the left side (lateral view), c) Internal view of the right thyroid gland (Rt), and the left thyroid gland (Lt) along with the right cranial thyroid artery (Cta), Thyroid gland (black arrow), the left parathyroid gland (black arrowhead), the rostral end of the gland (C), and the caudal end of the gland (Ca)

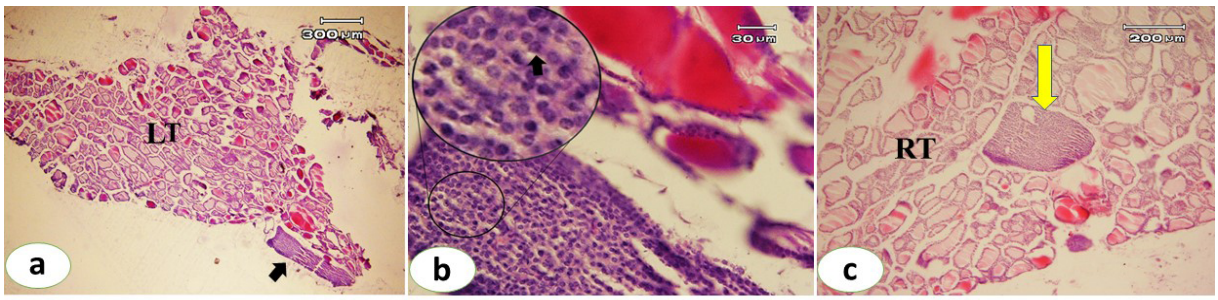

Figure 3. Micrograph of the thyroid and parathyroid glands from the Persian Squirrel, H&E staining

a) The left parathyroid gland is located at the base of the thyroid gland (LT) and consists of secretory cells with a rope-like arrangement (black arrow)

b) The main parenchyma of the parathyroid gland, which consists of secretory cells with acidophilic cytoplasm and round to oval euchromatic nuclei (black arrow)

c) The right parathyroid gland is observed within the parenchyma of the thyroid gland (yellow arrow)

Discussion

In the Persian squirrel (*S. anomalus*), the thyroid gland is composed of two lobes, which are located at the level of the second to third cervical vertebrae on both sides of the trachea, close to the base of the laryngeal cartilage. The gland extended to the second tracheal cartilage on the left and the third on the right. This positioning was similar to that observed in rats (Hadie et al., 2013) and African Giant Rats (Igbokwe, 2010). However, the thyroid gland extends from the larynx's caudal region to the trachea's sixth ring in guinea pigs (Yamasaki, 2016). No significant relationship was observed between the thyroid gland size and body size in the Persian squirrel, which aligns with findings reported in albino rats (Hall & Kaan, 1942).

According to Nakamura et al. (2019), the thyroid gland size can vary between different lobes, and in rats, the left lobe or one lobe can be absent. However, in the Persian squirrel, both thyroid gland lobes were present in the studied animals, with the right lobe larger than the left lobe. The sternothyroid muscles on the lateral and ventral sides cover the thyroid gland. From a dorsal perspective, it is located adjacent to the common carotid artery, internal jugular vein, and vago-sympathetic nerve (Mense & Boorman, 2018). This position is the same in the Persian squirrel.

The rostral end of the thyroid gland in rats is oval and butterfly-shaped, located below the thyroid cartilage and not connected to the tracheal cartilage from the dorsal aspect (La Perle et al., 2018; Tadjalli & Faramarzi, 2016).

The mean weight of the right thyroid gland in the Persian squirrel was 0.0082 g, and the mean ratio of the thyroid gland to the mean body weight was 0.039 (g/kg). In grasscutter, wild African, the mean weight of the thyroid gland was found to be 0.23 g, with a ratio of thyroid weight to body weight of 0.03 g/kg (Igbowe, 2010). In adult dogs, thyroid weight is approximately 1 g (Capen & Martin, 2003). In eutherian mammals, the relative thyroid weight to body weight ranges from about 0.07-0.24 g/kg, while in other marsupials, it ranges from 0.03-0.1 g /kg in other marsupials (Lawson & Carrick, 1998). In elephant seals, the ratio of total thyroid weight to body weight (relative thyroid weight) has been reported to be 0.175 g/kg. Thyroid weight varies between domestic and wild animals and depends on the size and weight of the animal.

In wild rats, the thyroid gland is long, thin, and brownish, while in domestic rats, it is short, round, and pink (Nur et al, 2023). In the adult Indian gray mongoose (Tadjalli & Faramarzi, 2016), the thyroid gland is described as dark brown (Tadjalli & Faramarzi, 2016). In the case of the Persian squirrel, the gland was long, thin, and brownish, resembling the characteristics observed in wild rats. Additionally, the absence of an isthmus and the separate nature of the two glands in the Persian squirrel is consistent with findings in other species, such as African grasscutters (Igbowe, 2010), guinea pigs (Yamasaki, 2016) and red pandas (Zhi-ping, 2004). Mota & Serkiz (2019) noted that the isthmus is sometimes absent in rats. An isthmus with glandular tissue exists in albino rats (Hall & Kaan, 1942), mongooses (Tadjalli & Faramarzi, 2016), and African giant rats (Enemali et al., 2016).

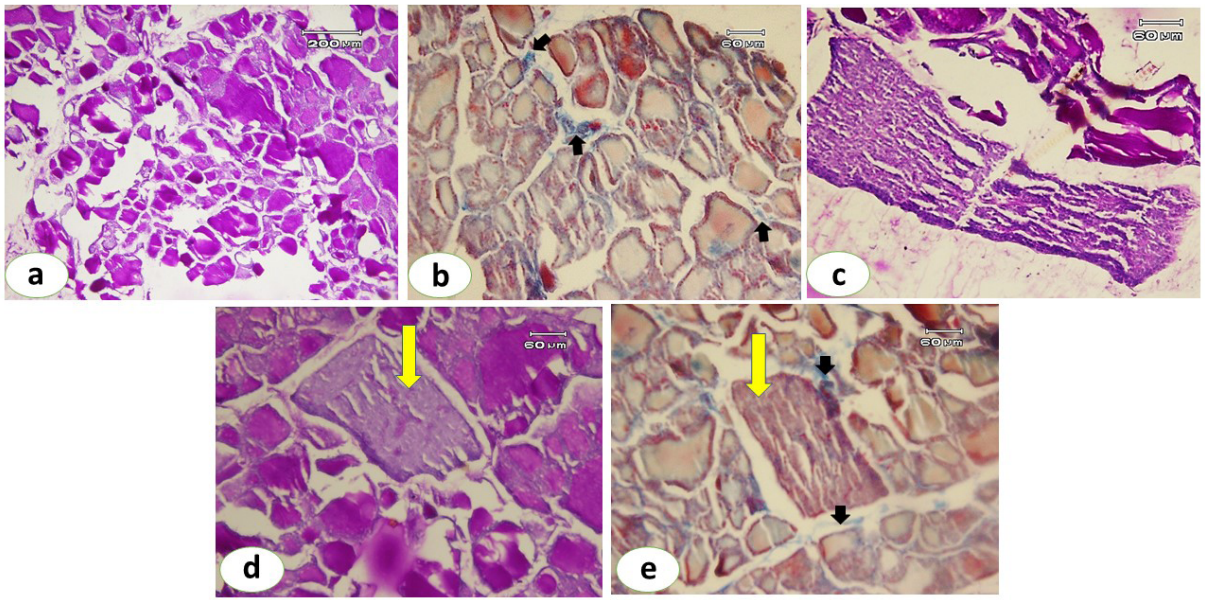

Figure 4. Micrograph of thyroid gland from the Persian squirrel

a) Follicles of the thyroid gland with a strong positive reaction to PAS staining

b) The colloidal substance of the follicles observed in Masson's trichrome staining from pale orange to deep orange

Between the follicles, the connective tissue contained collagen fibers (black arrow).

c) Micrograph of the left parathyroid gland

The parathyroid parenchymal secretory cells showed a weak positive reaction to PAS staining.

d) Micrograph of the right thyroid and parathyroid glands

The parathyroid parenchymal secretory cells (yellow arrow) were located within the parenchyma of the thyroid gland (with a strong reaction to PAS staining) and showed a weak positive reaction to PAS staining.

e) Micrograph of the right thyroid and parathyroid glands

The colloidal substance of the follicles appeared pale orange to deep orange on Masson's trichrome staining. The connective tissue between the follicles and the parathyroid gland (yellow arrow), contains collagen fibers (black arrow).

The histological characteristics of the thyroid glands of the Persian squirrel are similar to those observed in other mammals (Abdel-Margied et al., 2000). The capsule of the thyroid gland in the Persian squirrel consists of a single layer, which aligns with the results obtained in albino rats (Hall & Kaan, 1942). However, reports on guinea pigs (Yamasaki, 2016) and weasels (Al-Aamery & Dauod, 2017) indicate that the capsule in these species consists of two layers. Histological findings showed that the follicles in the thyroid gland of the Persian squirrel varied in shape and size, with some being large, medium, or small. These findings are consistent with those reported by`` in several mammals. In the Persian squirrel, the follicles exhibit cubic epithelial tissue in the resting state, while they appear cylindrical in the active state. This pattern is by observations in the albino rat (Hall & Kaan, 1942) and the mouse (Kaufman et al., 2016). However, this differs from reports on guinea pigs (Yamasaki, 2016) and African giant rats (Enemali et al., 2016), where the follicles are described as having squamous or cubic epithelium in the resting state and cubic epithelium in the active state. Variations in follicle size and the height of follicular cells indicate different activity levels among follicles and follicular cells. Thyroid activity, as measured by the amount of T3 secretion, differed between women and men, with significantly higher levels in women than in men (Abdel-Margied et al., 2000).

In Persian squirrels, parafollicular cells are primarily clustered between follicles and are rarely found individually in the wall. These cells exhibited bright cytoplasm, large euchromatin, and round to oval nuclei. The accumulation pattern of parafollicular cells and the shape of their nuclei are consistent with observations in albino rats (Hall & Kaan, 1942) and minks. However, a difference is observed in the distribution of parafollicular cells

between the Persian squirrel and albino rats. In albino rats, parafollicular cells are located only in the center of the gland, whereas in squirrels, they are found in all parts of the gland. According to previous reports on mice and African grasscutters (Igbowe, 2010), parafollicular cells are located individually in all parts of the thyroid gland between the follicles in guinea pigs (Yamasaki, 2016). Parafollicular cells are responsible for secreting a hormone called calcitonin, a physiological antagonist of parathyroid hormone, thereby reducing blood calcium levels by suppressing osteoclastic bone resorption (Igbokwe, 2010).

The Persian squirrel's follicles exhibited a positive reaction to PAS staining, indicating the active involvement of follicular cells in the formation of colloidal substances within the follicles. This result is consistent with observations in African wild mice (Igbokwe, 2010).

The parafollicular cells and colloid substance displayed a moderate-to-strong positive reaction to PAS staining. The capsule and trabeculae of the Persian squirrel thyroid gland showed a moderate reaction to Masson's trichrome and PAS, indicating the strong presence of collagen and carbohydrates in the capsule. In Masson's trichrome staining, the colloidal substance appeared to be varying shades of orange, with deeper orange colors indicating higher protein content. These results align with previous reports on guinea pigs (Yamasaki, 2016), albino rats (Hall & Kaan, 1942), rabbits (Parchami & Dehkordi, 2012; Moghadam et al., 2020; Raoofi et al., 2017) and African giant rats (Enemali et al., 2016).

Lappas et al. (2012) reported that the number and location of parathyroid glands can vary, and they can be found in different areas of the neck, thyroid parenchyma, or mediastinum. In mice (Taylor, 2014), the parathyroid gland is superficial and subcapsular and is an oval or lens-shaped organ located on the craniolateral edge of the thyroid gland. However, its position can vary (Flurkey et al., 2007; Kusmeirczyk et al., 2020). In female mongooses, the parathyroid gland is located on the medial side of the ventral edge of the thyroid gland, while in males, it is located on the posterior and lateral sides of each thyroid gland (Tadjalli & Faramarzi, 2016). In the Persian squirrel, the left parathyroid gland was on the anterolateral side of the thyroid gland and was oval with a light brown color. In contrast, the right parathyroid gland was deeper and was located within the parenchyma on the anterior side of the thyroid gland.

Histological studies of the Persian squirrel's thyroid gland revealed the presence of numerous small secretory cells arranged in a rope-like pattern within the parenchyma. These cells displayed acidophilic cytoplasm and round-to-oval euchromatin nuclei. PAS staining showed a weak positive reaction, indicating the presence of glycoproteins. No oxyphil cells were observed. The characteristics of the thyroid gland in the African giant rat were similar to those of the Persian squirrel, but oxyphil cells were present (Enemali et al., 2016).

The cranial and the caudal thyroid arteries provide blood supply to the glands. According to Vdoviaková et al. (2022), these arteries are separated from the common carotid artery in rats. However, conflicting reports suggest these arteries can also arise from the external and internal common carotid arteries. The parathyroid gland is supplied by the same arteries that supply the thyroid gland in rats (Allen & Fingeret, 2022), indicating a direct connection between the vascular bed and thyroid parenchyma. In mice, the cranial thyroid artery supplies the parathyroid gland (Abdreshov et al., 2019). In the Persain squirrel, only the cranial thyroid artery supplied blood to the thyroid and parathyroid glands. This artery originates from the common carotid trunk.

Conclusion

The Persian squirrel (*S. anomalus*) has two unequal thyroid glands; the right thyroid gland is larger and contains the right parathyroid gland within its parenchymal tissue. Parafollicular cells are predominantly in the form of cell accumulation and clusters between the follicles. The isthmus is absent in the thyroid gland of the Persian squirrel.

Ethical Considerations

Compliance with ethical guidelines

The animal procedures conducted in this study adhered to the standards of the University of Tabriz for the Humane Care and Use of Laboratory Animals. The study was also approved by the Research Ethical Committee of the Ministry of Health and Medical Education in Iran, following the Helsinki Protocol (1975) guidelines and adopted on April 17, 2006.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors thank the members of the Anatomical Section of the University of Tabriz, Tabriz, Iran, for their valuable support and help in preparing the present paper.

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