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

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Abstract

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

Objectives: The thyroid and parathyroid gland of the Persian squirrel duo to limited anatomical knowledge was considered in this study.

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

Result: The thyroid gland of the Persian squirrel composed of two unequal pale brown lobes without 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. Blood supply to the glands was facilitated by the cranial thyroid artery. The colloidal substances were Periodic acid schiff (PAS) positive, and 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 consisted of small secretory cells with a rope-like arrangement. These cells exhibited acidophilic cytoplasm, euchromatin round to oval nuclei, and displayed weak reaction to PAS staining.

Conclusions: This 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

Introduction

The thyroid gland has been receiving significant attention from researchers due to a wide range of disorders, including hypothyroidism and hyperthyroidism. Phylogenetically, this gland is known as one of the largest endocrine glands among vertebrate species (Onwuaso and Nwagbo,

2014). It plays a crucial role in regulating body growth, metabolism, and tissue development and differentiation (Choksi *et al*.,2003). These hormones include thyroglobulin, triiodothyronine, and thyroxine (Gartner, 2020). Evaluating the histology of the thyroid is crucial for assessing its function. The gland consists of numerous follicles of varying sizes, which form the functional and histological units of the gland. These units consist of three main components: follicular epithelial cells, parafollicular cells, and colloid cavity (Reece and Rowe, 2017). Thyroid hormones are synthesized by follicular cells, which constitute the major portion of the gland's parenchyma. These hormones are first stored in the follicular fluid, then broken down and converted into end products. Ultimately, they are released into the bloodstream (Baljit, 2017).

The parathyroid glands are responsible for producing 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 as well as the parathyroid IV, indicating their embryonic origin (König *et al*., 2020). 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 and Shabid, 2006), sheep, dogs, cattle (Greco and Davidson, 2017), goat (Joshi and Mathur, 2015), Bakerwali goat (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*) can be 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 a rise in their visits to veterinary clinics (Akbari and Kianifard, 2017).

Information available on naturally occurring thyroid and parathyroid diseases in small mammals is extremely limited. Although there is an abundance of research with 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. Overall, naturally occurring thyroid and parathyroid glands dysfunction is poorly documented in rodents, and more research is needed to explore these diseases (Thorson, 2014; Barber, 2004; Soltani and Dalimi, 2018; Mohammed Ibrahim *et al.*, 2022; Badi *et al.,* 2022; Sheikholeslami *et al.,* 2019). So, the morphological data can be useful to veterinary practitioner in thyroid disorder management. Due to the 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 research can be useful to diagnose the pathobiology and clinical treatment of endocrine diseases in Persian squirrels.

Materials and Methods

Five adult male Persian squirrels (*Sciurus anomalus*) were used to perform the study. These animals were euthanized by ketamine 10% overdose for reasons unrelated to endocrine disease,

either due to various causes in veterinary clinics in Tabriz or as a result of car accidents. animal procedures conducted in this study adhered to the standards of the University of Tabriz regarding 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 guidelines of the Helsinki Protocol (1975) and adopted on April 17, 2006. The research focused on the anatomical, histological, histochemical, and stereological aspects of the thyroid and parathyroid glands.

Anatomical study

First, to determine the blood supply of the thyroid and parathyroid glands, the common carotid trunk was carefully examined, the position, shape, and relationship of these glands with other structures were also observed. Additionally, 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, the samples were transferred to a 10% formalin buffer for further analysis.

Histological and histochemical study

Tissue samples, after tissue processing, were embedded in paraffin blocks and sliced into sections measuring 7 µm in thickness by microtome (LEICA, RM2145). These sections were stained with Hematoxylin and Eosin (H&E) for routine histological examination. Furthermore, 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.

Result

Anatomical result

The thyroid gland in Persian squirrels (*Sciurus anomalus*) is paired and situated on both sides of the caudal part of the larynx. It begins at the junction of the thyroid cartilage with the cricoid, at the end of the sternothyroid muscle, and at the level of the second and third cervical vertebra. The gland extends below these muscles to the third tracheal cartilage on the right side and the second tracheal cartilage on the left side. It appears as a thin, elongated structure with a brown color. The oval base of the gland was located in rostral, and its pointed tip was located in caudal direction. The isthmus of the gland was not present in Persian squirrel. The right thyroid gland was slightly larger than the left gland (Fig. 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 gland was 0.0082±0.00083 gr on the right side and 0.0058±0.00083 gr on the left side. In the rostral and lateral parts of the left thyroid gland, the parathyroid gland was observed. It exhibited an oval shape and a light brown color (Fig. 1b). However, the specific location of the left parathyroid gland was not determined during the anatomical studies. The blood supply to these glands was provided by the cranial thyroid artery. The correlation between the body weight and thyroid gland weight was 0.97 and significant at level of $p < 0.01$. However, there was no significant correlation 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 was surrounded by connective tissue that penetrated into the gland, dividing it into unknown parts. The main parenchyma of the gland consisted of thyroid follicles with irregular and polyhedral shapes and different sizes. These follicles contained follicular fluid (Fig. 2a, 2b) and were surrounded by follicular cells. The follicular cells were short to long cuboids, rarely cylindrical, with round to oval nuclei that appeared relatively heterochromatin (dark and pigmented) (Fig. 2b, 2c). The colloidal material reacted positively to PAS staining (Fig. 3a) and appeared pale orange to deep orange color in Masson's trichrome staining (Fig. 3b), which indicates the presence of glycoprotein with a high protein content in the more colorful colloids. Between the follicles, the connective tissue contained collagen fibers (Fig. 4b).

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

In the tissue sections of the left parathyroid gland, it was determined that this gland was located at the base of the thyroid gland. It consisted of small secretory cells with a rope arrangement (Fig. 3a). These cells had acidophilic cytoplasm, and their nuclei were euchromatin and round to oval in shape (Fig. 3b). The reaction of the parathyroid gland to PAS staining was low and weak (Fig. 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 (Fig. 3c). The cell

structure of the right parathyroid gland was similar to the left parathyroid gland. Both glands showed weak reactions to PAS and Masson's trichrome staining methods.

Discussion

In the Persian squirrel (*Sciurus 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 cartilages. The gland extends to the second tracheal cartilage on the left side and the third tracheal cartilage on the right side. This positioning is similar to what has been observed in rats (Hadie *et al*., 2013) and African Giant Rats (Igbokwe, 2010). However, in guinea pigs (Yamasaki, 2016), the thyroid gland extends from the caudal region of the larynx to the sixth ring of the trachea (Yamasaki, 2016). There was no significant relationship between the size of the thyroid gland and the body size in the Persian squirrel, which aligns with the findings reported in albino rats (Hall and Kaan, 1942).

According to Nakamura *et al*. (2019), the size of the thyroid gland can vary between different lobes, and in rats, it is possible for the left lobe or one lobe to be absent (Nakamura *et al*., 2019). However, in the Persian squirrel, both lobes of the thyroid gland were present in the studied animals, with the right lobe being larger than the left lobe. The thyroid gland is covered by the sternothyroid muscle from the lateral and ventral sides. From the dorsal perspective, it is located adjacent to the common carotid artery, internal jugular vein, and the Vago-sympathetic nerve (Mense and Boorman, 2018). This position is also the same in the Persian squirrel.

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

The mean weight of the right thyroid gland in the Persian squirrel was 0.0082 gr and the mean ratio of the thyroid gland to body weight mean was 0.039 (gr/kg). In grasscutter wild African, the mean weight of the thyroid gland was found to be 0.23 gr, with a ratio of thyroid weight to body weight of 0.03 gr/kg (Igbowe, 2010). In adult dog, the thyroid weight is approximately 1 g (Capen and 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 0.03-0.1g /kg in other marsupials (Lawson and Carrick, 1998). In elephant seals, the ratio of total thyroid weight to body weight (relative thyroid weight) is reported to be 0.175 g/kg. Thyroid weight varies between domestic and wild animals, and it 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 adult indian gray mongoose (Tadjalli & Faramarzi, 2016), the thyroid gland is described as dark brown (Tadjalli and Faramarzi, 2016). In the case of the Persian squirrel, the gland was reported to be 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 are consistent with findings in other species such as African grasscutters (Igbowe, 2010), guinea pigs (Yamasaki, 2016) and red pandas (Zhi-ping, 2004). Mota and Serkiz (2019) noted that in rats, the isthmus is sometimes absent. In albino rats

(Hall and Kaan, 1942), mongooses (Tadjalli and Faramarzi, 2016), and African giant rats (Enemali *et al*., 2016), an isthmus with glandular tissue exists.

The general histological characteristics of the thyroid glands in 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 findings in the albino rat (Hall and Kaan, 1942). However, reports on guinea pigs (Yamasaki, 2016) and Weasels (Al-Aamery and 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 vary in shape and size, with some being large, medium, or small. These findings are consistent with the report of Hartof-Nielsen *et al*. (2005) in several mammals (Hartof-Nielsen *et al*., 2005). In the Persian squirrel, the follicles exhibit a cubic epithelial tissue in the resting state, while they appear cylindrical in the active state. This pattern is in accordance with observations in the albino rat (Hall and Kaan, 1942) and the mouse (Kaufman, *et al*., 2016). However, it 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. The variation in follicles size and the height of follicular cells indicates different level of activity among follicles and follicular cells. Thyroid activity, as measured by the amount of T3 secretion, differs in women and men with significantly higher levels in women than in men. (Abdel-Margied *et al*. 2000).

In Persian squirrel, the parafollicular cells are primarily clustered between the follicles and are rarely found individually whitin the wall. These cells exhibit bright cytoplasm and possess large

euchromatin and round to oval nuclei. The accumulation pattern of parafollicular cells and the shape of their nuclei are consistent with observations in the albino rat (Hall & Kaan, 1942) and mink. However, there is a difference in the distribution of parafollicular cells between the Persian squirrel and the albino rat. In the albino rat, parafollicular cells are located only in the center of the gland, whereas in the squirrel they are found throughout all parts of the gland. According to the reports, in guinea pigs (Yamasaki, 2016), mice and African grasscutters (Igbowe, 2010), parafollicular cells are located individually in all parts of the thyroid gland between the follicles. Parafollicular cells are responsible for secreting a hormone called calcitonin, which serves as a physiological antagonist to parathyroid hormone, thereby reducing blood calcium levels by suppressing osteoclastic bone resorption (Igbokwe,2010).

The follicles in the Persian squirrel exhibited a positive reaction to PAS staining, indicating active involvement of follicular cells in the formation of the colloidal substance present within the follicles. This finding is consistent with observations in the African wild mouse (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's 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 as varying shades of orange, with deeper orange colors indicating a higher protein content. These results align with previous reports on guinea pigs (Yamasaki, 2016), albino rats (Hall & Kaan,

1942), rabbits (Parchami and Dehkordi, 2012; Moghadam *et al.,* 2020; Raoofi *et al.,* 2017) and African giant rats (Enemali *et al*., 2016).

Lappas *et al* (2012) mentioned 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 which is 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, they are located on the posterior and lateral sides of each thyroid gland (Tadjalli and Faramarzi, 2016). In the Persian squirrel, the left parathyroid gland was situated on the anterolateral side of the thyroid gland and was had an oval shape with a light brown color. On the other hand, the right parathyroid gland was deeper and 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 exhibited a weak positive reaction, indicating the presence of glycoprotein. No oxyphil cells were observed. In the African giant rat, the characteristics of the thyroid gland were similar to those of the Persian squirrel, but oxyphil cells were present (Enemali *et al*., 2016).

In general, blood supply to the glands is provided by the cranial thyroid artery and the caudal thyroid artery. According to Vdoviaková *et al*. (2022) in the rats, these arteries are separated

from the common carotid artery. However, there are conflicting reports suggesting that these arteries can also arise from the external and internal common carotid arteries. In rats, the parathyroid gland is supplied by the same arteries that supply the thyroid gland (Allen and Fingeret, 2022), indicating a direct connection between the vascular bed and the thyroid parenchyma. In mice, the parathyroid gland is supplied by the cranial thyroid artery (Abdreshov *et al*., 2019). In the Persain squirrel, only the cranial thyroid artery was responsible for supplying blood to both the thyroid and parathyroid glands. This artery originated from the common carotid trunk.

Conclusion

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

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Conflicts of interest

The author declared no conflict of interest.

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

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

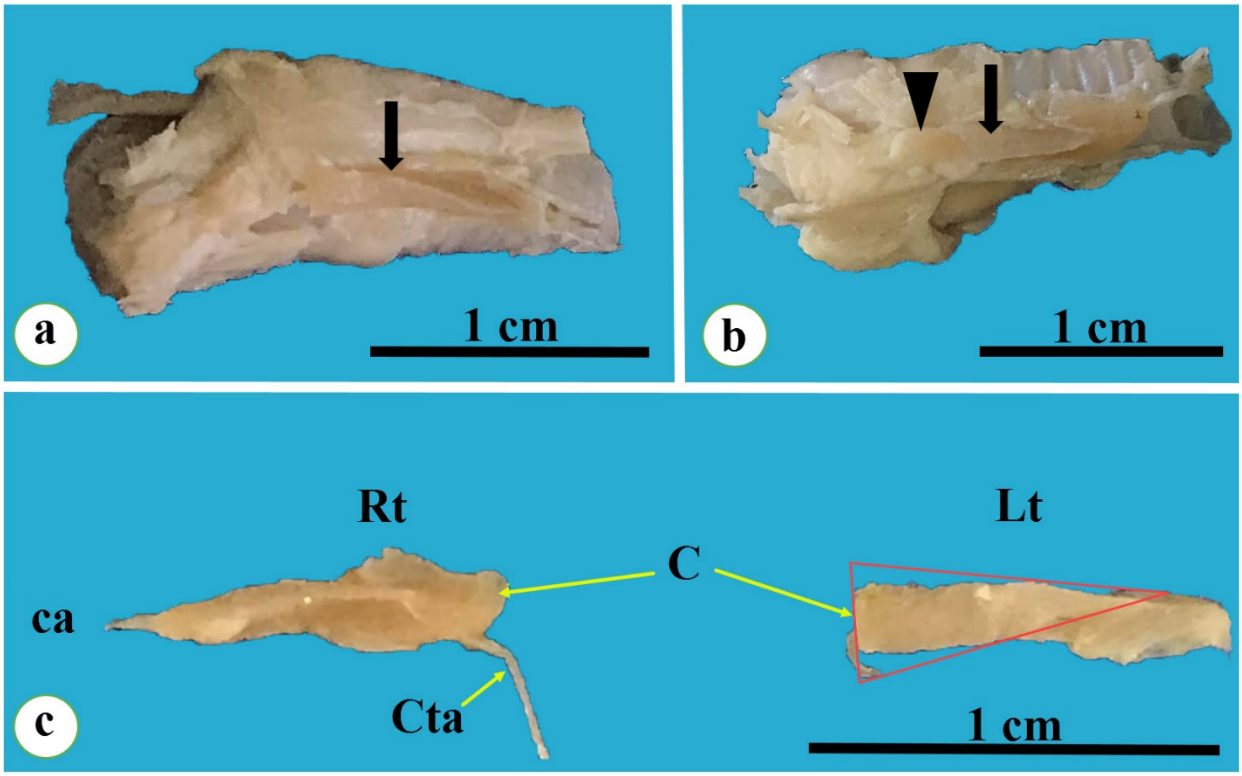

Figure 1: The gross photographs of the thyroid and parathyroid glands. **a**: The position of the right thyroid gland on the larynx and trachea (lateral view), **b**: It shows the position of the thyroid and parathyroid glands on the left side (lateral view). **c**: Internal view of the right thyroid 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).

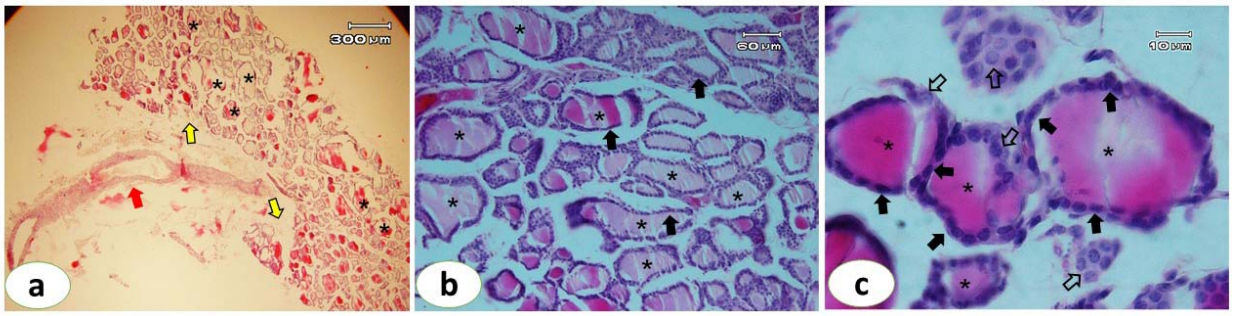

Figure 2: The micrograph of the thyroid gland from the Persian squirrel, H&E staining. **a**: The gland is surrounded by connective tissue, which penetrates the gland and divides it into unknown parts (yellow arrow), the cranial thyroid artery (red arrow) is visible. The main parenchyma of the gland 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 inside of the follicles is filled with colloid (*). Parafollicular cells are mainly observed as cellular aggregates between follicles and are rarely found within the wall of follicles, which have bright cytoplasm with a large and round to oval euchromatin nucleus (hollow arrow).

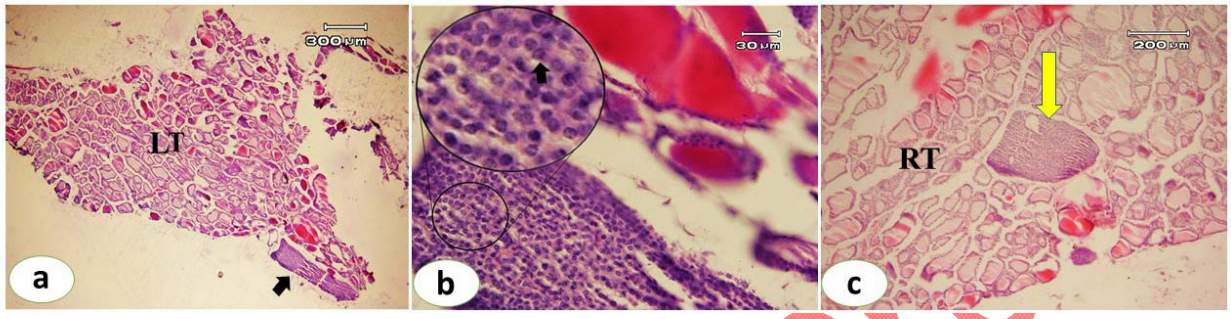

Figure 3: Micrograph of the thyroid and parathyroid glands from the Persian squirrel, H&E staining. **a**: The left parathyroid gland is located at the base of the thyroid gland (LT) and consists of secretory cells with a rope-like arrangement (black arrow). **b**: The main parenchyma of the parathyroid 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).

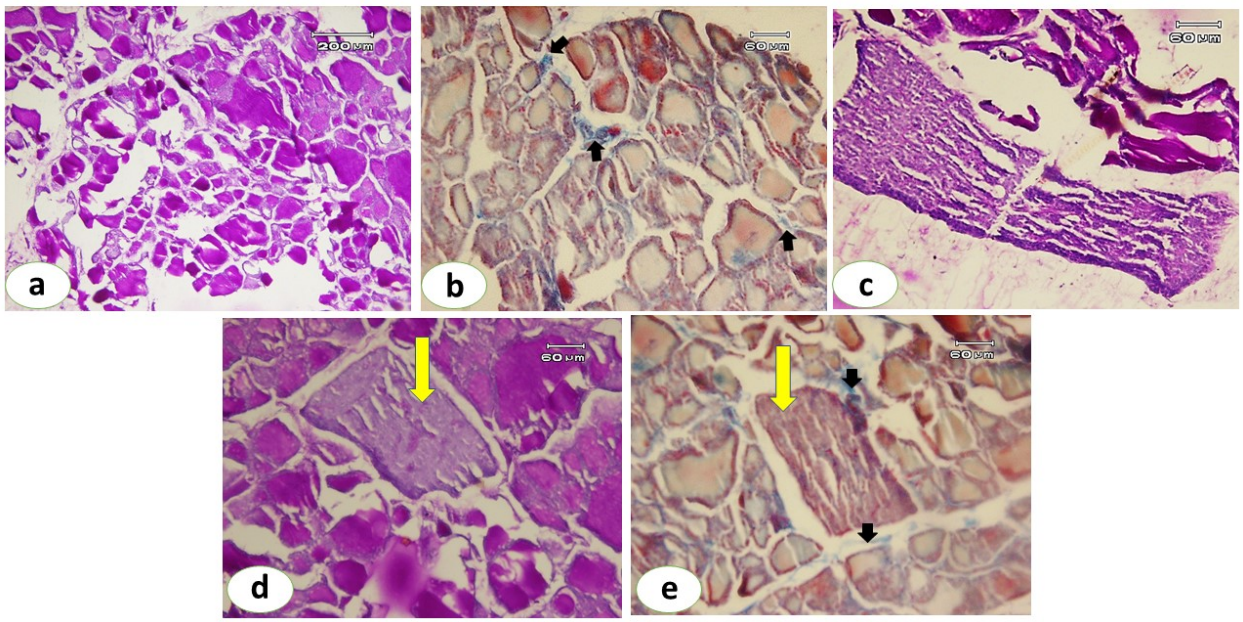

Figure 4: Micrograph of thyroid gland from the Persian squirrel. **a**: Follicles of the thyroid gland with a strong positive reaction to PAS staining. **b**: The colloidal substance of the follicles can be seen in Masson's trichrome staining from pale orange to deep orange. Between the follicles, the connective tissue contains collagen fibers (black arrow). **c**: Micrograph of the left parathyroid gland. The parathyroid parenchymal secretory cells show a weak positive reaction to PAS staining. d: Micrograph of the right thyroid and parathyroid glands. The parathyroid parenchymal secretory cells (yellow arrow) are located within the parenchyma of the thyroid gland (with a strong reaction to PAS staining) and show a weak positive reaction to PAS staining. **e**: Micrograph of the right thyroid and parathyroid glands. The colloidal substance of the follicles

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

بررسی آناتومیکی و بافت شناسی غده تیروئید و پاراتیروئید در سنجاب ایرانی(*Sciurus anomalus*)

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زمینه مطالعه: غده تیروئید به عنوان یکی از و بزرگترین غدد درون ریز در میان گونه های مهره داران شناخته می شود.

هدف: غده تیروئید و پاراتیروئید سنجاب ایرانی با توجه به کمبود اطلاعات آناتومیکی در این مطالعه مورد مطالعه قرار گرفت.

روش کار: برای انجام کار از پنج سنجاب ایرانی نر بالغ استفاده شد. پس ازآسان کشی، غدد تیروئید و پاراتیروئید تشریح و به صورت ماکروسکوپی مورد ارزیابی قرار گرفتند. سپس، نمونه‌های بافتی تثبیت شده و بعد از طی فرآیند بافتی، برای ارزیابی میکروسکوپی با هماتوکسیلین و ائوزین و رنگ های اختصاصی رنگ آمیزی شد.

نتایج: غده تیروئید سنجاب ایرانی از دو لوب قهوه ای کم رنگ نابرابر و بدون تنگه تشکیل شده است. لبه خلفی غده نوک تیز بود، در حالی که لبه قدامی محدب بود. غده پاراتیروئید چپ در لبه قدامی غده تیروئید و غده پاراتیروئید راست در داخل پارانشیم غده تیروئید قرار داشت. خون رسانی به غدد توسط سرخرگ تیروئید قدامی انجام می گرفت. مواد کلوئیدی پریودیک اسید شیف (PAS) مثبت بودند و در رنگ‌آمیزی تری کروم ماسون به رنگ صورتی تا نارنجی پررنگ به نظر می‌رسیدند که نشان‌دهنده وجود گلیکوپروتئین با محتوای پروتئین بالا در کلوئیدهای با رنگ شدیدتر است. سلول های پارافولیکولی عمدتاً به صورت تجمعات سلولی بین فولیکول ها قرار داشتند. غده پاراتیروئید از سلول های ترشحی کوچک با آرایش طناب مانند تشکیل شده است. این سلول‌ها دارای سیتوپلاسم اسیدوفیلی با هسته گرد تا بیضی شکل یوکروماتین بودند و واکنش ضعیفی به رنگ‌آمیزی PAS نشان دادند.

نتیجه گیری نهایی: این اطلاعات در مورد غدد تیروئید و پاراتیروئید می تواند برای دامپزشکان که با بیماری های مربوط به سیستم غدد درون ریز سروکار دارند بسیار مفید باشد.

کلمات کلیدی: آناتومی، هیستولوژی، غده پاراتیروئید، غده تیروئید، سنجاب ایرانی