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Presence of the Parafollicular Cells in the Thyroid Gland of the One-Humped Camel

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Abstract

The thyroid is an important endocrine gland that affects many organs of the body. A limited number of works have been done on the morphological and histological characteristics of this gland in the camel and controversial debates have been made on the presence of parafollicular cells. The aim of this study is to investigate the histological structure of the thyroid in the camel and determine the presence of parafollicular cells in this gland. This study was performed on 20 camels. The histological structure of the thyroid was studied using light microscope after preparing sections and staining it with Hematoxylin & Eosin, Verhoeff, and Toluidine blue. Thyroid gland has follicles of different sizes, follicular and parafollicular cells, and according to our results these cells are forming about 59.1% and 5% of the gland volume respectively. The large follicles are located in the peripheral part of the gland while the small follicles are seen in the central part of the gland. The central parts of the gland have a more extensive vascular bed than the peripheral parts. This study revealed that the thyroid gland in camel has parafollicular cells, but most of them are present in the central part of the gland.

Keywords: Camel, histology, parafollicular cell, thyroid

Introduction

Camel is a ruminant that is adapted to hot, dry, and inclement climates. Camel has many differences from animals that live in temperate climates. This animal can lose 25% of its body weight and they can concentrate their urine without illness. Camel's body temperature changes in a wide range. Stabilization of the internal situation of the camel's body depends on the endocrine system in hot weather and the thyroid gland influences many organs (Atoji et al., 1999). Thyroid gland contains both follicular cells that produce triiodothyronine (T3) and tetraiodothyronine (T4) hormones, and parafollicular cells, which produce calcitonin hormone. These hormones play an important role in the metabolism of animals (Abdel-Magied et al., 2000; Kausar and Shahid, 2006; Mason and Wilkinson, 1973).

Epithelial clusters that sprout from the caudal part of the fourth pharyngeal pouch are replaced by cells of the neural crest that are located around this pharyngeal pouch, making the ultimobranchial body. These cells incorporated into the thyroid gland and form parafollicular cells (Hyttel et al., 2010).

Yagil et al. (1978) showed that the thyroid gland affects the animal's compatibility with inclement weather. Thyroid gland structure has been studied by both light and electron microscopy in different mammals (Fujita, 1975; Kurihara et al., 1990). Few histological and anatomical studies have been performed previously on the thyroid gland of camels. Also, the focus of previous histological studies of the camel thyroid gland was on the ultrastructure of the follicular cells (Abdel-Magied et al., 2000; Atoji et al., 1999; Kausar and Shahid, 2006). Although the results of these studies were similar with respect to the general structure of the camel thyroid gland, there has been a disagree-

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ment on the presence of the parafollicular cells. Moreover, the results of preliminary studies performed in this area differ with some results of recent studies. The present study was conducted with an emphasis on the presence or absence of parafollicular cells on the camel thyroid gland.

Materials and Methods

This study was conducted in spring on 20 (10 males and 10 females) 6-10 years old one humped camels, whose age was determined by their teeth (Hillson, 2005). Samples were collected from the Semnan industrial slaughterhouse, Semnan Province, Iran. The slaughterhouse authorities gave permission to use the animals in this study. It was concluded that the study is in compliance with the Principles of Scientific Research and Publication Ethics according to the examination that was made by Semnan University Veterinary Faculty Sub-Committee of Ethics. First, the anatomical characteristics of the thyroid glands were examined and their dimensions were measured. Then, for histological investigation, both lobes of the thyroid were removed, weighed, divided into pieces with a thickness of 5 mm, and fixed in 10% buffered formalin solution for 72 h. After routine histological processing, thyroid samples were embedded in paraffin and 6-µm sections were prepared. The sections were stained with Hematoxylin & Eosin and Verhoeff for light microscopic investigation. Hematoxylin & Eosin stained sections were used for histological inspection and stereological procedures, and Verhoeff stained sections were used for revealing connective tissue (Bancroft and Gamble, 2008; Culling and Allison, 1985). For toluidine blue staining some specimens were fixed in phosphate buffered glutaraldehyde (pH 7.4) and post fixed in osmium tetroxide (Sigma-Aldrich Co. LLC, Germany) in the same buffer at 4°C. Then they were dehydrated and embedded in epoxy resin (Epoxy Embedding Kit, Sigma-Aldrich Co. LLC, Germany) and semi-thin sections (1 µm thick) were prepared and were stained with toluidine blue (Bancroft and Gamble, 2008; Culling and Allison, 1985; Glauert and Lewis, 1998).

Toluidine blue is an acidophilic metachromatic dye that selectively stains acidic tissue components such as sulfates, carboxylates, and phosphate radicals. Toluidine blue can be used to stain connective tissue mucins, especially acid mucins, mast cell granules, endocrine cells and frozen sections (Sridharan and Shankar, 2012). Lead-Haematoxylin is well used for staining most endocrine cells of the pancreatic islets, thyroid, pituitary gland and adrenal medulla. The two methods of Toluidine blue and Lead-Hematoxylin have similar results in staining of the thyroid endocrine cells (Solcia et al., 1969). In our study, Toluidine blue staining method has been used for identifying and showing parafollicular cells in the thyroid gland.

The volume densities of parafollicular and follicular cells were determined by using the point- counting method of Weibel (1979). For cell volume density calculations, sections were taken from eight different parts of the thyroid glands of each animal. In each section, 50 different areas were evaluated under × 400 magnification. In each area follicular and parafollicular cell numbers touching to the points of counting graticule (100 point graticule) were recorded.

Statistical analysis

For statistical analysis, Statistical Package for the Social Sciences 2003 (SPSS, IBM Corp.; NY, USA) software was used and the differences between groups were determined using a Student's t-test. Overall, p-values less than 0.05 were regarded as statistically significant.

Results and Discussion

The thyroid gland of camel were consisting of two oval shaped lobes connected together by a distinct non-glandular isthmus across the ventral surface of the trachea at the level of the second and third rings. The right lobe of the thyroid gland was located on the dorsomedial aspect of the trachea at the level of the first to fifth rings and the left lobe was located at the level of the second to sixth rings on the medial aspect of the trachea. Weight and dimensions of the thyroid gland were slightly higher in female camels compared with the male ones, though the difference was not statistically significant (Table 1).

The thyroid glands were surrounded by a thin capsule of dense irregular connective tissue, containing a large number of thin collagen fibers (Figure 1). Some trabeculae were penetrating from the capsule into the gland and producing some irregular lobules (Figure 2). Each lobule was consisting of a number of follicles with different sizes and shapes. There were many fibroblasts, blood capillaries and thin collagen fibers between the follicles. Each follicle was surrounded by an epithelium, varying from simple cuboidal to low columnar, lying on a basement membrane. The secretion were stored in the follicles as a homogeneous eosinophilic colloid. In each follicle, the size of the cells were the same.

Table 1. Dimensions and weight of the right and left lobes of the thyroid gland in one-humped camel (mean ± standard deviation)

	Camel	Right lobe	Left lobe
Length (mm)	Male	5.7±0.4	4.6±0.9
	Female	5.9±0.3	5.2±0.7
	Mean	5.8±0.35	4.9±0.8
Width (mm)	Male	2.4±0.7	2.1±0.8
	Female	2.8±0.2	2.5±0.4
	Mean	2.6±0.45	2.3±0.6
Height (mm)	Male	0.9±0.65	0.9±0.46
	Female	0.9±0.25	0.9±0.73
	Mean	0.9±0.45	0.9±0.6
Weight (gr)	Male	52.71±0.83	50.67±0.21
	Female	54.2±0.34	51.94±0.17
	Mean	53.46±0.59	51.31±0.19

Depending on their size, follicles were divided into two groups. Large follicles were located in the peripheral area and the small follicles were placed in the central part of the gland (Figure 1). Sometimes, the epithelium of the large follicles were containing low cuboidal to squamous cells, while the epithelium of small follicles were varying from cuboidal to low columnar. The colloid in the large follicles was completely acidophilic and thick, but in small follicles, it was less acidophilic, thinner and uniform. The follicular cells, which constitute the largest cell population of the gland, were cuboidal and have a relatively basophilic cytoplasm. A large nucleus was present at the base of the cells (Figure 3). Small follicles were forming the active follicular population, which were having vacuoles in their colloid.

The parafollicular cells were found in very small numbers in the camel thyroid gland. These cells, which were often found individually or collectively between follicles, were stained lighter and were larger than follicular cells. Parafollicular cells were commonly found between the small follicles in camel thyroid gland. They were rarely present between the large follicles or between follicular cells (Figure 3). Parafollicular and follicular cell volume densities are given in Table 2.

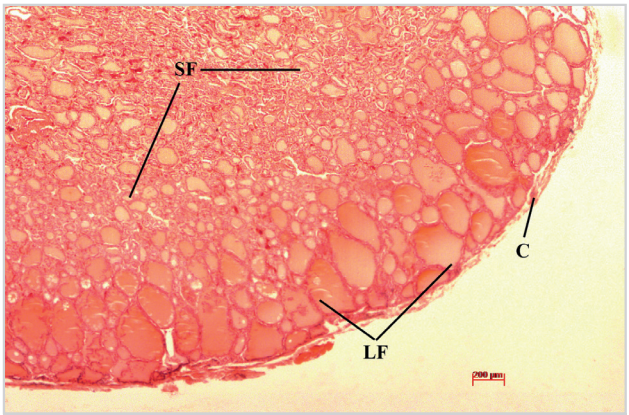

Figure 1. Thyroid gland of the camel. A thin collagenous capsule (C) surrounds the gland. The large follicles (LF) are located in the peripheral part of the gland and the small follicles (SF) in the central part of the gland. H&E.

Dimensions and weight of camel thyroid gland were not significantly different in the males and females. These results are similar to those of Kausar and Shahid (2006). The camel thyroid gland is located in a close contact with the trachea and on its lateral surface, as seen in other domestic mammals (Dyce et al., 1996; Nickel et al., 1979; Pousty and Adibmorady, 2003; Sisson et al., 1975). As Allen et al. (1998) have shown in their reports, the thyroid gland is surrounded by a capsule of collagenous connective tissue and penetration of trabeculae from the capsule into the gland divides it into various lobules. The lobules are made from follicles, which vary in size and shape. These features are also available in camels and are consistent with the findings of Kausar and Shahid (2006), Atoji et al. (1999), and Abdel-Magied et al. (2000). In contrary to the study of Kausar and Shahid (2006) reporting that the capsule of the camel's thyroid gland is thick, the results of this study show that the thyroid gland is surrounded by a thin capsule. Unlike other domestic mammals, in camel, the large (inactive) follicles are present in the peripheral part of the gland while the small (active) ones are located at the center of the gland (Abdel-Magied et al. 2000; Atoji et al. 1999; Pousty and Adibmorady, 2003). Loose connective tissue and vascular bed are

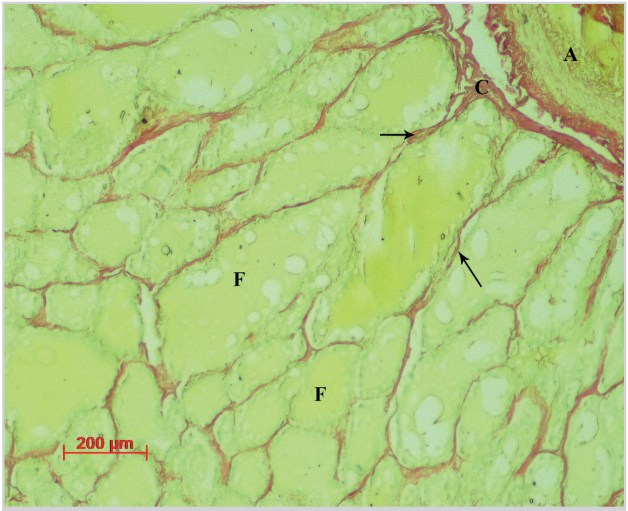

Figure 2. Thyroid gland of the camel. Penetration of trabeculae (arrow) from the capsule (C) into the gland. Artery: (A), Follicle: (F). Verhoeff.

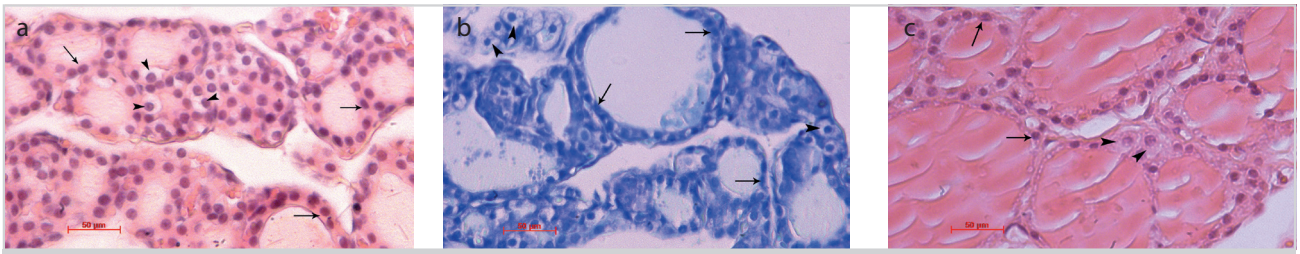

Figure 3. a-c. Thyroid gland of the camel. Hematoxylin & Eosin (a, c) and Toluidine blue (b); Follicular cells (arrows) that constitute the wall of the follicles. Parafollicular cells (arrowheads) with light cytoplasm are located between the follicles.

Table 2. Mean values (mean value ± standard deviation) of some stereological parameters of the thyroid gland in one-humped camel

Variable	Volume density
Follicular cell (%)	59.1±4.37
Parafollicular cell (%)	5.00±0.18
Colloid (%)	31.1±2.13
Stroma (%)	4.6±0.25

well developed in the central part of the gland, which is consistent with the activity of the central small follicles (Atoji et al., 1999; Kausar and Shahid, 2006; Pousty and Adibmorady, 2003). Camel is a ruminant, which is adapted to dry, very hot, and adverse climates (Atoji et al., 1999). In warm and dry areas, the internal condition of the camel's body is dependent on the endocrine system, and the thyroid affects many organs of the body (Banks, 1993). Reduction in thyroid function during dehydration in the summer helps to maintain water level by reducing pulmonary water loss and basal metabolism (Yagil et al., 1978). It seems that this specific arrangement of the follicles at the peripheral and central parts of the gland is effective in preparing the animal to cope with the hot and dry weather of the deserts. Although this condition has been observed in some rodents such as rats and guinea pigs (Pousty and Adibmorady, 2003), it has not been reported by researchers such as Abdel-Magied et al. (2000), Atoji et al. (1999) and Kausar and Shahid (2006) who studied camel's thyroid gland previously. Like other domestic mammals (Banks, 1993; Fujita, 1975; Mason and Wilkinson, 1973; Nickel et al., 1979), in the camel's thyroid gland, cuboid cells form the wall of the follicles and the inside of the follicles is full of colloid. These results are consistent with the findings of Abdel-Magied et al. (2000), Atoji et al. (1999) and Kausar and Shahid (2006).

Although there has been studies reporting that parafollicular cells are absent in camel's thyroid (Abdel-Magied et al., 2000; Atoji et al., 1999; Kausar and Shahid, 2006), this study showed that these cells are present in the camel's thyroid gland. The staining of these cells is paler than follicular cells. Also, they are brighter and larger than follicular cells and they are located between the follicles. These cells are significantly fewer than follicular cells and are more commonly seen between the small and active follicles of the central part of the gland, due to massive vascular bedding in this area. These cells are rarely present in the peripheral parts of the gland. The presence of ultimobranchial structure in the camel's thyroid gland has also been reported by Mubarak and Sayed (2005). In mammals such as horses, dogs, and cats, these cells are present as light and pale cells between the follicles and follicular cells and occupy a small number of thyroid cells (Banks, 1993; Manohar et al., 1995). In this study the presence of parafollicular cells in the thyroid gland of camel has been shown by routine histological methods, besides, further research is needed. They are located in the central part of the gland, between the small follicles. Like other domestic mammals, these cells form a small amount of the thyroid cells.

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Peer-review: Externally peer-reviewed.

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