Comparative histomorphological and histochemical study of thyroid gland in adult indigenous gazelle (*Gazella subgutturosa*) and sheep (*Ovis aries*)

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Abstract

The present work was carried out on 8 adult healthy indigenous gazelle and sheep (4 gazelle and 4 sheep) to study the histomorphological and histochemical features of thyroid gland in these two animals and to compare between them. The present findings showed that the histological structure of the thyroid gland in both sheep and gazelle was similar and appeared covered by a connective tissue capsule which send trabeculae that divide the glandular parenchyma into irregular lobules which made up of aggregation of a various shape and size of follicles that lined by a predominantly of simple cuboidal and simple columnar epithelium in gazelle, whereas in sheep lined by simple cuboidal to simple squamous epithelium. The para follicular cells were present inter epithelium and inter follicular, myoepithelial cells were also present in both thyroid gazelle and sheep. The colloid material found in the follicular lumen was PAS positive. The isthmus was glandular in both studied animals. The histomorphometrical analysis revealed there were a significant importance at $p<0.05$ between left and right lobes with the isthmus in both sheep and gazelle. There was a significant importance at $p<0.05$ in the height of the epithelium between sheep and gazelle.

Keywords: Gazelle, thyroid gland, isthmus, thyroid follicle, parathyroid cells

1. Introduction

The *Gazella subgutturosa* or as commonly Known as Goitered Gazelle is one of the important animals, it belongs to Bovidae family, Antilopinae subfamily, Genus Gazelle and Species subgutturosa. The Gazelle is a wide spread in Asia including Iraq and it found in desert and semi-desert habits. [$1,2$]

The Awasi sheep or *Ovis aries* it’s a very important ruminant and the dominant type in Iraq for meat, milk and wool, belongs to the family Bovidae, subfamily Caprine, Genus Ovis. [$3,4$]

The thyroid gland is one of the most important endocrine glands which secretes thyroglobulin hormones, thyroxin, triiodothyronine and Calcitonin hormones [$5$]. These hormones plays a central role in regulation of metabolic activities of the body and fetus development in mammals [$6,7,8$] regulation of nutrient, calcium absorption and calorigenesis [$9$] and normal reproductive function, milk and fibers production of domestic animals. [$10,11$] Also thyroid gland and thyroid hormones play a crucial role in nervous, immune and reproductive system and other systems as well as development and functions [$12$] and in mammalian adaptation to changes in the environmental conditions [$11$].According to these functions and others, a lot of studies had been done on this gland of different species of mammals despite of this fact, gazelle and sheep thyroid gland received little attention. Therefore the present investigation was aimed to: Throw a spot of light on the histological structure of the thyroid gland in indigenous gazelle (*Gazella subgutturosa*) and sheep (*Ovis aries*) and to compare between them.

2. Materials and Methods

Eight indigenous healthy adult Gazelle and Sheep (4 males Gazelle and 4 males sheep) were used in this study. The gazelle was collected from Baghdad governorate, AL-Madaen reservoir, while sheep obtained from AL-Shuala abattoir. Gazelle and sheep were humanly sacrificed and thyroid gland was dissected out following exposure then washed with distilled water, the specimens of the thyroid tissues from the...
cranial, middle and caudal part of the right, left lobes and the isthmus of the thyroid gland of gazelle and sheep were fixed at 10% neutral buffered formalin, dehydrated in a graded series of alcohol, cleared in xylene then embedded in paraffin wax. The blocks were sectioned at 5-6 μm thickness of slice using a rotary microtome. Paraffin sections were stained with haematoxylin and eosin and for histochemical studies PAS and Masson trichrome [13]. The sections were studied using Olympus light microscope with digital camera USB which connected with the computer slides and attachment at different magnification (4x,10x and40x) and the images were captured on a computer. An ocular micrometer, calibrated with a Fiji win 32 software, stage micrometer, was used for histometrical examination which include the diameter of different size follicles (small, medium and large sized follicles in μm [14]. The height follicular cells (μm) were determined for small, medium and large sized follicles. By using the Statistical Analysis System- SAS (2012) program the data were expressed as mean ± standard errors (SE) and p-value < 0.05 was considered statistically significance.

3. Results and discussion

The general histological structure of the thyroid gland in gazelle and sheep was similar and revealed a common pattern of the mammalian thyroid gland. 

This study revealed that the thyroid gland in two studied animals was covered by a capsule composed of an outer layer of adipose tissue interpose with of collagen fibers and inner dense irregular connective tissue contains thin collagen and elastic fibers with fibroblasts (Fig.1), this finding was in line with that found in camels [6] but disagree with observed in adult goats in which the capsule was consisted of three layers [15]. The present result showed that the thyroid capsule of the two lobes in gazelle was thicker than that in sheep but with no- significant importance.

The mean capsule thickness, of the right, left lobes and isthmus for both studied animals were shown in (Table1), the statistical analysis revealed no significant difference in capsule thickness between them in both sheep and gazelle. Clear connective tissue trabeculae containing vascular elements were extended from the capsule into the glandular parenchyma dividing it into various sizes of lobules (Fig.2). Both right and left lobes of thyroid in two studied animals were consisted of an aggregation of various shapes and size follicles (Fig.3) and each one was surrounded by a basement membrane with the thin connective tissue of collagen fibers and an extensive of a network of capillaries (Fig.4), this result was in agreement with that found on donkey, goat and sheep [16-18]. Thyroid gland is an endocrine gland that secretes T3,T4 and calcitonin hormones that play a key role in metabolism, thermoregulation of the body and quickly respond to the environmental condition changes, this action of thyroid allow the animals to adapt to the variation in environmental temperatures [19].

Various shapes of follicles were found; oval, round, irregular elongated and polygonal follicles and three sized of follicles were identified; small, medium and large sized follicles (Fig.3,5).There was a uniformly distribution of different size follicles through the sections, this result was similar to that reported in cattle [20], but in contrast with that found in camel in which the follicles were only two sizes, large and small sized follicles [21].

Three main sized of follicles were identified; the large, medium and small-sized follicles each thyroid lobe of gazelle and sheep, similar observation in buffalo [22] and in cat [23]. No significant important difference was found between gazelle and sheep thyroid in their follicles diameters.

The microscopic examination of sheep and the gazelle thyroid gland showed a homogenous distribution of variable sized follicles in thyroid sections, as observed in sheep [18], but at variance with who found that the small follicles were predominantly diffused at the periphery while the large follicles were found at the center [22].

The isthmus appeared glandular in both studied animals, showed different shapes and size follicles, fewer follicles density, more regular, large quantities of interstitial connective tissue than other part of the gland and they appeared mostly as oval and round shape follicles, each follicle surrounded by a numerous capillaries (Fig.5), this was in confirmation with the observation reported in bovine [24] and in Nubian goat [25], but in contrast with that found in fibrous isthmus and or entirely absent in sheep [26]. There was no significant variation in histological structure of thyroid gland between Gazelle and sheep, but the statistical analysis revealed no significant difference in a thickness of capsule, diameters of small, medium and large size follicles between left and right lobes in gazelle and sheep at p<0.05 but there was a significant difference between right and left lobes with isthmus (Table 1). Also there were no significant difference in a thickness of capsule, diameters of various size follicles in the left, right lobes and isthmus between Gazelle and sheep (Table 2).

The lining epithelium of gazelle thyroid follicles ranged from simple cuboidal epithelium to very few simple squamous epithelium (Fig. 6,7), while in Sheep, thyroid follicles were lined by simple cuboidal epithelium (Fig. 8), simple squamous epithelium (Fig.9) and occasionally simple columnar epithelium this may be indicating an inactive or resting stage, this observation was reported in goat [17, 27], there was no significant difference in the height follicular cells between the right, left lobes and the isthmus in both sheep and gazelle (Table1). Statistically, there were a significant difference in the height of follicular cells in both right, left lobe and the isthmus between sheep and gazelle at p <0.05 (Table 2), this finding indicate that the thyroid gazelle was in the active stage. The very active follicles are lined by simple cuboidal or simple columnar epithelium, while the resting or inactive follicles are lined by very low simple cuboidal to squamous epithelium [28].

When the gland is inactive, the follicles are large and the follicular cells are flat but when the gland is active, the follicles are small, the follicular cells are simple cuboidal or simple columnar and the edges of the colloid is scalloped forming many small reabsorptive lacunae [28,29].

The myoepithelial cells were found interposed between the follicular cells and the basement membrane (Fig. 6), similar finding was observed in buffalo [22].

Also this study showed a presence of para follicular cells or C-cells were appeared as large oval to round cells, exhibited more light stained cytoplasm with dense nuclei and they observed as a single or in a group of two or three cells, they positioned interfollicular cells (between the follicular cells) and some were located in space (between thyroid follicle) (Fig.10). In the isthmus the C- cells are also present as in thyroid lobes of both gazelle and sheep, this finding was similar to that found in buffalo [30] but was different from that found in dog and cats in which these cells were numerous [31], while these cells were missing in camel [14], the role of para follicular cells through calcitonin secretion which act in calcium metabolism [32].
A variable amount of eosinophilic colloid material was found in the intra follicular spaces of most sizes and shapes follicles in both studied animals. These material were observed as a homogenous substance (Fig.6), some follicles contains highly visible peripheral empty vacuoles appeared as a clear area and some follicles devoid of colloid (Fig.11). In both studied animals the colloid substance were PAS positive (Fig.12), due to its rich in glycoprotein substance \[33,34\], similar observation in sheep [21]. Some follicles contained faintly stained colloid substance which react weakly with PAS. Some follicles contains peripheral colloid vacuoles which indicate to metabolic activity of follicular cells, these result was agree with that reported in cattle [20].

**Table 1:** The histological parameters of thyroid gland in indigenous gazelle and sheep

<table>
<thead>
<tr>
<th>Histological parameter (µm)</th>
<th>Right lobe Mean±SE</th>
<th>Left lobe Mean±SE</th>
<th>Isthmus Mean±SE</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of capsule</td>
<td>199.36 ± 9.51</td>
<td>200.51 ± 12.63 a</td>
<td>210.18 ± 9.49</td>
<td>15,483 NS</td>
</tr>
<tr>
<td>Diameter of Small follicle</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>7,339 *</td>
</tr>
<tr>
<td>Diameter of Medium follicle</td>
<td>59.13 ± 3.96</td>
<td>60.11 ± 4.09</td>
<td>51.24 ± 2.97</td>
<td></td>
</tr>
<tr>
<td>Diameter of Large follicle</td>
<td>76.12 ± 3.09</td>
<td>74.11 ± 2.84</td>
<td>69.19 ± 2.98</td>
<td>5.002 *</td>
</tr>
<tr>
<td>Height of epithelium</td>
<td>143.09 ± 9.72</td>
<td>118.70 ± 8.02</td>
<td>23,417 *</td>
<td></td>
</tr>
</tbody>
</table>

*\( (P<0.05) \) Significant, NS: Non-Significant.

Means having with the different letters in same raw differed significantly.

**Table 2:** The histological parameters of thyroid gland in indigenous gazelle and sheep

<table>
<thead>
<tr>
<th>Part of gland</th>
<th>Histology parameter (µm)</th>
<th>Gazelle Mean±SE</th>
<th>Sheep Mean±SE</th>
<th>T-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right lobe</td>
<td>Thickness of capsule</td>
<td>199.36 ± 9.51</td>
<td>195.11±11.56</td>
<td>11,362 NS</td>
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<tr>
<td></td>
<td>Diameter of Small follicle</td>
<td>59.13 ± 3.96</td>
<td>59.87 ± 3.72</td>
<td>2,874 NS</td>
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<td></td>
<td>Diameter of Medium follicle</td>
<td>76.12 ± 3.09</td>
<td>78.79 ± 4.61</td>
<td>5,271 NS</td>
</tr>
<tr>
<td></td>
<td>Diameter of Large follicle</td>
<td>143.72 ± 8.37</td>
<td>150.03±11.06</td>
<td>13,712 NS</td>
</tr>
<tr>
<td></td>
<td>Height of epithelium</td>
<td>28.53 ± 2.39</td>
<td>17.83 ± 1.06</td>
<td>6,883 *</td>
</tr>
<tr>
<td>Left lobe</td>
<td>Thickness of capsule</td>
<td>200.51±12.63</td>
<td>199.55±11.85</td>
<td>8,467 NS</td>
</tr>
<tr>
<td></td>
<td>Diameter of Small follicle</td>
<td>60.11 ± 4.09</td>
<td>64.87 ± 3.65</td>
<td>5,774 NS</td>
</tr>
<tr>
<td></td>
<td>Diameter of Medium follicle</td>
<td>74.11 ± 2.84</td>
<td>77.68 ± 4.18</td>
<td>4,811 NS</td>
</tr>
<tr>
<td></td>
<td>Diameter of Large follicle</td>
<td>143.09 ± 9.72</td>
<td>145.86±10.41</td>
<td>8,209 NS</td>
</tr>
<tr>
<td></td>
<td>Height of epithelium</td>
<td>27.72 ± 2.41</td>
<td>17.75 ± 0.94</td>
<td>6,358 *</td>
</tr>
<tr>
<td>Isthmus</td>
<td>Thickness of capsule</td>
<td>210.18 ± 9.49</td>
<td>219.69±14.73</td>
<td>13,924 NS</td>
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<td></td>
<td>Diameter of Small follicle</td>
<td>51.24 ± 2.97</td>
<td>51.00 ± 2.42</td>
<td>3,648 NS</td>
</tr>
<tr>
<td></td>
<td>Diameter of Medium follicle</td>
<td>69.19 ± 2.98</td>
<td>70.19 ± 2.39</td>
<td>5,031 NS</td>
</tr>
<tr>
<td></td>
<td>Diameter of Large follicle</td>
<td>118.70 ± 8.02</td>
<td>125.82 ± 7.51</td>
<td>6,226 NS</td>
</tr>
<tr>
<td></td>
<td>Height of epithelium</td>
<td>25.36 ± 1.87</td>
<td>17.48 ± 1.14</td>
<td>4,072 *</td>
</tr>
</tbody>
</table>

*\( (P<0.05) \) Significant, NS: Non-Significant.
Fig 1: Histological section of gazelle thyroid gland (left lobe) showing: a- capsule, b-outer layer of capsule (adipose tissue), c- inner layer of capsule, d- thyroid follicle, e- interstitial connective tissue PAS x10

Fig 2: Histological section of sheep thyroid gland (left lobe) showing: a-thyroid lobule, b-capsule, c-trabeculae, d-thyroid follicle, PAS stain x10

Fig 3: Histological section of gazelle thyroid gland (right lobe) showing different size and shaped of thyroid follicle: a-polygonal follicle, b-oval shape follicle, c-rounded shape follicle, d- irregular shape thyroid follicle, H & E stain x10

Fig 4: Histological section of gazelle thyroid gland (right lobe) showing: a- inter follicular connective tissue (collagen fiber arrow), b-red blood cells in capillaries, c-sinusoid d-lumen of thyroid follicle, Masson tri chrome x40

Fig 5: Histological section of isthmus in thyroid gazelle showing: a-capsule, b-interstitial connective tissue, c-large sized follicle, d-medium sized follicle, e- small sized follicle, f- colloid substance, (arrows), H&E stain x 10

Fig 6: Histological section of gazelle thyroid gland (left lobe) showing: a-high simple columnar epithelium, b- RBC inside the capillaries, c- nucleus of myoepithelial cells, H & E x4

Fig 7: Histological section in gazelle thyroid gland (right lobe) showing: a-simple cuboidal epithelium, b-nucleus of cell, c-colloid material, d-Interstitial connective tissue, H and E x40

Fig 8: Histological section of sheep thyroid gland (left lobe) showing: a-thyroid follicle, b- simple cuboidal epithelium, c- para follicular cells, d- empty thyroid follicle, e- adipose tissue of capsule, H & E x10
4. Conclusion
The current finding was the first recorded in indigenous sheep and gazelle. The histological structure of the thyroid gland in both, gazelle and sheep was similar to that pattern found in most domesticated animals. The isthmus of the thyroid gland appear as glandular tissue and has a similar structure to the other part of thyroid gland (thyroid lobes) observed as a glandular tissue in both sheep and gazelle. The colloid substance filling the thyroid follicles was reacted positively with PAS. No accessory thyroid lobes was observed in both sheep and gazelle.

5. Acknowledgment
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6. References


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