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Original Research Article

MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON PARATHYROID GLAND OF ADULT MALE GOAT *(CAPRA HIRCUS)*

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Abstract: The parathyroid glands are essential endocrine glands as they produce hormones that maintain calcium within the normal level in blood through secretion of parathormone. The removal of parathyroid glands leads to fatal levels of hypocalcemia. The number of parathyroid glands is species specific. The current investigation was performed on 20 healthy freshly slaughtered adult male goats. Fifteen specimens were immediately fixed in 10% buffered neutral formalin, then processed for histological and immunohistochemical examination. Other 5 specimens were handled for transmission electron microscope. The present work was conducted to study the morphological and histological characteristics of parathyroid glands of goat. Parathyroid glands comprised of two pairs, each pair formed from external and internal glands. External parathyroid gland was rounded or oval in shape. Its location was varied in the same animal where it may be located cranial to thyroid gland or beside the submandibular salivary gland. Internal parathyroid gland was embedded inside thyroid tissue. It appeared as pale rounded area at the end of cranial part of the right and left thyroid lobe. The histological findings demonstrated that, each parathyroid gland is surrounded by a thin capsule of dense irregular connective tissue. The glandular parenchyma is divided by short thick septa into ill distinct compartments. Each compartment had numerous numbers of chief cells. The active chief cells were polygonal in shape with oval nuclei. Immuno-histochemical findings revealed that they are positively reacted against chromogranin antibody. Electron microscope revealed that the cytoplasm have abundant mitochondria, rough endoplasmic reticulum, evenly distributed golgi apparatus and numerous secretory granules. On the contrary, the inactive chief cells have more vacuolated cytoplasm which contains less cell organelles.

Key words: parathyroid gland; chief cells; ultrastructure; chromogranin

Introduction

Goats play an important role in the food and nutritional security of the rural poor people (1). The economic returns of goat are rising every year in developed countries (2).

Parathyroid glands are the last real organ recognized in humans (3). They are essential for life in most animals and human (4). They assume an extraordinary part in the direction of the calcium metabolism inside the body (5,6).

The glands' parenchyma consists of densely packed cellular structure that are arranged in

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various forms including follicles or anastomosing cords and nests of polygonal cells. They are isolated by single or double layered collagen and reticular fibers with fibrocytes. The parathyroid cells are classified according to the presence of glycogen and lipid droplets into darkly stained active secreting and lightly stained inactive resting cells (7).

The active chief cells are polygonal in shape. They have light, spherical or oval indented nuclei. The inactive chief cells are polygonal outlines and have less cell organelles than the active cells. They have more vacuolated cytoplasm and central nucleus (8). In rat the staining properties of the cytoplasm reflects different functional status of the chief cells (9).

In mouse, chief cells have some morphologic changes as indicated by various phases of the secretory cycle (10). In *Rattus rattus*, the chief cells are oval in shape (11), the active stage is polygonal in shape, the plasma membranes of adjacent active chief cells have complex interdigitations, while, the inactive chief cells are cuboidal and have uncomplicated interdigitations between adjacent cells (12).

Immunohistochemistry revealed that the parathyroid glands contain two major kinds of cells; chief cells and oxyphilic cells. Chief cells are considered as the functional and the most well-known cells (13). The immunocytochemical staining revealed that chief cells contain less secretory granules than other endocrine cells (14). They have densely stained diffuse fine granules containing PTH (parathyroid hormone) and Chromogranin in their cytoplasm (15). The parathyroid cells are stained focally or diffusely to chromogranin antibodies (16).

Chromogranin A is a major protein of the parathyroid glands which is co-stored and co-secreted with parathyroid hormone. Chromogranin A is dispersed essentially in endocrine and neuroendocrine cells (17). Chromogranin A and other subclasses of chromogranin have been found in most endocrine cells with secretory granules in human being (18,19) and animals (20, 21).

The present work aimed to throw more light on the morphology and histological structure of the parathyroid glands of goat and differentiate their different cell types by light and transmission electron microscopes. Also, to investigate localization of chomogranin A immunoreactive cells by immunohistochemical studies.

Material and methods

Animals and Tissue Samples

The current study was conducted on 20 healthy freshly slaughtered adult male goats "*Capra hircus*" taken from Minia El-Kamh abattoir, Sharkia Governorate, Egypt. Fifteen samples were used for the routine histological studies and five samples were used for the electron microscopical studies. The parathyroid glands were dissected from the healthy animals immediately after slaughter. The shape and color of the glands were studied immediately after being dissected.

Tissue preparation

The specimens were immediately fixed in 10% buffered neutral formalin, then dehydrated followed by clearing in Xylol. All specimens were infiltrated with soft melted paraffin in the hot air oven and were embedded in hard paraffin. Using rotary microtome, sections of 5-7 μm thickness were cut. The paraffin sections were stained with Harris's Hematoxylin and Eosin (H & E) stain as a routine staining method to demonstrate the general histological structure. Crossmon's trichrome stain was used for the collagen and muscle fibres (22).

Tissue preparation for immunohistochemistry

Avidin biotin peroxidase method was used. The sections used were mounted on charged slides, then deparaffinized by xylene, and re-hydrated in graded ethanol then washed in phosphate buffer saline (PBS) at pH 7.2 for 5 minutes. To block endogenous peroxidase activity, the sections were immersed in 0.3% hydrogen peroxide in water. The sections were then washed in distilled water several times and then washed in PBS. The sections were then washed in 10% normal rabbit serum (blocking reagent) in a humid chamber for 30 min to reduce non specific binding of immunoglo-

bulins. The sections were incubated with antisera containing the specific primary antibody (chromogranin rabbit monoclonal antibody RM-9112-R7, Thermo scientific, Thermo Fisher Scientific). The sections were then incubated in a humidified chamber at room temperature overnight. Excess reagent was thrown off and the slides were washed in four changes of PBS, 5 min each. Then, the sections were incubated at horse reddish peroxidase polymer for 15 min at room temperature. The slides were rewashed in four changes of PBS, 5 min each. Diaminobenzidine (DAB) was used as chromogen and sections were incubated for 2-4 min at room temperature. Sections were washed in distilled water for 5 min, then were counter stained with Mayer's haematoxylin, dehydrated in ascending grades of alcohol, cleared in xylene and then mounted in Canada Balsam (22).

All the stained sections were examined with a standard light microscope (Olympus BX 21, Objective X 4,10,40,100 and Ocular X 10) and photographed by a digital Dsc-W 800 super steady cyper shot camera (Sony-Japan) at the Department of Histology and Cytology, Zagazig University).

Transmission electron microscopic (TEM) studies

Pieces of tissue about 1 mm^3 were fixed immediately in a buffered glutaraldehyde - formaldehyde fixative (GA/FA) that consists of (1% glutaraldehyde and 10 % formaldehyde in 0.1 M Phosphate buffer at pH 7.4 at 4ºC**)** for 2 h, then fixed in 1% osmium tetroxide. After rinsing in the osmium tetroxide, they were dehydrated in ascending grades of ethanol ending with propylene oxide, and embedded in Epoxy resin. Semi thin sections were cut on a MT2 Sorvall microtome and stained with toluidine blue. Ultrathin sections were cut on a RMC MT6000_ XL ultramicrotome, mounted on copper grids and stained with uranyl acetate and lead citrate. The ready ultrathin sections were examined and photographed at Faculty of Agriculture, Mansoura University by a JOEL electron microscope (JEM 1200 EX II) operating at 80KV (23).

Results

Morphology of parathyroid glands

Parathyroid glands right and left lobes consisted of two pairs; external pair (right and left) and internal pair (right and left). External parathyroid gland was rounded or oval in shape. It had pale reddish color and consisted of right and left lobes. The gland was located anterior to the cranial pole of thyroid gland. External parathyroid gland was embedded inside the remnants of thymic tissue. Internal parathyroid gland consisted of right and left parts. It was embedded inside thyroid tissue. Internal parathyroid gland was very small to be seen in fresh samples where it appeared after making serial segments in the formalinized thyroid lobes. It appeared as pale rounded area at the end of cranial part of the right and left thyroid lobe (Figure 1).

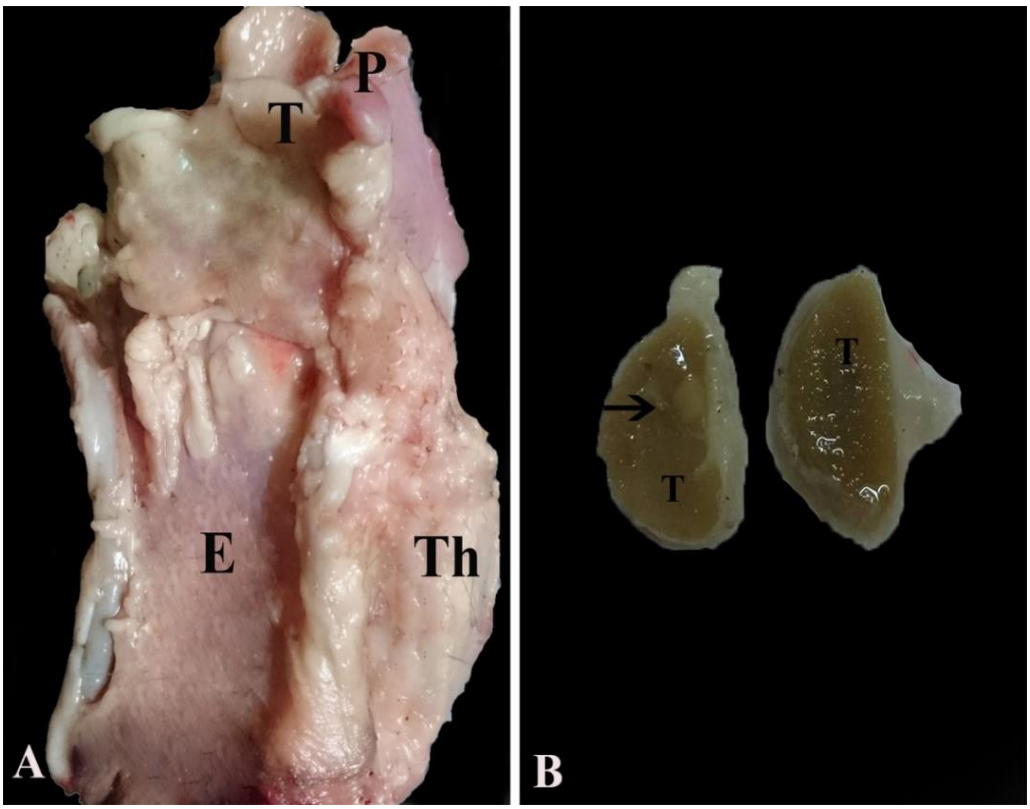

Figure 1: Photomacrograph of goat parathyroid glands. (A) showing "T" remnant of thymus gland, "P" external parathyroid, "Th" thyroid gland and "E" esophagus. (B) showing the less observed internal parathyroid "arrow" embeded within thyroid gland lobe "T" after its separation at the cranial end

Light microscopy

External parathyroid gland was surrounded by thin connective capsule, which consisted of connective tissue fibers and cells. The capsule arises from which irregular short and thick septa dividing the parenchyma into incomplete compartments (Figure 2A). These compartments had densely packed cellular structure of only one cell type of chief cells, which present near the blood capillaries (Figure 2B). In goat parathyroid glands, no oxyphil cells were observed. Chief cells were abundant and evenly distributed inside the lobules. They were separated by interstitial connective tissue including collagen fibers and blood capillaries (Figure 2C). In semi thin section, many vacuoles of different sizes were observed and the chief cells were distributed all over the parenchyma beside the blood capillaries (Figure 2D).

Internal parathyroid gland was embedded inside thyroid tissue (Figure 3A) and separated from them by connective tissue septa containing collagen fibers (Figure 3B).The chief cells were classified into two types according to the staining affinity; darkly stained cells and lightly stained cells. Chief cells were round or oval in shape with pale acidophilic cytoplasm. It had rounded vesicular and centrally located nuclei (Figure 3C). Immunohistochemical observation revealed that the chromogranin immunoreactive cells gave a strong positive reaction with anti-chromogranin, the expression was strongly observed in the cytoplasm of chief cells (Figure 3D).

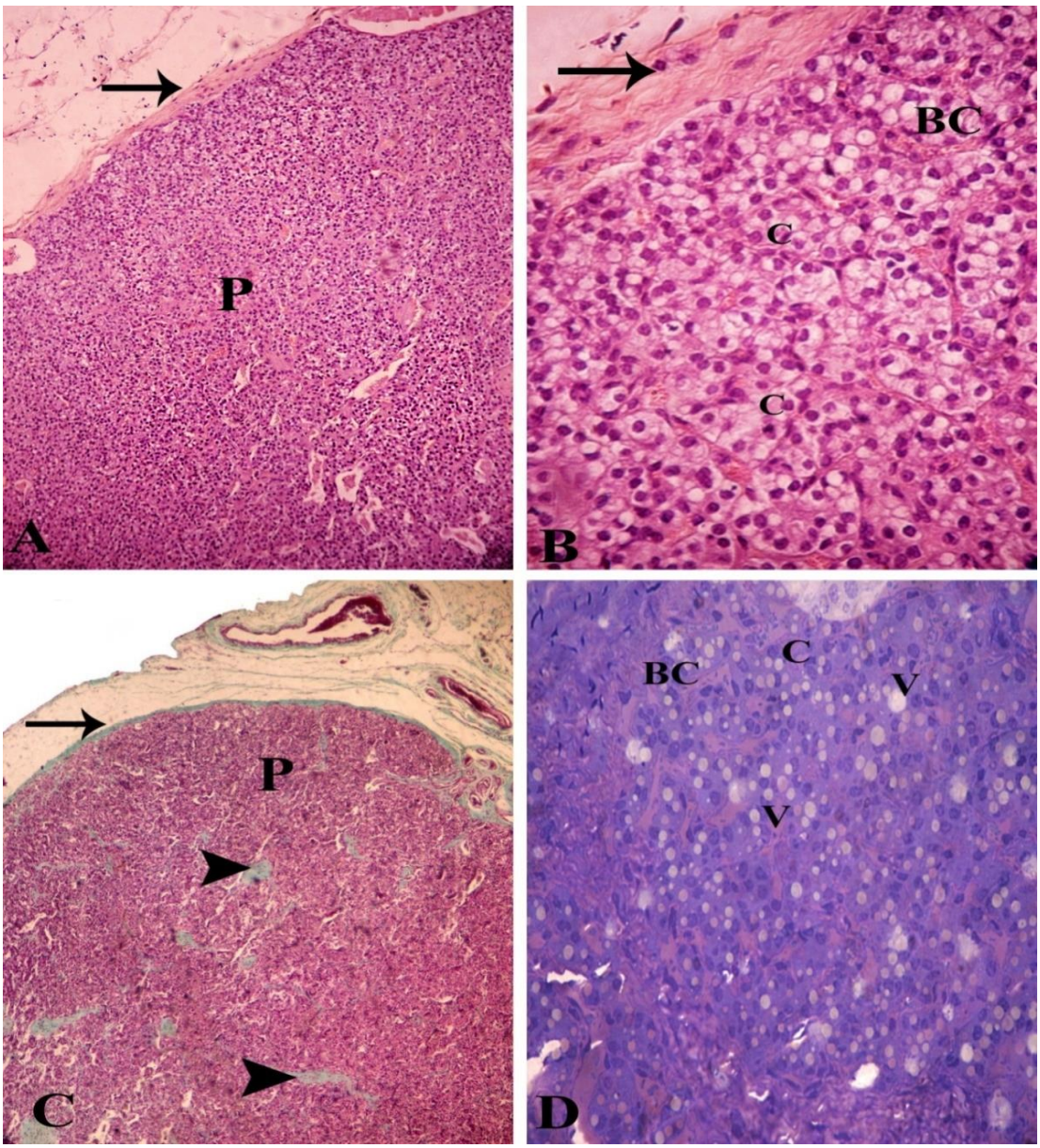

Figure 2: Photomicrograph of the goat's external parathyroid gland showed (A) the glands' connective tissue capsule "arrow"and the parenchyma "P". (B) the glands' capsule "arrow", the parenchyma consists of chief cells "C" which present near to blood capillary "BC". (C) showed the glands capsule had collagen fiber " arrow" the parenchyma "P" consists of chief cells which separated by interstitial connective tissue including collagen fibers (arrow heads) (D) semi thin section of external parathyroid showed many vacuoles "V" of different sizes and the chief cells (C) are distributed all over the parenchyma beside the blood capillaries (BC). (A&B): H&E stain, (C) : Crossmon's trichrome, (D): Toluidine blue x. 100 in (A) x. 50 in (C) and x. 400in (B,D)

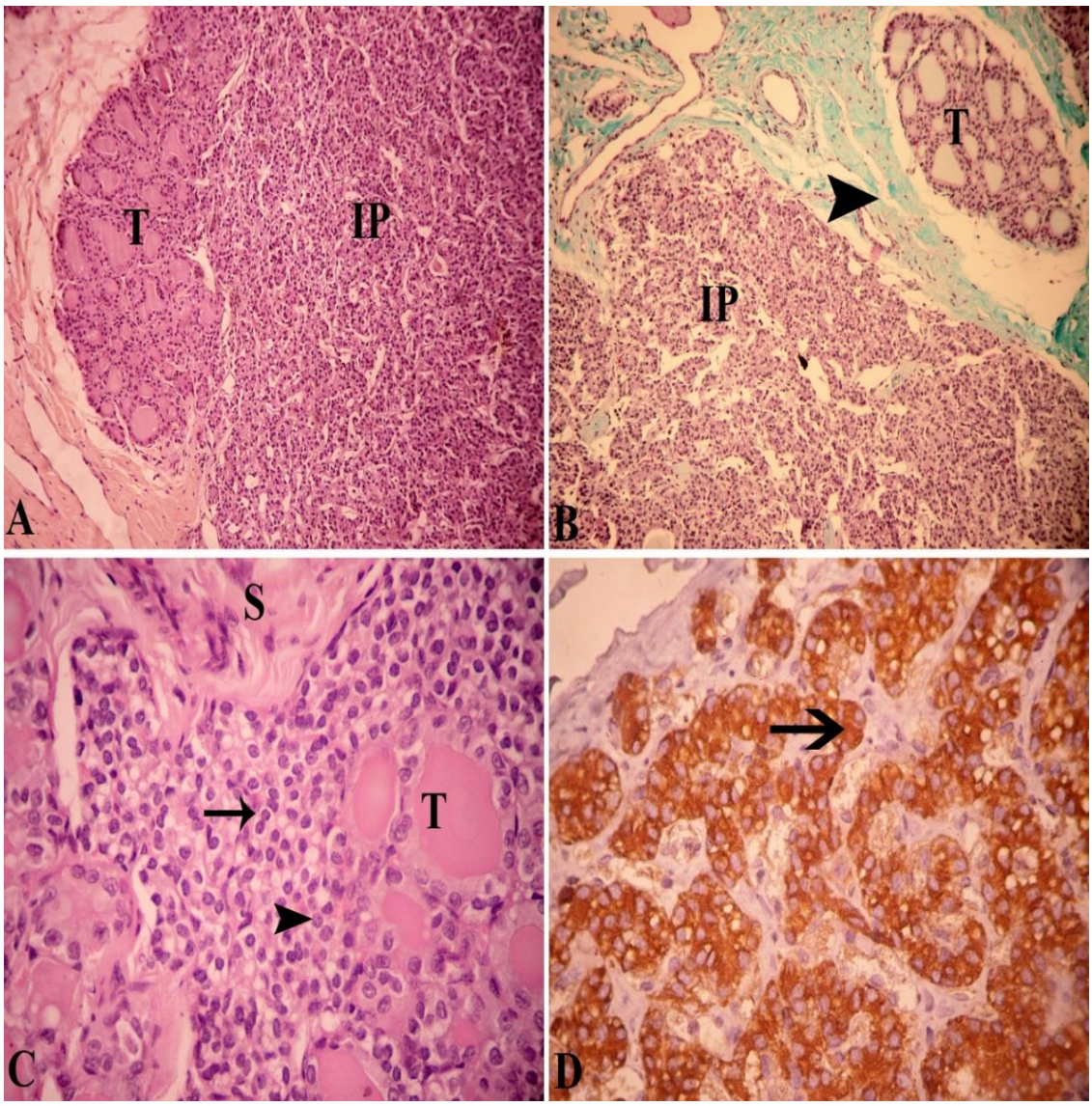

Figure 3: Photomicrograph of the goat's internal parathyroid gland showed (A) the internal parathyroid gland "IP" is embedded inside thyroid tissue "T". (B) the internal parathyroid gland "IP" is embedded inside thyroid tissue "T" and separated by connective tissue septa containing collagen fibers "arrow head". (C) internal parathyroid gland showed the gland is surrounded by connective tissue septa "S" The gland's parenchyma is consisted of light "arrow head" and dark chief cells "arrow". (D) External parathyroid gland showed the immune-reactivity of chromogranin A. The chief cells are positively reacted against chromogranin antibody "black arrow" Stain: (A&C): H&E, (B) : Crossmon'strichrome, (D): IHC staining x. 100 in (A,B) and x. 400in (C,D)

Ultrastructure

The active chief cells were polygonal in shape with oval or round nuclei. Their nuclei were large and light with peripheral heterochromatin spots. The nuclear envelope was indented with many nuclear pores. Their cytoplasm had abundant mitochondria, randomly distributed rough endoplasmic reticulum, evenly distributed golgi apparatus and numerous dense secretory granules. The inactive chief cells had polygonal outlines with more vacuolated cytoplasm which contain less cell organelles and more central nucleus (Figure 4). The inactive chief cells had rounded nucleus and moderate number of mitochondria. They were located beside blood capillary that contain red blood corpuscle (Figure 5).

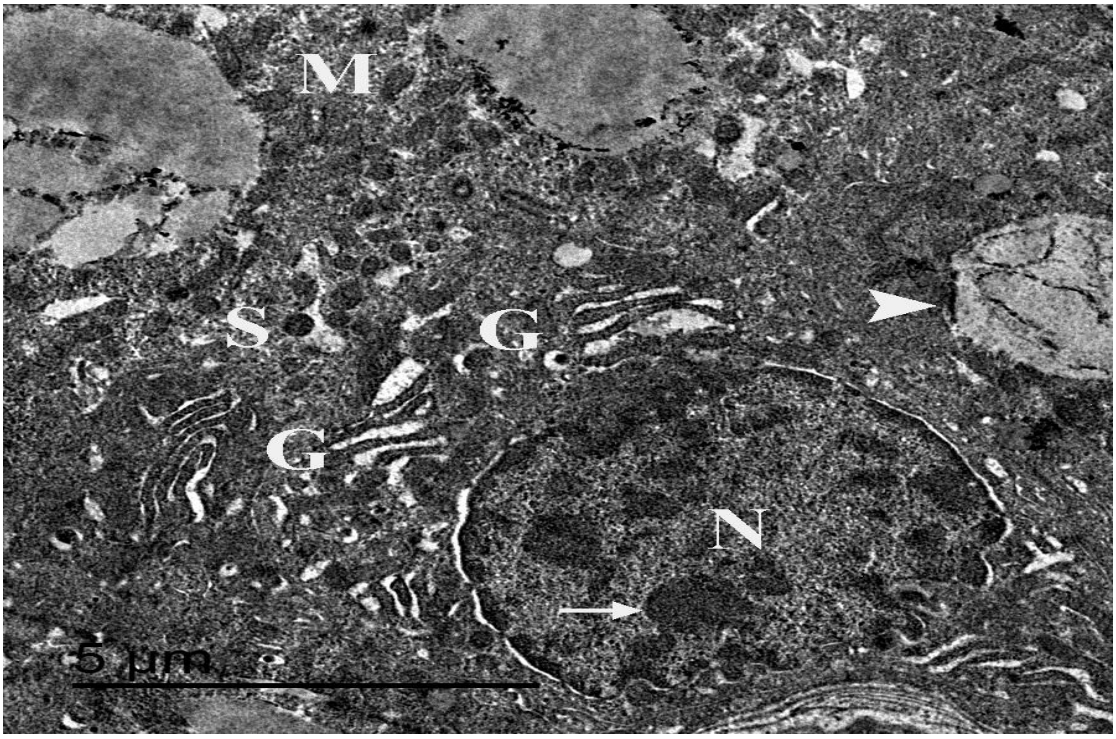

Figure 4: Transmission electron micrograph of goat's parathyroid gland. Ultrathin section showed that the active chief cells have rounded nucleus "N". Their nuclei are large with electron dense heterochromatin spots "arrow". The cytoplasm of these cells have abundant mitochondria "M", evenly distributed golgi apparatus "G" and several secretory granules "S". The inactive chief cells have more vacuolated cytoplasm "arrow head"

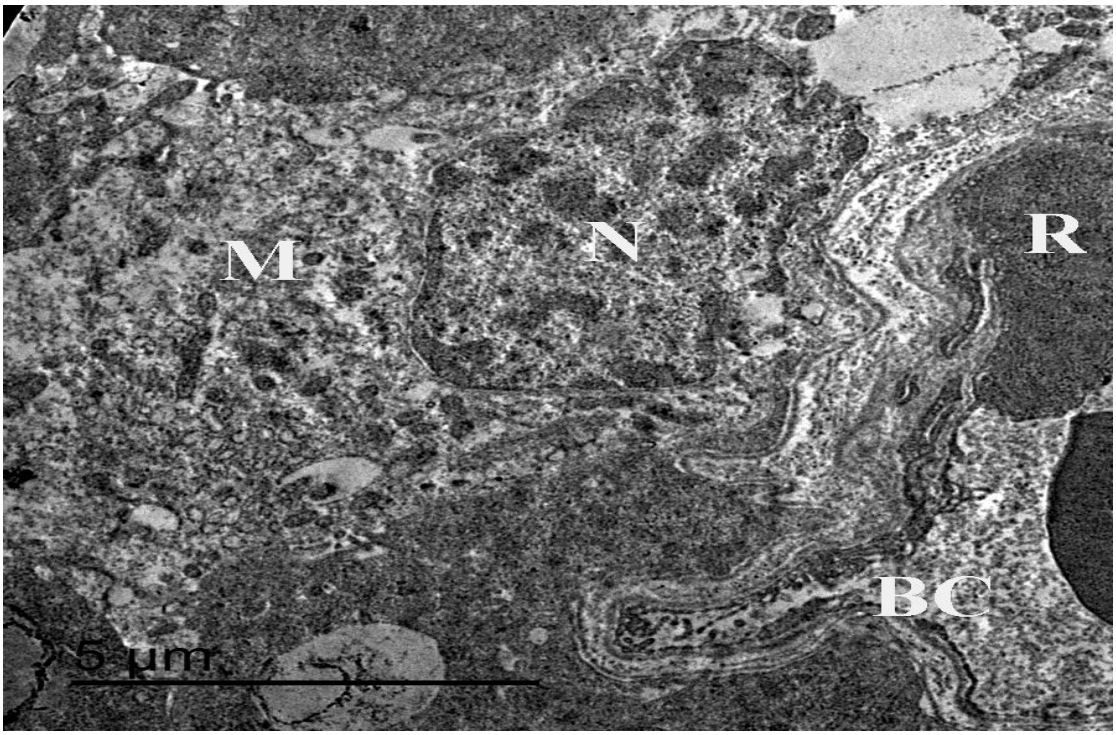

Figure 5: Transmission electron micrograph of goat's parathyroid gland. Ultrathin section showed that the inactive chief cells have rounded nucleus "N". The cytoplasm of these cells has moderate number of mitochondria "M". They are present near blood capillary "BC" containing red blood corpuscle "R"

Discussion

The parathyroid glands vary in number and location in several vertebrates (24). The parathyroid glands are small pale brown flattened oval disk (12). In goat, the parathyroid glands have two embryological origins. The external parathyroid and the internal parathyroid originate from III and IV pharyngeal pouches respectively. It is organized bilaterally. The external parathyroid gland lies ventral to the wing of the atlas. However, the internal parathyroid gland is buried in the medial portion of the cranial half of the thyroid gland (25), our study confirmed the results of above mentioned authors. Besides, in the present work, the location of the external parathyroid gland varied in the same animal for example; it may be located cranial to thyroid gland embedded within remnant of thymic tissue, also it may situated beside submandibular salivary gland.

The present study revealed that the parathyroid glands of goat consisted of external and internal pairs. The external and internal parathyroid glands have been recorded in large ruminants; buffalo (26) and camel (8). External parathyroid gland of camel was located anterior to the cranial pole of the right and left lobe of thyroid gland or 9-16cm dorsal to the dorsal pole of the thyroid gland (8). In our study the internal parathyroid gland was buried inside thyroid lobe. t couldn't be seen in fresh samples. However, it appeared after making serial segments in the formalinized thyroid lobes rather than in camel the internal parathyroid was not circumscribed and cannot be seen by naked eye (5).

The current work indicated that external parathyroid gland was surrounded by thin connective capsule which sent irregular short and thick septa dividing the parenchyma into incomplete compartments. Similar results were reported in buffalo (26). In goat the parathyroid compartments had densely packed cellular structure of only one cell type called chief cell. The same results were confirmed in rats (27). However, the parathyroid gland of horse consists of two types of cells including chief cells and oxyphilic cells (28) which were not detected in goat's parathyroid parenchyma under investigation in current study. Also these cells are absent from the parathyroid glands of the rat and many species of lower animals (11). Its number increases by aging (29). While in human (30) and in camel (8) the oxyphil cells are larger than chief cells. Their cytoplasm is filled with numerous large mitochondria as a need for energy production.

Chief cells were classified into two types; darkly and lightly stained cells. They undergo morphologic changes corresponding to different stages of the secretory cycle (10). They were round to oval in shape with pale acidophilic cytoplasm. Chief cells had round, vesicular and centrally located nuclei. Similar results were recorded in horse by Babu et al. (28) .

Parathyroid hormone (PTH) 3 and chromogranin A (CgA) represent the two major proteins of the parathyroid gland whose secretion is controlled by the concentration of extracellular Ca2+ (31). The most hypotheses for the role of CgA, is that it is a precursor protein for autocrine or paracrine peptides that modulate stimulated endocrine cell secretion (31,32). Using ABC immunohistocheistry; our results confirmed previously mentioned findings through detection of positively reacted chief cells with chromogranin antibody (33).

The active chief cells were polygonal in shape with oval nuclei. Their nuclei were large and light with peripheral heterochromatin spots. The cytoplasm of these cells have abundant mitochondria, randomly distributed rough endoplasmic reticulum, evenly distributed golgi apparatus and numerous dense secretory granules which indicate active protein synthesis. On the contrary, the inactive chief cells have more vacuolated cytoplasm which contains less cell organelles. Similar results were recorded in previous studies in camel (8) and (12). Chen et al. (12) added that these vacuoles may be due to numerous lipid droplets, lysosomes or glycogen particles

Conclusion

Parathyroid glands of adult goat consisted of external and internal pairs. The location of the

external parathyroid gland varied in the same animal. The internal parathyroid gland was buried in the thyroid gland. It had only one cell type called chief cells without oxyphil cells. Two types of chief cells were observed; active and inactive suggesting different stages of activity according to calcium level in the blood. These cells were positively reacted to chromogranin antibody which has an important role in its endocrine function.

Conflict of interest

There is no conflict of interest.

References

1. Kumar S, Rama Rao CA, Kareemulla K, Venkateswarlu B. Role of goats in livelihood security of rural poor in the less favoured environments. Indian J Agric Econ 2010; 65 (4): 1–22.
2. Sahlu T, Goetsch AL. A foresight on goat research. Small Rumin Res 2005; 60 (1-2): 7–12.
3. Modarai B, Sawyer A, Ellis H. The glands of Owen. J R Soc Med 2004; 97 (10): 494–5.
4. Bienzle D., Jacobs RM, Lumsden JH. Relationship of serum total calcium to serum albumen in dogs, cats, horses and cattle. Can Vet J 1993; 34 (6): 360–4.
5. Metwally MA, Attia HF. Anatomical and Histological Studies on the Parathyroid gland of the Camel (Camelus Dromadrius). In the International Scientific Conference on Camel. Saudi Arabia 2006 May, pp: 1484–94.
6. Ramasamy I. Recent advances in physiological calcium homeostasis. Clin Chem Lab Med 2006; 44 (3): 237–73.
7. Ross MH, Romrell LJ, Kaye GI. Histology.3rd ed.1995 Williams and Wilkins Sydney. Tokyo. PP: 113.
8. Al-Ramadan SY, Ali AM, Al-Zghoul MB, Althnian TA, Alzayer MA. Macro-and Micro-Morphological Studies on the Parathyroid Glands of Dromedary Camel. Pak Vet J 2016; 37: 59–64.
9. Capen CC, De Lellis RA, Yarrington JT. Endocrine system. In: Handbook of Toxicologic Pathology, Vol 2 (Haschek WM, Rousseaux CG, Wallig MA, eds). Academic Press 2002; New York, 681–783.
10. Chen H, Emura S, Yao XF, Shoumura S. Morphological study of the parathyroid gland and thyroid C cell in senescence-accelerated mouse (SAMP6), a murine model for senile osteoporosis. Tissue Cell 2004; 36 (6): 409–15.
11. Emura S, Tamada A, Hayakawa D, Chen H, Shoumura S. Electron Microscopic Study of the Parathyroid Gland of Rattus rattus. Okajimas Folia Anat Jpn 1999; 76 (1): 71–80.
12. Chen H, Senda T, Emura S, Kubo K. An update on the structure of the parathyroid gland. Open Anat J 2013; 5 (1): 1–9.
13. Tomita T. Immunocytochemical staining patterns for parathyroid hormone and chromogranin in parathyroid hyperplasia, adenoma and carcinoma. Endocr Pathol 1999; 10 (2): 145–56.
14. Lu M, Kjellin H, Fotouhi O, Lee L, Nilsson I, Höög A, Lehtiö J, Larsson C. Molecular Differences Between Oxyphilic Cell and Chief Cell Parathyroid Adenoma. In endocrine reviews 2014, (Vol. 35, No. 3). 2055 L ST NW, suite 600, washington, dc 20036 USA: endocrine Soc.
15. Gonzalez Campos A. Isolation of human parathyroid cell type as a tool for investigating the mechanisms of human primary aldosteronism. Ph.D. thesis, Universita Degli Studi Di Padova, 2014.
16. Oka T, Onoe K, Matsumiya K, Takaha M, Yoshioka T, Koide T, Sonoda T, Kimura M, Sakurai M. Light microscopical immunohistochemical study on parathyroid adenoma in primary hyperparathyroidism. Urol Int 1994; 52: 121– 5.
17. Cohn DV, Fasciotto BH, Zhang JX, Gorr SU. The chemistry and biology of chromogranin A (secretory protein-I of the parathyroid and other endocrine glands. In: The Parathyroids (Bilezikian, J. P., Levine, M., Marcus, R., eds.), 1994 pp. 107–19. Raven Press, New York, NY.
18. Cotesta D, Caliumi C, Alò P, Petramala L, Reale M G, Masciangelo R, Signore A, Cianci R, D'Erasmo E, and Letizia C. High plasma levels of human chromogranin A and adrenomedullin in patients with pheochromocytoma. Tumori 2005; 91 (1): 53–8.
19. Welin S, Stridsberg M, Cunningham J, Granberg D, Skogseid B, Oberg K, Eriksson B, Janson ET. Elevated plasma chromogranin A is the first indication of recurrence in radically operated midgut carcinoid tumors. Neuroendocrinol 2009; 89 (3): 302–7.
20. Escribano D, Soler L, Gutie´rrez AM, Martı´nez-Subiela S, Cero´n JJ. Measurement of chromogranin A in porcine saliva: validation of a time-resolved immunofluorometric assay and evaluation of its application as a marker of acute stress. Anim 2012; 7 (4): 640–7.

21. Colombo B, Curnis F, Foglieni C, Monno A, Arrigoni G, Corti A. Chromogranin A expression in neoplastic cells affects tumor growth and morphogenesis in mouse models. Cancer Res 2002; 62 (3): 941–6.

22. Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier Health Sciences; 2008.

23. Glauert AM, Lewis PR. Biological specimen preparation for transmission electron microscopy. 1998; Volume 17, Portland press; London.

24. Manley NR. Embryology of the Parathyroid Glands. In Hypoparathyroidism 2015; (pp. 11-18). Springer, Milano.

25. Seethala RR, Virji MA, Ogilvie JB. Pathology of the parathyroid glands. In: Barnes L, 3rd ed. surgical pathology of the head and neck, vol. III. New York, NY: Informa Health Care 2009; 1429–72.

26. Hussin AM, Al-taay MM. Histological study of the thyroid and parathyroid glands in Iraqi buffalo 'bubalus bubalis' with referring to the seasonal changes. Bas J Vet Res 2009; 8 (1): 26–38.

27. Capen CC, Mohr U. Anatomy, histology, ultrastructure, parathyroid, rat. 2nd ed., Springer Verlag, Berlin, Heidelberg, New York, 1996; 330–33.

28. Babu A P, Dhyana VR, Rajasekhar U, Ramayya PJ. Microanatomical studies on the parathyroid glands of horse. Intl J of Sci 2016; 5 (2): 1238–42.

29. Wardle EN, Kurihara I, Saito T, Obara K, Shoji Y, Hirai M, Soma J, Sato H, Imai Y, Abe K, Yuasa S. Oxyphil cell function in secondary parathyroid hyperplasia. Nephron 1996; 73 (4): 580–6.

30. Ritter CS, Haughey BH, Miller B, Brown AJ. Differential gene expression by oxyphil and chief cells of human parathyroid glands. J Clin Endocrinol Metab 2012; 97 (8): E1499–505.

31. Cohn DV, Fasciotto BH, Reese BK, Zhang JX. Chromogranin A: a Novel Regulator of Parathyroid Gland Secretion. J Nutr 1995; 125 (suppl 7) :2015S -19S.

32. Huttner WB, Gerdes HH, Rosa P. The granin (chromogranin/secretogranin) family. Trends Biochem Sci 1991; 16 :27–30.

33. Schmid KW, Hittmair A, Ladurner D, Sandbichler P, Gasser R, Tiitsch M. Chromogranin A and B in parathyroid tissue of cases of primary hyperparathyroidism: an immunohistochemical study. Virchows Archiv A. 1991;418 (4): 295–9.