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Comparative histomorphological and immunohistochemical study of parathyroid gland in two Iraqi mammals (Weasel, *Herpestes javanicus* and long-ear hedgehog, *Hemiechinus auritus*)

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Abstract

The present study aimed to investigate the anatomy, histology, and immunohistochemistry of parathyroid gland in two Iraqi mammals (Weasel, *Herpestes javanicus* and Long-ear hedgehog, *Hemiechinus auritus*) as a comparative study. A total of (20) animal for each species were used in the present study. Animals collected were immediately anesthesia and dissected to get the parathyroid gland. Methods of Humason and Bancroft and Stevens were employed for histological techniques. Different stains were used (Hematoxylin- Eosin stain-(H & E), Periodic Acid Schiff stain-(PAS), Azan stain, and Methyl Blue stain-(MB)) for staining the histological sections. Anti-calcitonin, code140778 marker used for immune-histochemical study. Results of the present study revealed that parathyroid glands in both animals under investigation were small, oval or irregular shape glands located in attached with thyroid gland. Histologically parathyroid gland in both studied animals were surrounded by thin connective tissue capsule, which represented as an extension from thyroid gland capsule, the capsule extended inside the gland tissue to separated it into several lobules. Three types of cells were recognized in the gland tissue represented by chief cells, oxyphil cells, and water clear cells. The immunohistochemical study of parathyroid gland in both species under investigation revealed response to interaction with calcitonin, as the cells appeared in brown color giving an evidence for the immune reaction in cytoplasm and plasma membrane of the cells, but the reaction in *H. auritus* was weaker than that in *H. javanicus*.

Keywords: **Parathyroid gland, Morphology, Histology, Immunohistochemistry, Weasel, Long-Eared Hedgehog.**

Introduction

Parathyroid gland is a small oval shaped endocrine gland located in attached with thyroid gland in most vertebrates. Generally in human there are (4) parathyroid glands, while the number were (2-6) in other mammals other than human (Seethala *et al*., 2009). The glands are located at dorsal side of thyroid gland (Bloom and Fawcett, 1970; Al-Mukhtar and Al-Rawi, 2000; Chen, *et al*., 2013). Parathyroid gland originates from endoderm of pharyngeal pouches (Larsen *et al*., 2001; Sheffield, 2002; Mohebati and Shaha, 2012). The main function of parathyroid gland is secreting parathyroid hormone (PTH), which is a polypeptide formed from (84) amino acids, and it is responsible for regulation of calcium level in blood (Akerstrom *et al*., 1984; Gensure *et al*., 2005; Chen *et al*., 2013). Parathyroid gland surrounded by a thin connective tissue capsule, which is represented by an extension of thyroid gland capsule, the capsule extended inside the gland tissue as a trabiculae which separated the gland tissue into lobules (Eroschenko, 2005; Johnson, 2010). The gland architecture is formed from mass or robs of epithelial cells, which supported by reticular fibers. In most mammals the gland tissue contains two types of differentiated cells represented by the Chief or Principle cells which are the most abundant cells and responsible for the secretion of parathyroid hormone, and the Oxyphil cells which are not appeared in human embryo and their number increase with the increasing age, the function of this type is not clear yet (Bloom and Fawcett, 1970; Al-Mukhtar and Al-Rawi, 2000; Al-Abdulla, 2012). Previous studies recorded another type of cells which were Water- Clear cells, which recorded in hamster, rabbit, Opossum, and echidna (Haynes, 1995; Emura *et al*., 1990). This type of cells not found or rare in human and its appearance

related with parathyroid adenoma (Roth, 1970), they increased their number with advance age or due to changing the level of parathyroid hormone (PTH) (Liang *et al*., 2010; Chen *et al*., 2013). Kanter(1996), Oksanen(1998), and some other researcher recorded Parathyroid Syncytial cells, in human, rats and dogs. They were multinucleated giant cells, originated from chief cells, and their appearance related with age and hyperparathyroidism (Kanter, 1996; Oksanen, 1998; Meuten *et al*., 1980). Review of the previous researches revealed that few work have been done on the parathyroid glands of Iraqi mammals, and no work have been done on the species under investigation, this situation supported our suggestion for the current study. It is important to note that this study is a part of an extensive study conducted to investigate the anatomy, histology, histochemistry, immunohistochemistry, biometry, and amino acid analyses of thyroid and parathyroid glands in the above two Iraqi mammals Al-Aamery and Dauod, 2016 a&b; 2020; 2021).

Materials and Methods

A total of (40) animals (20 of each specie) were used to investigate the anatomy and histology of parathyroid glands. Animals were collected and classified according to the field guide of Iraqi wild mammals (Al-Sheikhly and Haba, 2014).Animals anesthesia and dissected to collect the specimens of parathyroid glands and fixed in (10%) formalin. Methods oh Humason (1979) and Bancroft and stevens (1986) were used for histological techniques. Different stains were used to stain the histological section (Hematoxylin- Eosin (H-E), Periodic Acid Schiff (PAS), Azan stain, and Methyl Blue stain (MB)). For immunhistochemical study rabbit polyclonal calcitonin (anti-calcitonin, code 140778) were used.

Results and Discussion

Morphological description: Results of the present study revealed that parathyroid glands in both animals under investigation appeared as a small oval, rounded or irregular shaped, located in attached with thyroid gland, either at apical-terminal or lateral-terminal position (Figure 1, 2, 3).This results agree with the results of several previous studies (Bloom and Fawcett, 1970; Ross *et al*., 1995; Junqueira and Carneiro, 2005; *inter alia*), and the similarity of the results probably due to the interaction and coordination between the thyroid and parathyroid glands in their functions. The number of parathyroid glands are differ in different mammals, in human (4) parathyroid gland were recorded in other mammals the number was (2-6) glands (Seethala *et al*., 2009). In the present study the number recorded were (2) pairs in both studied species, this results agree with the above previous recorded results.

Histological structure: Histologically results showed that parathyroid glands in both animals under investigation embedded within thyroid gland tissue and surrounded by a thin connective tissue capsule, which formed from collagen fibers, elastic fibers, and few smooth muscle fibers, in addition to the blood vessels and nerve fibers, the capsule represented an extension from thyroid gland capsule surrounded the parathyroid gland and separated it from thyroid gland (Figure 4, 5, 6). The connective tissues of parathyroid gland capsule in both studied species extend inside the tissue of the glands as trabeculae to separate it into small lobules (Figure7). Gland architecture appeared as a mass or robs of cells separated by trabeculae. In *H. auritus* the tissue formed (2-4) oval or round lobules (Figure 3). This result revealed an agreement with previous results recorded by (Bloom and Fawcett, 1970; Eroschenko, 2005; Kunnik *et al*., 2008; Johnson, 2010; *inter alia*). Results of this study recognized three types of differentiated cells within the gland tissue of both species under investigation represented by chief (principal cells), oxyphil cells, and water-clear cells (Figures 8, 9, 10, 11). Review of the previous studies stated that there were some differences in the types of cells in parathyroid gland, and gave them different names, Munger and Roth, (1963), stated that there was only one type of cell in Virginia deer represented by a chief cells, and in human there were chief cells and oxyphil cells. Shoumura, *et al*., (1993) also found in hamster only one type of cells represented by chief cells. On the other hand several workers recognized two types of cells in *Camellus dromadirus* (Metwally and Attia, 2006); in brown bat recorded two types (chief cells and water-clear cells (Kwiecinki *et al*., 1987). Meuten *et al*., (1987) recognized two types named them, chief cells and multisyncytial cells, and in Iraqi buffalo there were four types of cells represented by light principle cells, dark principle cells, syncytial cells, and water-clear cells (Hussain and Al-Taay 2009). Results of the present study were in consistent with these results. The agreement may be due to the fact that parathyroid glands of mammals are analogous structure, while the differences in the names of cells perhaps related with the concentration of the secretion.

Immunohistochemical study: Results of the present study by using rabbit poly clonal calcitonin (Anti-calcitonin receptor-antibody “ab140778” for the

parathyroid gland in *H. javanicus* and *H. auritus* revealed clear response for the immunohistochemical reaction, as the cells of the gland (cytoplasm and plasma membrane of chief cells) (Figure 12, 13, 14, 15, 16), but the reaction was more clear in *H. javanicus*. It is important to note that there were no differences in the reaction in male and female. Results of the previous studies revealed clear differences in the immunohistochemical reaction of parathyroid gland of different animals. This differences suggested that the responses depend on the type of cells reacted and the hormone secreted (Okada *et al*., 1995; Kitazawa *et al*., 2002; *Inter alia*). It is important to note that we didn't find previous works dealt with the parathyroid gland of the animals under investigation from the Iraqi environment to compare our results with them.

Figure (1): Section in thyroid gland of *H. javanicus*, shows the terminal position of parathyroid gland (Pa), (Azan stain) (100X.

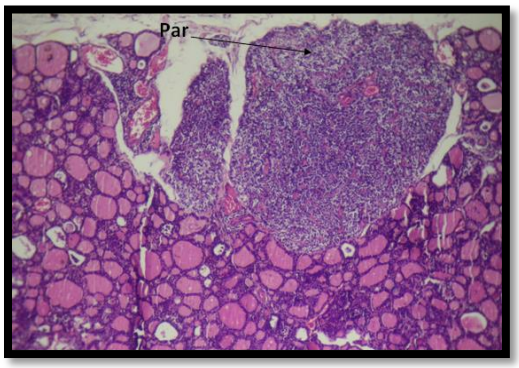


Figure (2): Section in thyroid gland of *H. javanicus* shows the lateral terminal position of parathyroid gland (Par), (PAS stain) (400X).

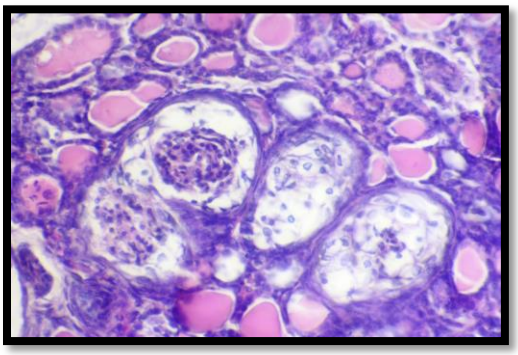


Figure (3): Section in parathyroid gland of *H. auritus*, shows the lobulation of the gland (4 lobs), (PAS) (400X).

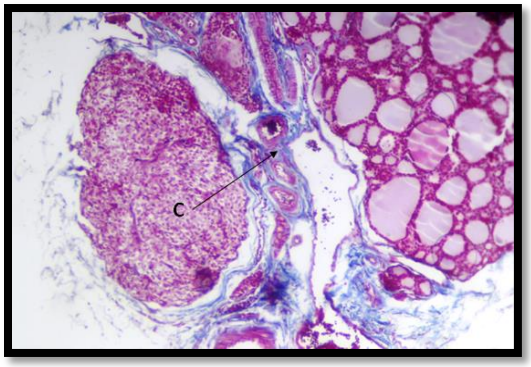


Figure (4): Section in parathyroid gland of *H. javanicus*, shows the capsule of the gland (C), (Azan stain (100X).

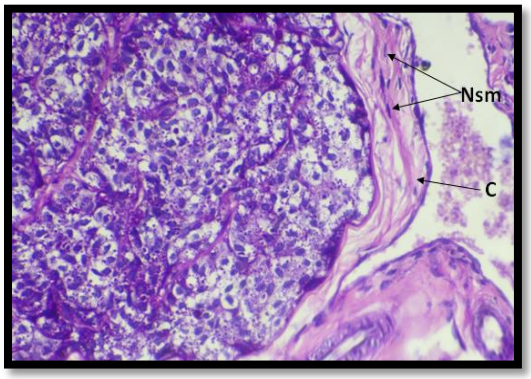


Figure (5): Section in parathyroid gland of *H.javanicus* shows the the capsule (C) and nuclei of capsule's smooth muscle (Nsm), (PAS), (400X).

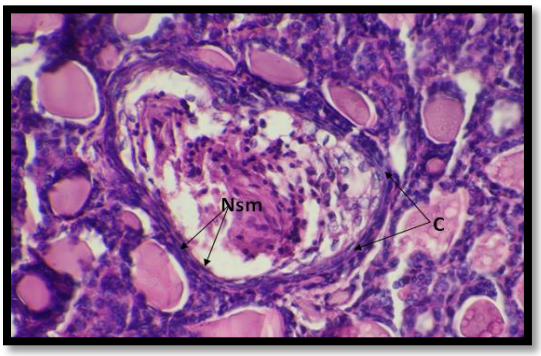


Figure (6): Section in parathyroid gland of *H. auritus* shows the capsule of the gland *H.auritus* (C) and the nuclei of smooth muscle fibers within the capsule (Nsm , (PAS), (400X).

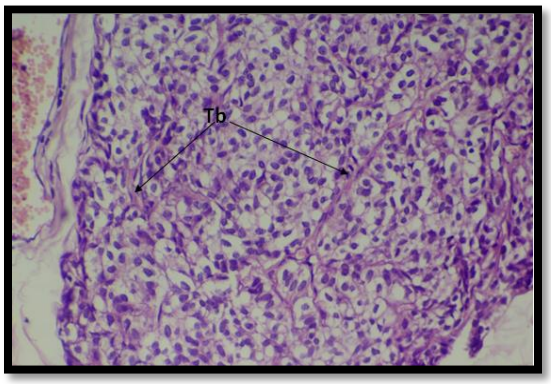


Figure (7): Section in parathyroid gland of *H. javanicus*, shows the trabeculae (Tb) within the tissues of the gland, (H&E), (400X).

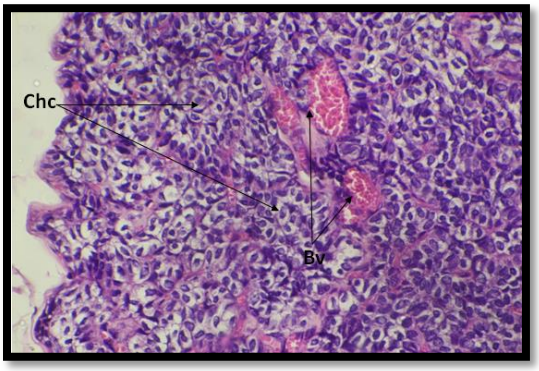


Figure (8): Section in parathyroid gland of *H. javanicus* shows chief cell (Chc) and blood vessel (Bv)(PAS),(400X).

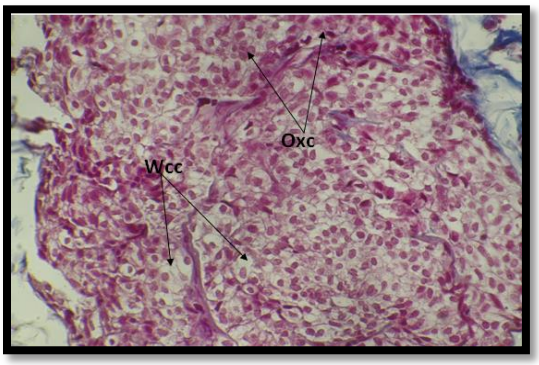
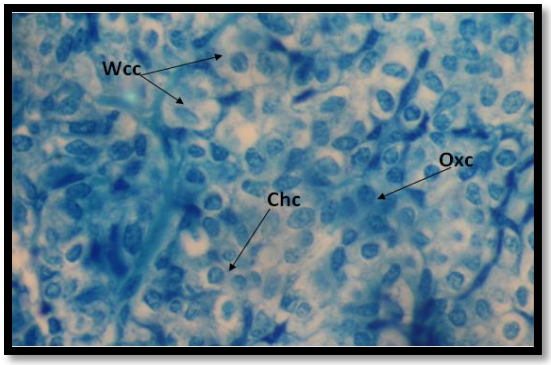


Figure (9): Section in parathyroid gland of *H.javanicus* shows oxyphil cell (Oxc) and water-clear cell (Wcc) , (Azan stain) (400X).



(Figure (10): Section in parathyroid gland of *H.javanicus* shows the chief cell (Chc) , oxyphil cell (Oxc) and water-clear cell (Wcc),(MB stain), (1000X).

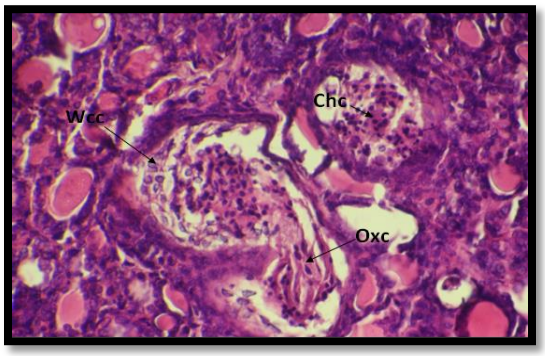


Figure (11: Section in parathyroid gland of *H.auritus* shows the chief cell (Chc) , oxyphil cell (Oxc) , and water-clear cell (Wcc) ,(PAS stain) (400X).

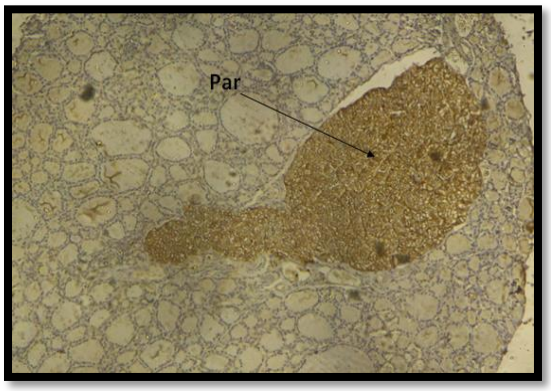


Figure (12): Section in parathyroid gland of *H. javanicus* shows the response of the immunohistochemical reaction (anticalcitonin) (100X).

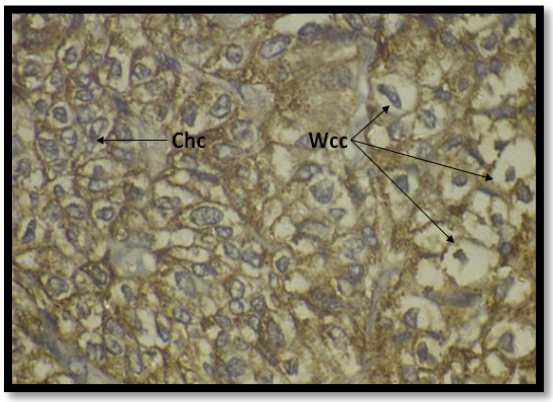


Figure (13): Section in parathyroid gland of *H. javanicus* shows the response of the reaction, (Chc) chief cell; (Wcc) water clear cell (anticalcitonin) (1000X).

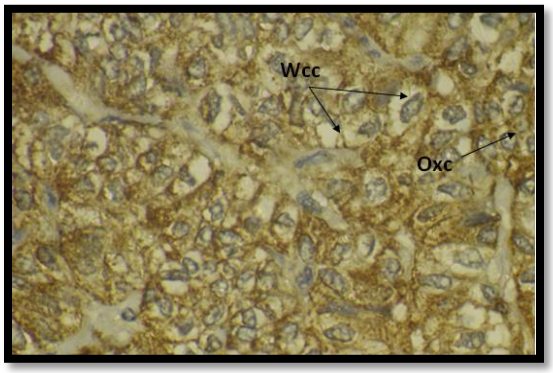


Figure (14): Section in parathyroid gland of *H. javanicus* shows the reaction f the water clear cell (Wcc), and oxyphil cell (Oxc), (anticalcitonin) (1000X).

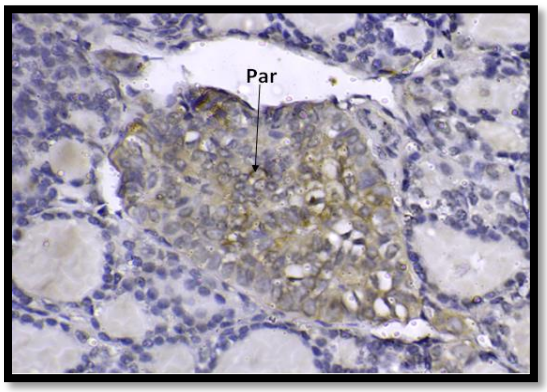


Figure (15): Section in parathyroid gland of *H.auritus* shows the immunohistochemical reaction of the gland's tissue, (anticalcitonin),(400X).

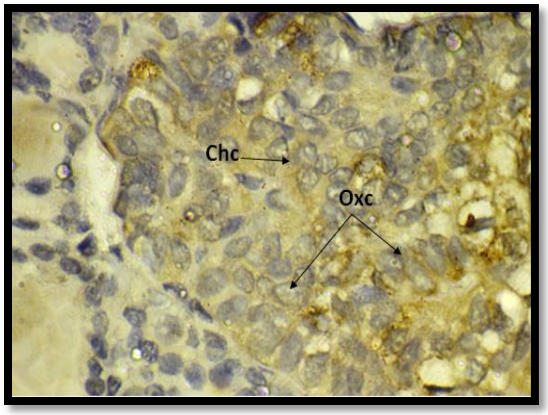


Figure (16): Section in parathyroid gland of *H.auritus* shows the chief cell (Chc) and oxyphil cell (Oxc) (anticalcitonin),(1000X).

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