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RESEARCH ARTICLE

Macro- and Micro-Morphological Studies on the Parathyroid Glands of Dromedary Camel

Al-Ramadan SY[1,*], Ali AM[1], Al-Zghoul MB[2], Althnian TA[1] and Alzayer MA[3]

[1]Anatomy Department, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia; [2]Department of Basic Medical Veterinary Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan; [3]Pathology Department, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia
*Corresponding author: salramadan@kfu.edu.sa

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A B S T R A C T

The present study was performed on ten adult dromedary camels of both sexes. The morphological features of the parathyroid glands which are one of the important endocrine glands in controlling calcium level were described. The parathyroid glands formed of external and internal parathyroids. The external parathyroid located at the region between the origin of occipital and lingual branches of common carotid artery, while the internal parathyroid located in most of the examined cases in a navel-like depression on the cranial or craniolateral border of the thyroid glands. Each parathyroid gland is encapsulated with a thin capsule of dense irregular connective tissue from which fine connective tissue septa extending to divide the gland into small lobules. The parenchyma of parathyroid glands is composed of three types of cell: chief cells, oxyphil cells and water-clear cells. However, the chief cells could be subdivided into light and dark cells at the ultrastructure level. The results of the present study will add information which will open a field of studies for the various biological and medical researchers, as well as, it may add a new knowledge for the role for the several cellular structures of the parathyroid in addition to its relation with pathological conditions.

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INTRODUCTION

The parathyroids are usually represented by four, relatively small, yellowish-brown glands lie on a close relationship to the thyroid lobes. Functionally, these glands respond to low serum calcium levels by releasing parathyroid hormone (PTH) which increases serum calcium levels through direct action on bone and the kidneys (Danks *et al*., 2011). Therefore, parathyroid glands are essential for life of animal and their removal could lead to fatal levels of hypocalcemia due to a condition called hypoparathyroidism (Potts, 2005; Hoorn and Zietse, 2013). Accordingly, the knowledge of this gland in all its aspects is a vital necessity for practitioners in the field of veterinary medicine and life sciences.

During embryonic development, the endodermal epithelium of the dorsal wing of the 3rd pharyngeal pouch gives rise to the parenchymal cells of the parathyroid III or the external parathyroid because of their extracapsular relationship to the thyroid gland. While the endodermal epithelial cells of the dorsal wing of the fourth pharyngeal pouch form the parenchymal cells of the parathyroid IV or the internal parathyroid because their final location is inside the capsule of the thyroid gland (Grevellec and Tucker, 2010). The external parathyroid gland maintains its relationship to the thymus and, with it, migrates caudally to become located lateral to the thyroid cartilage (in the horse and carnivores) or at the division of the common carotid artery (in ruminants and pigs), while he internal parathyroid gland which, like its external counterpart, also migrates caudally with the thymus and becomes located lateral to the thyroid cartilage, embedded in the thyroid gland (Hyttel *et al*., 2010).

The parathyroid parenchyma is composed of two cell types, the principal (chief) and oxyphil cells (Samuelson, 2007). However, another two types of cells were also described in various animal species, water-clear cell and multinucleated cell (Chen *et al*., 2006; Bai *et al*., 2012; Chen *et al*., 2013).

Our laboratory has been encountered with the parathyroid glands of camel as early as 1998; the works has not been directed primarily toward the parathyroids but has been done on the thyroid gland of the camel (Al-Ramadan, 1998). That work mentioned the parathyroids

as a small structure located at the craniodorsal corner of each thyroid lobe. Later, Metwally and Attia (2006) had described the histological features of the parathyroid glands without any further information if they are describing internal or external parathyroid glands. However, our laboratories have established thorough searches for any parathyroid issue within thyroid glands but all we found were remnants of ultimobranchial tissues, the origin of the calcitonin cells, (Al-Ramadan, 2013). Our laboratories also published ultrastructural features of these glands, utilizing the parathyroid tissue that is located at the craniodorsal corner of thyroid gland (Al-Zayer *et al*., 2013). Accordingly, an in-depth research has to be done on this very important gland to pave the way for subsequent physiological and clinical studies in this animal. In this study, the morphometric, macroscopic, microscopic and ultrastructural features of both external and internal parathyroids will be profoundly presented and discussed.

MATERIALS AND METHODS

Animals and tissue collection: Samples of this study were collected from Al-Omran Slaughter House, Al-Ahsa, Saudi Arabia. Ten adult (four males and six females) apparently healthy dromedary camels have been utilized to finish this study. These animals were derived from herds maintained in open yards outside the metropolitan area.

Immediately after the camels were slaughtered, parathyroid glands (internal and external) were dissected out and morphologically described. Samples for electron microscopic and histological study were collected and placed in suitable fixatives.

Gross and histological study: After dissection, gross anatomical features of the parathyroid glands were recorded and confirmed later with microscopical examinations. Any structure taken from the same area and proven not to be parathyroid gland has been excluded from the study. For search of any internal parathyroid tissue within the thyroid glands, freehand sectioning of the thyroid lobes followed by microscopic examination has been performed.

For conventional microscopic study sample were fixed in 10% neutral buffered formaldehyde solution. Fixed tissues were further processed by routine paraffin-embedment techniques, cut into 5 µm sections. For general histological features, standard hematoxylin and eosin were performed according to protocol described by Bancroft and Gamble (2007).

Ultrastructural study: Small pieces (about 1-3 mm) from both external and internal parathyroid glands were fixed by immersion in 2.5% gluteraldehyde in 0.1 M. cacodylate (Cac.) buffer, pH 7.4 at a temperature of 4°C. for 1 hour and were washed in Cac. buffer. The tissue blocks were then post- fixed with 1% OsO4 in Cac. buffer for 90 minutes at room temperature, dehydrated in acetone and embedded in Epon Araldite (502 kit, Pelco, USA). Sections were obtained in a Leica EM UC6 ultramicrotome, then placed on copper grids and stained with 0.5% uranyl acetate and 3% lead citrate. Examination of the sections were carried out using a JEM 1011 (Joel, Tokyo, Japan) electron microscope at 80 kv.

RESULTS

Macroscopical study: The parathyroids in the dromedary camel consist of two pairs of external and internal glands. The external parathyroid pair located at the area extending from the bifurcation of the common carotid artery all the way down to the ramification of the occipital artery, which represent the distance 6-10cm ventral to the ventral border of the mandibular salivary gland or 9-16cm dorsal to the dorsal pole of the thyroid gland. Nevertheless, the external parathyroids might get confused with the medial retropharyngeal lymph node which is paler in color, firmer in texture, and larger in size. However, our data further confirmed by histological analysis of both structures (Fig. 1A, 1B & 1C). While the internal parathyroid glands occupy positions closely related to cranial or craniolateral pole of thyroid glands. In most of the cases examined they are located in a navel-like depression on the cranial or craniolateral border of the thyroid glands. In some occasions they are located at the posterior surface of the thyroids or 2-4mm cranial to the cranial pole of the thyroid glands. Within the same vicinity, the deep cranial cervical lymph node could be seen but they are much larger and firmer than internal parathyroid (Fig. 2A & 2B). Serial free hand sectioning of the thyroid glands followed by histological examinations resulted negative for any parathyroid tissue.

Microscopical study: Each parathyroid gland is encapsulated with a thin capsule of dense irregular connective tissue. Fine connective tissue septa extending from this capsule and dividing the gland into small lobules (Fig. 3A). Accompanying these septa, blood vessels, lymphatics and nerves could be detected. Internal parathyroid, may share the thyroid its capsule in some cases (Fig. 3B). However, the parenchyma of both glands separated with wide septum.

Microscopic analysis of the parathyroid glands showed no clear differences between the external and internal parathyroid glands at the microscopic level. The parenchyma of both parathyroid glands in adult dromedary camel is composed of three types of cell: chief cells, oxyphil cells and water-clear cells. These cells arranged in clusters, cords or follicles. The chief or principal cells are small, polygonal with light spherical nucleus and acidophilic cytoplasm (Fig. 3A-C; 4A). They represent the majority of the cells within the parathyroid gland. Other type of cells is the oxyphil cell. They are larger than chief cells with deeply acidophilic cytoplasm (Fig. 4B). Oxyphil cells occur in groups of few cells or single cell scattered between chief cells. Water-clear cells are also constantly detected within camel parathyroid glands. They are large cells and their nuclei vary in size, shape and intensity of their chromatin staining. These cells have clear or vacuolated cytoplasm, hence their name (Fig. 4C). Water-clear cells occur as isolated cells or as group of cells. However, in certain locations the whole lobule is infested with these cells.

Ultrastructural study: Based on the electron microscopic data, the parenchymal cells of the parathyroid glands of the adult camel could be divided into four cell types: active chief cells, inactive chief cells, oxyphil cells and water-clear cells. The active chief cells were tall or polygonal in shape with spherical, oval or indented nuclei. Their nuclei were large and light with peripheral heterochromatin spots. The cytoplasm of these cells have diffusely scattered ribosomes and abundant evenly distributed mitochondria, some Golgi apparatus, lipid droplets and rER as well (Fig. 5A). The inactive chief cells have polygonal outlines, less cell organelles than the active cells but more vacuolated and more central nucleus. However, the activity of chief cells was independent of color intensity. The plasma membranes were straighter than active cells and had occasional uncomplicated interdigitations with adjacent chief cells (Fig. 5B).

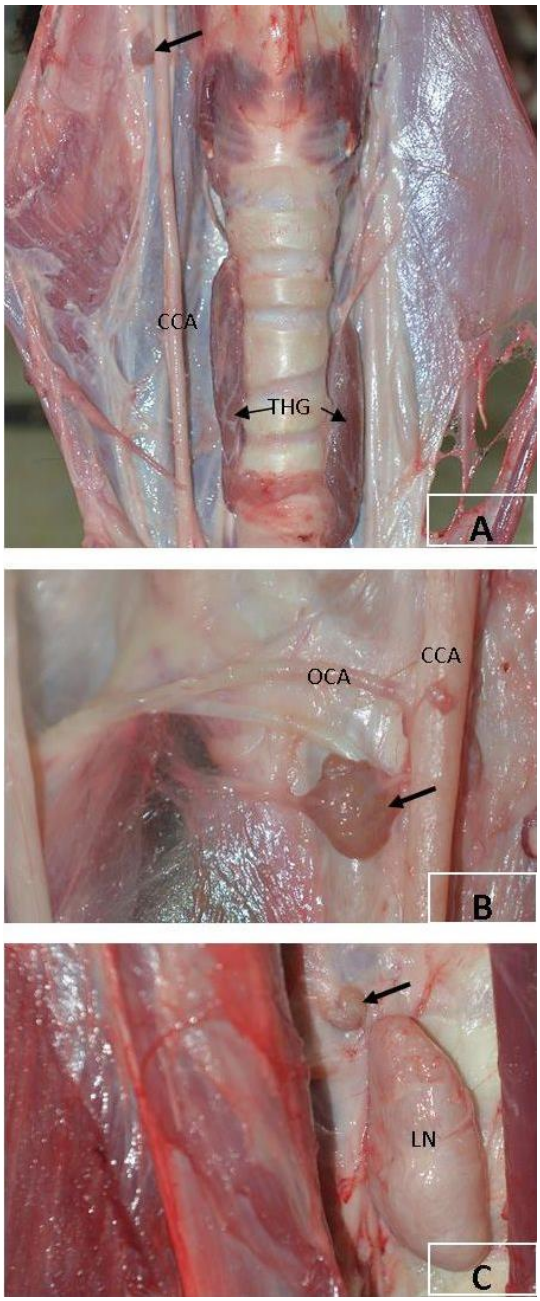

Fig. 1: A, B & C. Showing: External parathyroid gland (Arrow), common carotid artery (CCA), occipital artery (OCA), thyroid gland (THG) and medial retropharyngeal lymph node (LN).

Infrequently, some oxyphil cells in the parenchyma of parathyroid gland characterized by more mitochondria than active chief cell, few secretory granules and some lipid droplets (Fig. 6A).

The fourth type of cells was the water-clear cells. Cytoplasm of these cells was occupied by many membrane-limited vacuoles. Those vacuoles, having electron-lucent spaces with some electron dense materials. The nuclei of these cells were irregular or indented, however, spherical or oval could be detected (Fig. 6B). Secretory granules, rER, mitochondria, lipid compartments, scatted ribosomes were occasionally seen within these cells (Fig. 6C). In all samples no multinucleated cells could be detected.

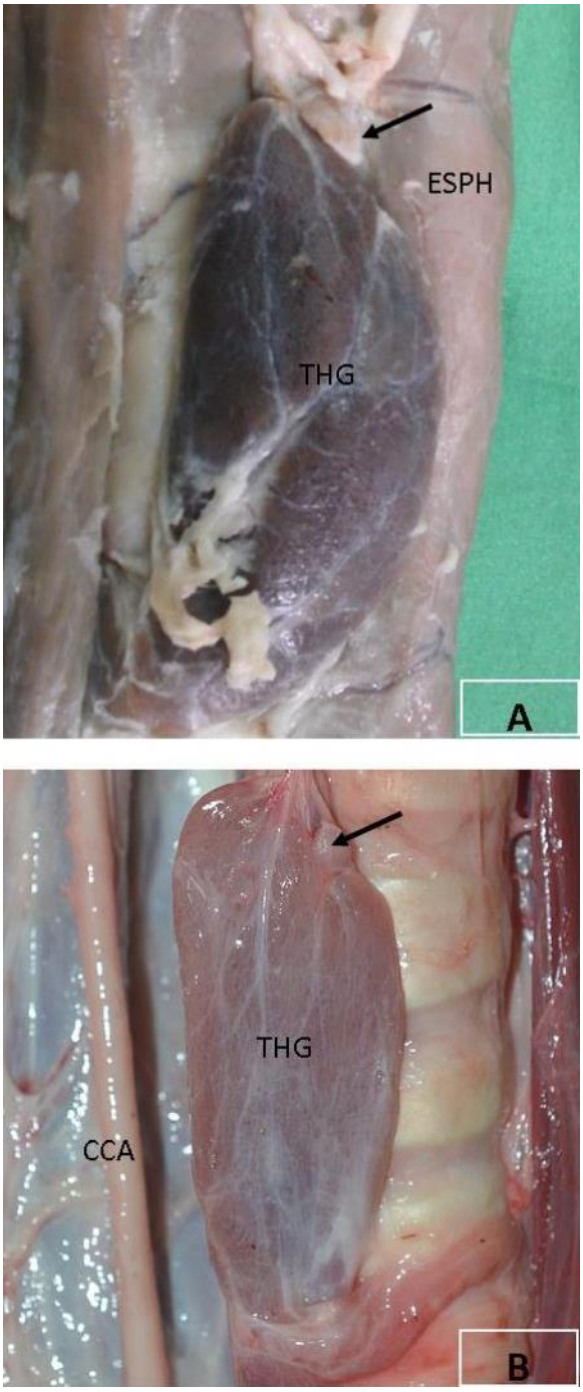

Fig. 2: Showing: (A), anterior surface of thyroid gland (THG) and the internal parathyroid gland (Arrow). (B), showing the posterior surface of thyroid gland (THG) and the interior parathyroid (Arrow) at craniodorsal of thyroid gland which was flipped over.

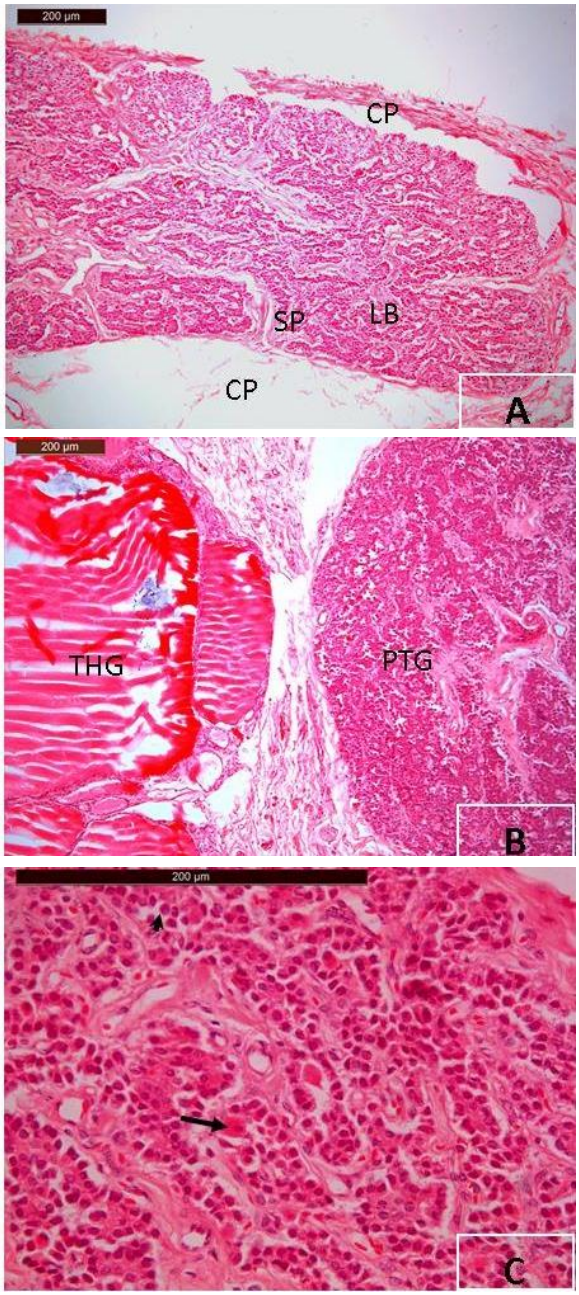

Fig. 3: (A), Light micrograph of internal parathyroid gland of camel showing: connective tissue capsule (CP), septum (SP), parathyroid lobules (LB). (X200). (B), Light micrograph of thyroid gland (THG) and internal parathyroid (PTG). (C), lobules of parathyroid glands with some oxyphil cells (Arrow) and water-clear cells (Arrowhead). (X200).

DISCUSSION

The present study showed that the parathyroid glands of dromedary camel consist of two pairs of external and internal parathyroids. To the best of the authors knowledge, this is the first time; both glands are recorded in the dromedary camel. With the exception of pig which has only the external parathyroids (Swindle *et al*., 2012; Hyun *et al*., 2015), the external and internal parathyroid glands have been recorded in cat (Bichard, 2006), Buffalo (Hussain and Al-Taay, 2009), Dog (Liles *et al*., 2010) horse and all other domestic ruminants (Konig and Liebich, 2004). Contrary to the previous statement, Lahav *et al*. (2015) could not find the internal parathyroids in cattle but that might be due to the technique they have used, X-ray fluorescence-based differentiation, to detect the glands.

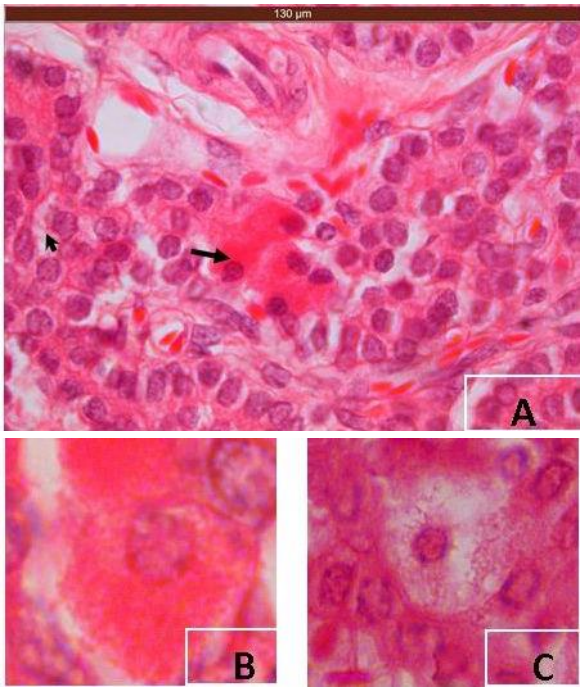

Fig. 4: (A), Light micrograph of external parathyroid gland of camel showing: Oxyphil cells (Arrow) and water-clear cell (Arrowhead). (X200). (B) Light micrograph of oxyphil cell. (X630). (C) Light micrograph of water-clear cell (X630).

Our finding revealed that the external parathyroids located at the area extending from the bifurcation of the common carotid artery all the way down to the ramification of the occipital artery, which represents the distance 6-10cm ventral to the ventral border of the mandibular salivary gland or 9-16cm dorsal to the dorsal pole of the thyroid gland. To date, no study has reported the existence of the external parathyroid gland in dromedary camel. The variation in the position of the external parathyroid glands is common to human and all domestic farm animals and it is always a challenge for the surgeons to locate the external parathyroid glands (Hyun *et al*., 2015). In horse, external parathyroids are located along the trachea close to the caudal deep cervical lymph nodes, dorsal to the medial edge of thyroid all the way caudal as far as 15 cm rostral to the 1st rib. This variation in the position of equine parathyroids might be the reason why Davies *et al*. (2010) could not detect any of the parathyroid glands in the horses they have examined. Another reason was the size of the glands which might be beyond the resolution of the scintigraphy and the ultrasonographic techniques they have utilized. However, in dog the external parathyroids are located close to the cranial pole of the thyroid gland (Fukui *et al*., 2015). In the small and large ruminants, the external parathyroids are found medial to the bifurcation of the common carotid artery (Konig and Liebich, 2004).

In all examined camels, we found the internal parathyroid in close relation with the thyroid glands but not within its parenchyma. In canines, the internal parathyroids are embedded within the caudal aspect of each thyroid lobe (Liles *et al*., 2010). In camel, internal parathyroid is located in a navel-like depression on the cranial or craniolateral border of the thyroid glands. In some occasions they are located at the posterior surface of the thyroids or 2-4 mm cranial to the cranial pole of the thyroid glands. Similar observation has been recorded in the domestic ruminants and horse (Konig and Liebich, 2004).

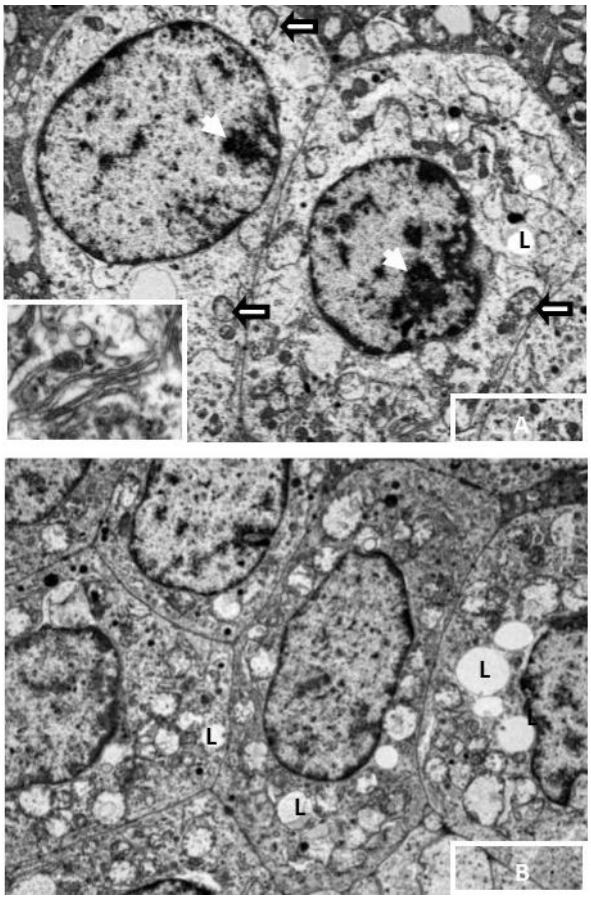

Fig. 5: (A), Transmission electron micrograph of active chief cells. (A) Active light cells with evenly distributed mitochondria (arrows), the nuclei are large with heterochromatin spots (arrowhead), there is also some membrane-limited lipid droplets (L) (X 8,000). Insertion, showed a Golgi stack (X 20,000). (B) Inactive chief cells with straight plasma membrane separating between adjacent cells, more lipid droplets within the cytoplasm (arrow) and less organelles are seen in this cell (X 8,000).

In the present study the structure of the parathyroid gland is in accordance with previous description about the stroma of parathyroid glands in the domestic mammals. Moreover, the blend of septa between thyroid and parathyroid glands was also recorded in other animals (Samuelson, 2007).

With conventional microscopy, we were able to record three types of cells within the parenchyma of parathyroid glands: chief cells, oxyphil cells and water-clear cells. The chief cells are constant components of the parathyroid glands in all domestic animals while other cell type's notably water-clear cells were not recorded in all animal species. Oxyphil cells were recognized in the large ruminant and horse but not regularly seen in other domestic animals (Samuelson, 2007). Similar to our previous findings within the internal parathyroids (Al-Zayer *et al*., 2013), we also report here the presence of water-clear cells in the external parathyroids of adult dromedary camel. These cells are constantly present in all samples examined. They are large cells and their nuclei vary in size, shape and intensity of their chromatin staining. These cells have clear or vacuolated cytoplasm. This type of cells is extremely rare or absent in normal humans and their presence is usually linked with parathyroid hyperplasia or parathyroid adenoma (Chen *et al*., 2013). However, other authors refer to these cells as light inactive chief cells (Gartner and Hiatt, 2007; Samuelson, 2007).

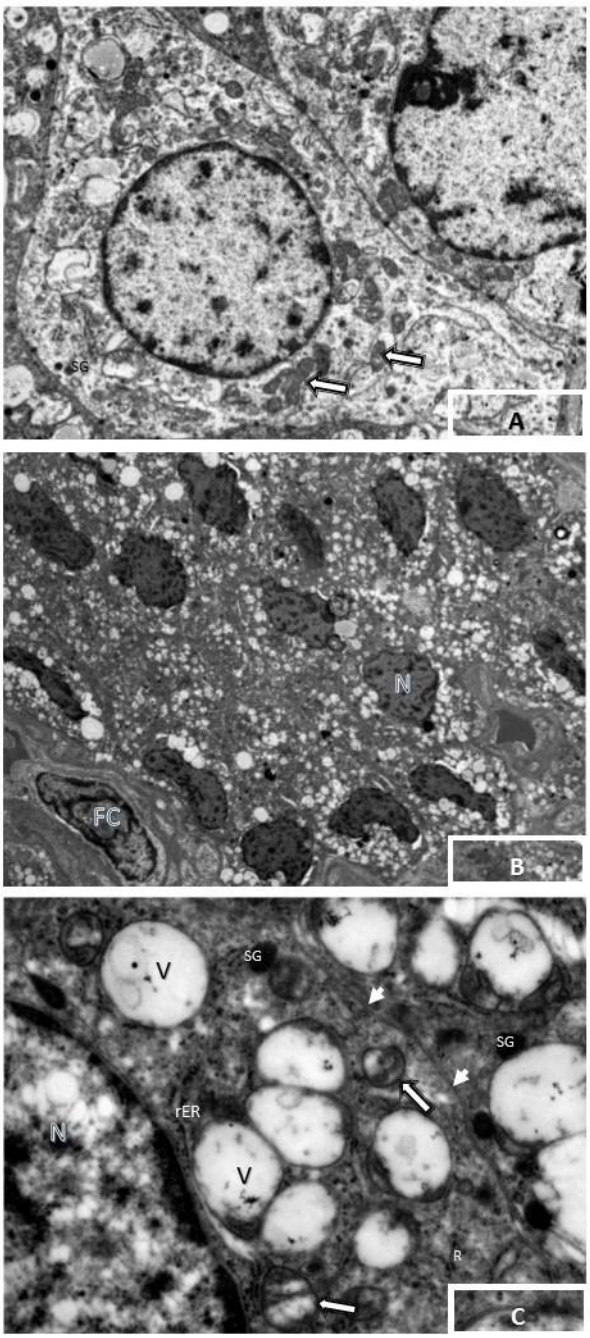

Fig. 6: (A) Transmission electron micrograph of oxyphil cell, the cytoplasm has numerous mitochondria (arrows) with different forms and few secretory granules (arrowhead) (X 10,000). (B) Group of water-clear cells (W), showing vacuolated cytoplasm and irregular nuclei (N), a fibroblast cell (FB) and connective tissue fibers (X 2,000). (C) Part of water-clear cell, showing the membrane-limited vacuoles (V), some mitochondria (arrows), scattered ribosomes (R), secretory granules (SG), rough endoplasmic reticulum (rER), part of its nucleus (N) and part of a cell membrane (arrowhead) (X 30,000).

In the present study we are able by aid of transmission electron micrographs to subgroup the chief cells into light and dark cells. Therefore, the cells that had been described early as light chief cells (Samuelson, 2007) might be merely water-clear cells. However, this claims needs further investigation and more evidences. In this respect, some literatures attributed the variations in the color to different functional phases, diet or even to the procedures of tissue preparation (Matsushima *et al*., 2005).

In the adult camel, we could recognize numerous water-clear cells as isolated cell, group of cells, or widely distributed within certain lobules. These cells are vacuolated with electron-lucent vacuoles occasionally some electron dense materials, thread like could be observed within these vacuoles. Water-clear cell was reported in many species (Chen *et al*., 2013). Increasing attention was directed toward this cell because of the link between its presence and parathyroid hyperplasia or parathyroid adenoma (Chen *et al*., 2006).

Conclusions: The data presented here showing the detailed morphological features of parathyroid glands in the adult dromedary. Moreover, we are recording, for the first time, the location of the external parathyroid glands and the presence of water-clear cells in adult camels.

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Author's contribution: SYA and MAA designed and carried out most of the work and writing. AMA, MBA and TAA contributed equally in the collection of the samples, lab work and help the first author in the revision of the manuscript and approved the final version.

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