Detection of Calcitonin Cells in Camel (*Camelus dromedarius*) Parathyroid Glands

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ABSTRACT

Both thyroid and parathyroid glands are derivatives of the pharyngeal pouches. During early embryological stages, calcitonin cells (C-cells) migrate from neural crest to the fourth pharyngeal pouch before they are incorporated within thyroid gland and become an integral postnatal component of this gland. This study was conducted to investigate the presence of C-cells in the parathyroid glands of adult dromedary camels. Ten parathyroid glands from adult camels of both sexes were investigated using immunohistochemical technique. Calcitonin cells were markedly detected as several cells groups within the internal parathyroid glands, whereas few cells were reacted to the anti-calcitonin antibody within the external parathyroid glands. In conclusion, the C-cells could be found in derivatives of fourth pharyngeal pouches other than thyroid gland like the internal parathyroid glands.

Key Words: Camel, C-Cell, Parathyroid glands, Pharyngeal pouched.

INTRODUCTION

The calcitonin cell is present in the thyroid gland and it is a part of neuroendocrine system. Based on histochemical properties, they have been classified as Amine Precursor Uptake and Decarboxylation cells. According to Das *et al*. (2017) they have been called by variety of names for example; C cell, parafollicular cell, neurohormonal cell, giant-light cell, argyrophil cell, light cell, and mitochondria-rich cell by various researchers. They secrete calcitonin, a hormone that is involved in maintaining calcium homeostasis (Das *et al.,* 2017).

In a comparative early study, Carvalheira and Pearse (1967) concluded that the calcitonin or C-cells are not exclusively thyroid cells, and that they may be quite widely distributed through the thyroid-parathyroid complex. This conclusion was not extravagant, given that both C-cells and parathyroid glands are derivatives of the pharyngeal (or branchial arches) pouches, which appear as transient structures in early embryonic life. However, the development of the pharyngeal derivatives depends on the interaction and integration of various cell populations, including the surface ectoderm, foregut endoderm, paraxial mesoderm and neural crest cells (Kameda *et al.,* 2013). Calcitonin cells develop from a subpopulation of cephalic neural crest cells that broadly populate the branchial arches in all animals of the vertebrate series (Johansson *et al.,* 2015). Although the origin of C-cell precursors is controversial, it is well known that the ultimobranchial bodies bring the parafollicular C-cells to the thyroid during thyroid development of higher vertebrates (Fagman and Nilsson 2010).

Moreover, the endodermal epithelium of the dorsal wing of the third 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 parathyroids because their final location is inside the capsule of the thyroid gland (Latshaw, 1987).

This study was designed to investigate the presence of C-cells within the external and internal parathyroid glands of the dromedary camel.

MATERIALS AND METHODS

Animals and tissue collection

Samples of this study were collected from Al-Omran Slaughterhouse, Al-Ahsa, Saudi Arabia. Tissues from ten adult (four males and six females) apparently healthy dromedary camels were used in the study. Immediately after slaughter, parathyroid glands (internal and external) as well as thyroid gland were dissected out, and then placed in 4% paraformaldehyde in PBS (pH 7.3). After 48 hours, all parathyroid lobe samples were serially sliced into several slices, about 5mm thick, and embedded in paraplast (Leica Microsystems, St. Louis, MO).

Conventional Histological staining

Standard hematoxylin and eosin (H and E) protocol according to Bancroft and Cook (1994) was used to investigate general histological structures of the parathyroid glands.

Immunohistochemistry

The expression of calcitonin was detected in paraformaldehyde- fixed, and paraffin-embedded, internal, and external parathyroid glands as well as thyroid gland. Cross-sections (4 µm thick) were prepared using procedures described previously (Al-Ramadan, 2013). Briefly, calcitonin was detected with primary antibody (types, sources, antibody dilutions, host, reactivity, and clonality are shown in Table (1). Sections were dewaxed rehydrated and incubated overnight with primary antibodies at 4°C while the rest of incubations were performed at room temperature. After several washings, sections were then incubated with secondary biotinylated antibody for 30 - 60 min. After washing with PBS, the sections were incubated with streptavidin-HRP conjugate (HSS-HRP) for 30 min. and finally washed. Visualization was achieved by immersing sections in freshly prepared AEC chromogen solution until desired stain intensity was developed.

Histological images were obtained with Leica DM6000-B microscope and Leica DEC-420 digital camera (Leica Microsystems, Germany). For negative controls, primary antibody was substituted with normal rabbit serum, while the rest of procedures were maintained. Sections from normal camel thyroid glands were used as a positive control for calcitonin.

The labeling intensity of cells was scored on a subjective scale of: Negative; (-) Weak; (+) Moderate; (++) Strong; (+++) and very strong (++++).

Table 1. Information of the antibody used in the study

Antibody	Company	Cat. No.	Host	Dilution	Reactivity	Clonality
Anti-Calcitonin	Dako	A0576	Rabbit	1/200	Human	Polyclonal

RESULTS

Internal parathyroid:

Both right and left internal lobes of the parathyroid gland showed some specific cells that reacted strongly (+++) to anti-calcitonin antibodies. However, the distribution showed no patterns in term of central versus peripheral but we were able to differentiate between high infested lobules versus low infested ones (Figure 1).

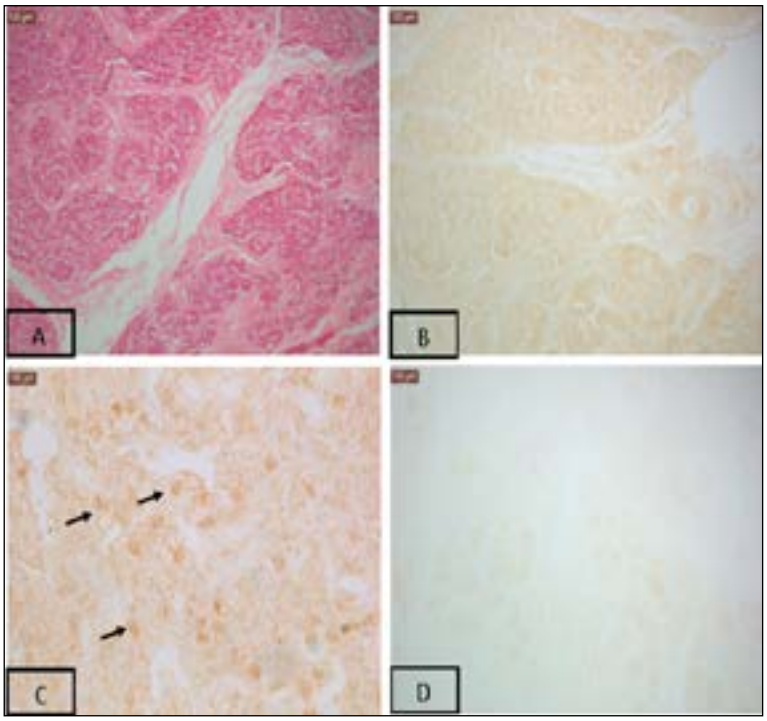

Figure 1. Histological feature and immunohistochemistry for calcitonin of camel internal parathyroid glands. (A) Shows the general structural features of the internal parathyroids (H&E, X10). (B) Shows the internal parathyroid glands stained with anti-calcitonin antibodies, there is moderate intensity of the stain (+++) (X10). (C) Shows higher magnification of internal parathyroid, showed specific reaction of anti-calcitonin (arrows) in some cells (X100). (D) Represent the negative control where the primary antibody is replaced with normal rabbit serum (X100).

External parathyroid:

Few cells reacted positively to anti-calcitonin antibody. However, the intensity of the reaction was low (+) to moderate (++) in comparison to the internal parathyroid. Moreover, some animals revealed negative interaction to the immunohistochemistry (Figure 2).

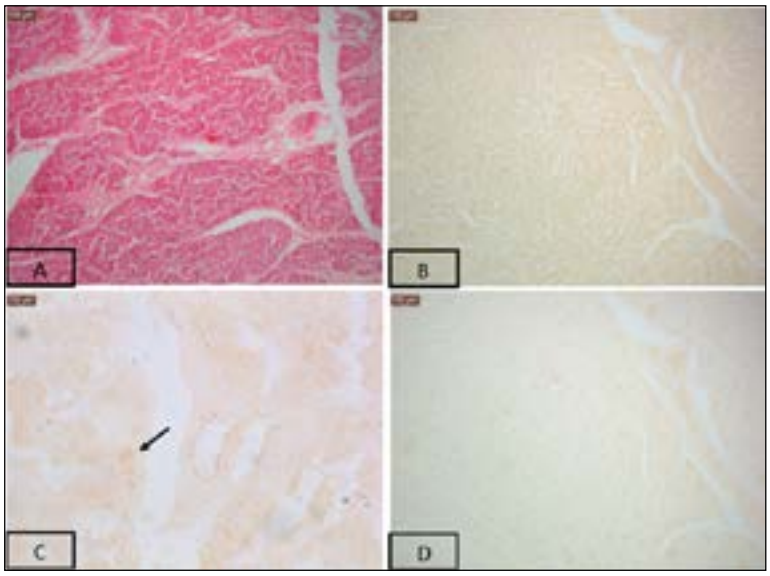

Figure 2. Histological feature and immunohistochemistry for calcitonin of camel external parathyroid glands. (A) Shows the general structural features of the external parathyroids (H&E, X10). (B) Shows the external parathyroid glands stained with anti-calcitonin antibodies, showing weak reactivity toward anti-calcitonin (+) (X10). (C) Higher magnification of internal parathyroid, showing specific reaction of anti-calcitonin (arrows) in a few cells (X100). (D) Represent the negative control where the primary antibody is replaced with normal rabbit serum.(X100).

Thyroid glands:

The thyroid gland of camel showed very strong (++++) signal for the calcitonin within the parafollicular cells, while the thyroid sections that were treated as negative control shows no positive signals (Fig.3).

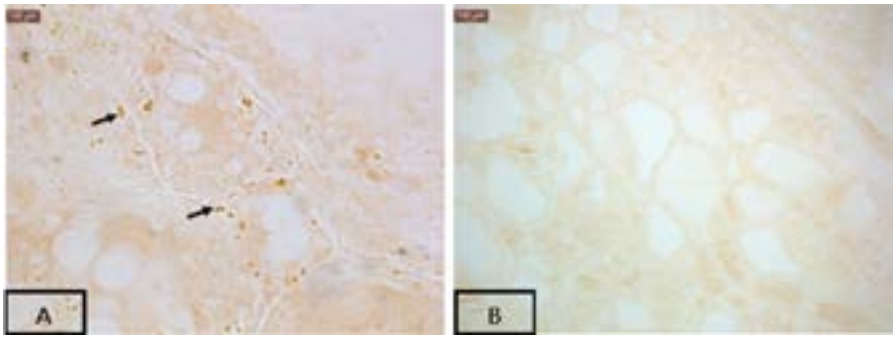

Figure 3. Positive control for Immunohistochemistry for calcitonin of camel thyroid gland. (A) Shows thyroid glands stained with anti-calcitonin antibodies, very strong specific reaction toward anti-calcitonin antibody (arrows) in the parafollicular cells (X100). (B) Represent the negative control where the primary antibody is replaced with normal rabbit serum.(X100).

DISCUSSION

The parathyroid glands are four tiny glands, located in the neck, that control the body's calcium levels. With exception of the pig, all the domestic mammals have four glands representing the internal and external parathyroid glands. In the pig, however, the internal parathyroid pair is the only parathyroid detected in this species so far (Hyun *et al.,* 2015). Until recently, the location of the external parathyroid of camel was not determined. Al-Ramadan *et al.* (2017) were able to determine the position of the external pair of the parathyroid glands. In camels, the external parathyroid glands 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 6-10 cm ventral to the ventral border of the mandibular salivary gland or 9-16 cm dorsal to the dorsal pole of the thyroid gland. While the internal parathyroid glands occupy positions closely related to cranial or craniolateral pole of thyroid glands (Al-Ramadan *et al.,* 2017). The determination of each of the parathyroid sites opens the door for those interested to conduct in-depth research on each pair separately. From this perspective, our research team first began to conduct research on a comparison of the cellular components of each pair individually. The internal and external parathyroid glands have similar physiology in terms of production of parathyroid hormone and both show no clear differences at the conventional and electron microscopic levels. 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 (Al-Ramadan *et al.,* 2017). However, the internal and external parathyroid glands originated from two different pharyngeal pouches (Neves and Zilhao, 2014). Therefore, we hypothesized that both pairs have different cellular components. To the best of the authors' knowledge, there is no previous work to identify C-cells within the parathyroid glands of the dromedary camels using immunohistochemistry.

Comparison between internal and external parathyroid glands showed that the internal infested with the C-cells while the presence of this type of cell in external parathyroids is very limited. During embryogenesis, the endodermal epithelium of the dorsal wing of the third pharyngeal pouch gives rise to the parenchymal cells of the parathyroid III or the external parathyroid. 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 parathyroids (Grevellec and Tucker, 2010). Similar mechanism of development has been reported in the camel, in which the primordia of internal parathyroid glands (parathyroids IV) develop from fourth pharyngeal pouch while the external parathyroid glands (parathyroids III) arose from third pharyngeal pouch (Helal, 2007). In addition, there are interaction and integration of various cells populating the pharyngeal pouches that might be reflected on the cellular subpopulation of the organs (thyroid, thymus, and parathyroids) which derived from this region. In this respect, the C-cells develop from a subpopulation of cephalic neural crest cells that broadly populate the branchial arches at the level of fourth brancheal pouch (Johansson *et al.,* 2015; Kameda *et al.,* 2013). Accordingly, the presence of cells positive to calcitonin in the internal parathyroid but not external parathyroid is understandable because of the close developmental relation between the thyroid glands and the parathyroid IV (internal parathyroid).

In conclusion, C-cells are not strictly a thyroid component and might be detected in other organs derived from pharyngeal pouches specifically those derived from the fourth pouch. However, further studies are needed to investigate the histogenesis of the parathyroid glands and to track the pathways of different cellular components in the camel and other domestic animal species. Moreover, the physiological link between these cells and the other components of the parathyroid glands need to be clarified.

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الكشف عن خلايا الكالسيتونين في الغدد الجار درقية في الإبل

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الملخص

كل من الغدة الدرقية والغدد جار الدرقية تشتق من الجرابات البلعومية خلال المراحل الجنينية المبكرة. تهاجر خلايا الكالسيتونين من القمة العصبية إلى الجراب البلعومي الرابع قبل اندماجها ضمن الغدة الدرقية؛ حيث تصبح مكونًا ثابتًا من مكونات الغدة الدرقية بعد الولادة. تمثل الهدف الأساس من هذه الدراسة في تعرف وجود خلايا الكالسيتونين في الغدد جار الدرقية في الإبل العربية البالغة، وقد أجريت هذه الدراسة على عشر من الإبل البالغة من كلا الجنسين باستخدام التقنية النسيجية الكيميائية المناعية.

تم تحديد وجود خلايا الكالسيتونين بشكل ملحوظ في عدة مجموعات في الغدد جار الدرقية الداخلية، بينما كان تفاعل أنسجة الغدد جار الدرقية الخارجية ضعيفًا وفي عدد محدود من الخلايا فقط، وكنتيجة لهذه الدراسة فإن خلايا الكالسيتونين يمكن أن تتواجد خارج الغدة الدرقية؛ حيث يمكن تواجدها في مشتقات الجرابات البلعومية الرابعة مثل الغدد جار الدرقية الداخلية.

الكلمات مفتاحية: الإبل، الجرابات البلعومية، خلايا الكالسيتونين، الغدد جار الدرقية.