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MORPHOLOGICAL STUDY OF ULTIMOBRANCHIAL REMNANTS IN THE THYROID GLAND OF DONKEY (*EQUUS ASINUS*)

(With 12 Figures)

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دراسة مورفولوجية على بقية الجسم الخلصمى الأخير بالغدة الدرقية في الحمار

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تحتوى الغدة الدرقية بالثدييات على تركيب ثانوى ينشأ من بقية الجسم الخلصمى الأخير ، حيث توجد مكوناته مندمجة بداخل الغدة الدرقية بين الجريبيات الدرقية أو توجد بجوارها مكونة ما يسمى بخلايا الكالسيتونين الدرقى ، وقد تبدو الخلايا الأخيرة في صورة جريبيات أو تجمعات خلوية مصمتة أو مجوفة تسمى بالأعشاش الخلوية المصمتة أو الأعشاش الخلوية المتحوصلة. وتتمثل أهمية الجسم الخلصمى الأخير بالثدييات في كونه المصدر الرئيسى لخلايا الكالسيتونين الدرقى ودوره في التطور الطبيعي للغدة الدرقية وتمييز خلايا الكالسيتونين ، كما أنة يعتبر منشأ لبعض الأورام أو السرطانات الدرقية. أظهرت هذه الدراسة أن بقية الجسم الخيشومي (الخلصمي) الأخير بالغدة الدرقية للحمار يتكون من تجمعات من جريبيات غريبة ومتميزة من حيث الشكل والحجم ، حيث ظهرت بشكل حويصلات أو كيسيات متعرجة أو منتظمة في الشكل في بعض الأحيان. وقد شوهدت بعض الجريبيات الخلصميه الأخيرة مكتسبة أشكالاً أخرى، متمثلة في صورة قنيات أو إنبيبات أو أكياس خيشومية كهفيه ذات متاهات، واحتوت تجاويف بعض هذه التركيبات على مواد غروانية مخزنه، وربما بدت فارغة في مواطن أخرى. وقد ظهرت هذه التركيبات متناثرة خلال النسيج الغدي الدرقي الأصيل، خاصة تحت الغلاف الغدي، وكذا بداخل الحواجز بين الفصيصات ، وقد شوهدت في بعض الحالات أعداد غير قليلة من الجريبيات الخلصميه متناثرة في النسيج الخلالى بين الجريبيات الدرقية الأصيلة. وقد أوضحت الدراسة الحالية أن هذه الجريبيات الخلصمية تكون مصحوبة ببعض الجريبيات الثانوية الصغيرة وكذا بعض الجيوب أو الزوائد الظهارية المنبثقة من جدران هذه الجريبيات ، هذا بالإضافة إلى وجود تجمعات خلوية مصمتة أو ذات تجاويف. كما شوهدت عدة جريبيات أو كيسيات مختلطة ومبطنة بخلايا الكالسيتونين بالإضافة إلى الخلايا الفارزه لهرمون الثيروكسين. تكونت البطانة الظهاريه للجريبيات الخلصميه الأخيرة من طبقة واحدة من خلايا مكعبة أو مفلطحة الشكل أحياناً. بينما أبدت جريبيات أخرى جداراً ظاهرياً مرتصفا كاذباً، أو مكتسبة بنوعين من الأنسجة الظهاريه؛ أحدهما بسيط والآخر مطبق أو

مضفف كاذب. وتمثلت المكونات الخلوية للجريبيات الخلصمية الأخيرة من خلايا الكالسيتونين وكذا الخلايا الفارزة لهرمون الثيروكسين الدرقي. أما تلك الخلايا المكونة للأعشاش الخلوية المصمتة والمتحوصلة فتمثلت في خلايا رئيسية متعددة الأضلاع ذات هيولي محب للصبغة الحمضية هذا بالإضافة إلى أعداد ضئيلة من خلايا الكالسيتونين الدرقى ذات هيولي رائق. وباستخدام الماسح الإلكتروني ظهر السطح الطليق للخلايا الظهارية المبطنة للجريبيات الخلصميه الأخيرة مزوداً بخميلات امتصاص دقيقة ذات كثافات وأطوال مختلفة، هذا بالإضافة إلى وجود هدباً مركزياً وحيداً بسطح كل خلية. وقد شوهدت عدة نشاطات إفرازية متآكلة في بعض الخلايا المكونة للأكياس أو الجريبيات الخلصميه الأخيرة ، حيث تميز الغشاء الخلوي العلوي بوجود قمم حلميه الشكل ذات أحجام مختلفة، هذا بالإضافة إلى وجود مناطق متآكلة أو ملساء.

SUMMARY

The mammalian thyroid gland contains a secondary structure, the ultimobranchial body remnants (UBBRs), which is incorporated into thyroid parenchyma and remains there as scattered parafollicular and interfollicular calcitonin secreting cells or gathered into follicle-like structures and cellular clusters, termed solid cell nests (SCNs) and cystic cell nests (CCNs). The UBBRs of present study have been revealed as ectopic structures of various forms and sizes alongside with the usual thyroid follicles which demonstrated in form of large follicles or cysts, large ducts or branching tubules, irregular or labyrinth-like follicles locating under the capsule or deeply situated in the interstitial connective tissue of donkey's thyroid gland. In addition, several small secondary or differentiating follicles, epithelial outgrowths, as well as various cellular aggregates that described in form of SCNs or CCNs were also demonstrated in close vicinity with the aforementioned structures. Mixed ultimobranchial follicles (UBFs) might be found to be admixed with SCNs which composed of solid cell nests cells and follicular cells, forming lumen-like pattern were also demonstrated. The lining epithelium of UBFs was mostly cuboidal in appearance, but sometimes varied from squamous to pseudostratified or stratified in form. The cellular components of UBFs were consisted of usual follicular cells and clear or calcitonin cells. Meanwhile, the SCNs were comprised of main polygonal cells with eosinophilic cytoplasm and large sized calcitonin cells (C-cells) with pale staining cytoplasm which accounts a minor cellular proportion. Surface of most ultimobranchial (UB) follicular cells was almost hexagonal in shape, studded with sparse or dense pleomorphic microvilli, which were denser at the cellular borders. Each follicular cell bore single cilium that projecting over the cell surface.

Interestingly, bleb-like apocrine protrusion of the apical cytoplasm of some UB follicular cells was frequently demonstrated. These apocrine blebs (aposomes) were varied in shape, size and showed smooth or irregular surface. In addition to such aposomes, deep to shallow circumscribed or eroded areas of the apical cell surface was frequently demonstrated. These features indicate that the UB follicular cells in donkeys are similar to those of buffaloes and exhibit an apocrine activity alongside with the usual merocrine mode of secretion. In conclusion, although minor in mass, UBB contribution to the thyroid is important because this structure has been cited as the source of C-cells in mammals including human. Despite the biologic significance of UBBRs remains disputable, the heterogenous expression of antigens in SCNs has indicated that the cells comprising these structures have different biologic functions; at least some C-cells and follicular cells would originate in SCNs. Furthermore, it has been suggested that these remnants might constitute the origin of some ectopic structures described in the thyroid gland including some types of thyroid neoplasms.

Abbreviations used in this study: APUD cells, amine precursor uptake and decarboxylation cells; C-cells, Calcitonin cells; CCN(s), cystic cell nest(s); SCN(s), Solid cell nest(s); UB, ultimobranchial; UBB, ultimobranchial body; UBF(s), ultimobranchial follicle(s); UBBR(s), ultimobranchial body remnant(s).

Key words: *Thyroid, ultimobranchial body, apocrine secretion, thyrocalcitonin cells, follicular cells*

INTRODUCTION

The donkey (*Equus asinus*) is one of farm animals, which adapt to a desert environment of torrid heat and extreme desiccation. This animal, however, played an important role in the Egyptian heritage and culture at the prehistoric or ancient periods, and even today they occupied the priority as a traditional mean for transportation and draft works. The physiology of donkey is might different in some aspects from those of other farm animals that live in mild climates. In hot arid areas, maintenance of internal homeostasis is closely linked to endocrine systems, in particular the thyroid gland which affect many organs in the body (Wilson, 1989). In addition, the thyroid hormones play a significant role in the metabolism of the living organism.

The thyroid gland is derived mainly from a central anlage composed of proliferating endodermally derived epithelium of the

pharyngeal floor (Sadler, 1990) giving rise to follicular cells synthesizing thyroid hormones (T3 and T4) (Kameda *et al.,* 2007). In addition, there is a lateral anlage, the ultimobranchial body, that arises in the fifth pharyngeal pouch, which in humans is considered to be part of the fourth (Sugiyama, 1971). The UBB is composed of cells and mesenchyme of neural crest origin. It migrates caudally and fuses with the lateral lobes of the thyroid during embryonic life (Sugiyama, 1971; Sadler, 1990) giving rise to C-cells producing calcitonin (Kameda *et al.,* 2007). Additional substances produced by C-cells include somatostatin, substance P and histaminase (Santa *et al.,* 1988).

In mammals, the UBB is incorporated into the thyroid parenchyma and remains there as scattered calcitonin or parafollicular cells (Nunez and Gershon, 1978; Srivastav and Rani, 1988), whereas in non-mammalian vertebrates, it is present as a discrete organ (Dacke, 1979; Robertson, 1986).

The UBB consists of two epithelial components- (i) follicles of various sizes around a large central lumen and (ii) cell clusters scattered in the loose connective tissue between the follicles (Clark, 1971; Khairallah and Clark, 1971). Currently, it is accepted that the SCNs, cystic cell nests and the so-called mixed follicles are indeed UBB remnants (Sayed *et al.,* 2005; Kameda *et al.,* 2007). The lining epithelium of the follicles may be squamous, cuboidal, or pseudostratified (Clark, 1971; Khairallah and Clark, 1971; Takagi and Yamada, 1982). The lumen of these follicles may be either empty or filled with some colloidal substance with cellular debris (Clark, 1971; Khairallah and Clark, 1971).

Solid cell nests were defined as aggregates of polygonal or spindle-shaped cells forming isolated areas of solid tissue. The SCNs are composed of main cells, C-cells, and the so-called mixed thyroid follicles (Sadler, 1990). The main cells are smaller than C-cells and exhibit round or ovoid nuclei with dense chromatin and posessed deeply eosinophilic cytoplasm, but the C-cells, which account for a minor proportion of solid cell nest population and characterized by clear cytoplasm (Sugiyama, 1971; Thomas *et al.,* 1987; Sadler, 1990). However, the mixed follicles may be admixed with solid cell clusters, forming the so-called cystic cell nest, composed of main cells and follicular cells, forming a follicular lumen-like pattern containing colloid-like material, acid mucins, cell debris and granular material (Sadler, 1990; Pearse and Polak, 1971). UBB contribution to the thyroid is important because this structure has been cited as the source of calcitonin-producing cells in mammals

including human (Polak *et al.*,.1974). It has been suggested that the UBB remnants might constitute the origin of some ectopic structures described in the thyroid gland, as well as some types of thyroid neoplasms (Santa *et al.*, 1988; Williams *et al.*, 1989). However, no reports on the occurrence of such UB structures in the donkey thyroid could be traced. Therefore, in the present study, we examined the occurrence as well as the histomorphological features of UBBRs in the thyroid glands of donkey with special reference to the surface ultrastructural features of UB follicular cells to understand its morphology and physiology more precisely.

MATERIALS and METHODS

Thyroid glands from eight male donkeys (*Equus asinus*) aging from 6 months to 7 years were freshly removed under deep anaethesia at dissection room, Faculty of Veterinary Medicine, Assiut University, Egypt. For light microscopy, one thyroid lobe from each animal was cut transversely at 2-4 mm intervals, fixed in a solution formed of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). After fixation, specimens were dehydrated, cleared, embedded in paraffin and cut into transverse serial sections of 4-5 µm thick. Serial sections representing the whole thyroid gland were stained with hematoxylin-eosin for general purposes and topographical localization of ultimobranchial tissue in the thyroid gland. Other small blocks (1-2 mm3) were post-fixed in a 1% OsO4 solution of 0.1 M phosphate buffer (pH 7.3) for 2-3 hours at 4 °C, dehydrated through a graded ethanol series, and embedded in epoxy resin. Thin sections (1 µm thick) were cut with an ultramicrotome, stained with toluidine blue and examined with a transmission light microscopy.

For scanning electron microscopy, small blocks of thyroid tissue from the other lobe were freshly obtained from the region surrounding the parathyroid IV, immersed in a formaldehyde-glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.3) as described by Karnovsky (1965) for 24 h at 4 °C. Thereafter, the tissues were post-fixed in a 1% OsO_4 solution of 0.1 M phosphate buffer (pH 7.3) for 2-3 h at 4 °C and dehydrated in a series of graded ethanol. After critical point drying and ion sputter coating with gold, specimens were examined in a JEOL (JSM-5400LV) scanning electron microscope at 25Kv.

RESULTS

1. General findings:

The thyroid gland of growing and adult donkey was encapsulated with a capsule of dense connective tissue, give rose to several septa which divide the thyroid parenchyma into various sized lobules. Each lobule was formed of numerous typical thyroid follicles, separated with a delicate interfollicular connective tissue enriched with blood and lymph vessels. The maximal diameter of thyroid follicles was 120.43 ± 7.23 µm and the minimal diameter measuring about 22.19 ± 3.12 µm. The follicular epithelium of these usual follicles was formed of one layer of cubical to columnar cells, containing rounded vesicular nucleus located in the most central portion of cytoplasm.

Apart from the typical thyroid tissue, the light microscopic as well as the three-dimensional observations of thyroid tissue revealed the coexistence of ectopic structures consisting of various unusual follicles closely similar to those described in the UB body. These structures of ultimobranchial body remnants varied greatly in shape, size, structure and function in comparison with those of normal thyroid follicles. They had been observed in form of follicles, cysts, ducts or tubules which lied under the capsule, as well as in the vascular interlobular septa or randomly intermingled with the thyroid tissue proper (Figs. 1-10). In addition, lumenless cellular masses (SCNs), epithelial outgrowths, as well as secondary (differentiating) follicles were frequently associated with these UB structures (Figs. 1-8). Parafollicular cells, singly or accumulated in clusters, forming the so-called SCNs were also demonstrated in the interfollicular tissue between usual thyroid follicles (Figs. 1-8).

2. Histomorphological study:

In the present histomorphological investigation of ultimobranchial body remnants in the thyroid gland of donkeys, some UB follicles were located peripherally, singly or in groups under the capsule of thyroid, while others were present deeply in the perivascular connective tissue within thyroid gland lobules. Some of these follicles exhibited large diameters, more or less irregular outlines and corrugated lumen which showing labyrinth-like cystic structure (Fig. 1). They randomly distributed alongside with the usual follicles, almost near to the glandular capsule (Fig. 1) or deeply situated within parenchyma. The lining epithelium in these structures was varied from simple squamous to stratified cuboidal variety. The nuclei in such epithelial elements were

elongated hyperchromatic in the former type of epithelium, but rounded or spherical and centrally located in the second variety (Fig. 1). The lumen of these follicles was almost containing colloid-like substance which exhibited weaker reaction than that expressed in adjacent usual thyroid follicles (Fig. 1). In addition to the previously described UB follicles, some associating secondary derivatives including epithelial outgrowths, differentiating secondary small follicles as well as solid cellular clusters, composing of basophilic polygonal to squamoid cellular formations were also revealed in close association with the main UB follicular wall (Figs1, 5& 7).

Notably, bi-lobed or invaginated cystic follicles with incomplete divided lumens (Figs. 2 and 4), multilobed cystic follicles (Fig. 3) and or ovoid to spherical shaped follicles (Fig. 2) were also observed as usual components of UBBRs in the thyroid gland of present study. Such follicles were lined by simple cuboidal epithelium which might be changed into stratified formations in some regions of the follicular wall (Fig. 2 and 3). However, other UB follicles showed attenuated epithelial formations (Fig. 4). The follicular epithelial wall composed of follicular cells associating with light clear cells (Figs. 2 and 3). The cavities of these UB follicles were filled with a homogenous pale eosinophilic substance (Fig. 2) or contained thick colloid-like or gelatinous material (Fig. 4). The present investigation of UBBRs in donkey thyroid also showed duct-like UB follicles located within the vascular interlobular connective tissue of thyroid gland (Figs. 5 and 7). These duct-like UBFs had irregular outlines and sometimes they give tubular-like branching within the surrounding connective tissue (Fig.7) and lined by simple cubical or attenuated epithelial formations which might changed into stratified epithelial variety at some follicular regions (Figs. 5 and 7). They had wide slightly folded lumens that appeared free from any colloid substances or cellular debris (Figs. 5 and 7). In addition, small irregular tubular and rounded follicles, solid and cystic cell nests that filled with colloid-like materials were frequently demonstrated in close association with the follicular wall of these duct-like UBFs (Fig. 7). Furthermore, long branching tubule-like follicles were present in the interlobular connective tissue (Fig. 8). This tubule and duct-like UB follicles contained empty lumen and showed finger like tubular branches that lined mostly by cubical epithelial formations together with few clear C-cells (Fig. 8). Another form of UBB remnants was also seen within the interstitial tissue of the thyroid gland which appeared as an aggregation of irregular small sized UB follicles in close association with cell clusters

of solid cell nests (Fig. 6). Some of the UB follicles exhibited tubular or cystic appearance that lined with epithelium composed of simple to stratified cuboidal variety and sometimes merged with cells of SCN. The lumen of some of these follicles contained little amount of pale or deeply stained eosinophilic colloid-like material, while that of other UB follicles was empty (Fig. 6).

In addition to the ectopic UB follicles, the present study revealed various solid and cystic cell nests and mixed follicles as UBB derivatives within the thyroid gland of donkey, in close proximity to the main UB follicles. Well defined solid cell nests were frequently demonstrated as clusters or aggregates of cellular masses composed of distinctive main cells which are polygonal to elongate or even spindle-shaped basophilic formations with centrally located rounded to oval nuclei and eosinophilic cytoplasm (Figs. 1-8). In addition, few large lightly-stained, clear or C-cells account for a minor cellular proportion of SCNs and characterized by clear or translucent cytoplasm and large rounded central nuclei (Fig. 3). Varying degrees of cystic changes were seen in some solid cell nests, forming the so-called cystic cell nests that filled with an eosinophilic dense material in their lumens and showed various sizes, shapes (Figs. 2 and 5-7). The cystic cell nests forming a follicular lumen–like pattern which occasionally presented narrow colloid-containing primordial or growing differentiating follicles. The SCNs were occasionally seen attached to the wall of the adjacent follicles, merged or appeared in continuity with their lining epithelium, forming the mixed follicular component of UBB (Figs. 2, 6). The cells of SCNs had been also observed between the usual follicles (parafollicular) in the thyroid gland of the present study (Figs. 1, 5 and 7).

A large multiloculated cystic-like UB follicles encircled with a vascular fibrous tissue and lined by follicular epithelium consisting of cuboidal follicular cells and light clear C-cells was revealed in the UBB of the present study. The follicular wall of this cyst was merged with main and light cells of adjacent SCN which showed continuity with the cyst epithelium or sometimes located parafollicularly (Fig. 3).

3. Scanning or three dimensional studies:

Three dimensional observation of the thyroid tissue revealed the coexistence of several UBFs that were randomly distributed alongside with the proper thyroid follicles, almost near to the capsule, as well as in the interlobular septa or randomly intermingled with the proper thyroid follicles (Fig. 9). These follicles exhibited large diameters, more or less irregular outlines and slightly folded lumen (Figs. 9-10). The surface of

ultimobranchial follicular cells was almost flat, hexagonal-shaped and studded with sparse to dense pleomorphic microvilli. Such microvilli were much denser at the intercellular borders than that of remaining cell surface (Figs. 11 and 12). The cellular borders were further demarcated by a shallow intercellular depression, which clearly delineated adjacent cells (Figs. 11 and 12). Density and/or length of microvilli varied considerably between UB follicles, as well as from cell to cell in a given follicle. In addition, each follicular cell bores a single cilium projecting over the cell surface (Figs. 11 and 12). This cilium apparently was less distinct at the surface of follicular cells exhibiting a dense microvillus border or those cells showing apocrine activities. Interestingly, bleb-like apocrine protrusion of the apical cytoplasm of some cells was frequently demonstrated (Figs. 10 and 12). The apocrine blebs were varied in size and shape, which showed smooth, eroded or irregular surface. In addition to these aposomes, deep to shallow eroded or circumscribed areas of the apical cell surface were frequently demonstrated (Fig. 12).

LEGENDS

Fig. 1: Thyroid gland of adult male donkey showing subcapsular irregular labyrinth-like UBFs (*Asterisks*) with various sizes and shapes associating with secondary derivatives formed of solid cellular masses (S) together with differentiating secondary small follicles (*Double arrowheads*). The UBFs exhibit an irregular outlines and corrugated lumens occasionally containing moderately-stained vacuolated colloid-like substances. Some of these follicles are lined on one side with a single layer of cubical to flattened cells possessing rounded to compressed nuclei, and on the other side the epithelial membrane is formed of pseudostratified to stratified formations (*Arrowheads*). Notice the epithelial outgrowths (*Arrows*) associating with the UBFs. C; connective tissue capsule; TF, thyroid follicles. Haematoxylin and eosin stain. X100

Fig. 2: Thyroid gland of 1.5 year old male donkey showing several UBFs possessing various sizes and shapes and epithelial lining. The larger UBFs are spherical, ovoid or bi-lobed and lined partially with simple cubical cells containing rounded central nuclei and pseudostratified or stratified epithelial foci (*Arrowheads*) in some follicular places. Notice the solid cellular masses in association with the UBFs with various stages of cystic formation (*CN*) lined by cuboidal epithelium and filled with colloid-like substances in

their lumens (*Double arrowheads*). Various SCNs attaching to the wall of some follicles and merged with their lining epithelium, forming mixed follicles (MF). Haematoxylin and eosin stain. X100

Fig. 3: Semithin section of UB tissue in the thyroid gland of adult male donkey showing several UBFs (*Asterisks*) lined with normal dark follicular epithelium (F) in addition to large lightly-stained clear cells (C), possessing clear or translucent cytoplasm and rounded central nuclei. Notice the solid cell masses (S) in association to the follicular wall forming mixed follicles (MF) or located parafolliculary. The solid cell nests composed of both clear cells (C) and main cells (M) containing dark cytoplasm and ovoid to rounded nuclei with dense chromatin. The solid arrow indicates a multilocular cystic-like UB follicle surrounded by a vascular connective tissue. Some UBFs shows pseudostratified or stratified epithelial foci in their lining epithelium (*Arrowhead*). Toluidine blue stain. X400

Fig. 4: Thyroid gland of adult donkey showing a large bi-lobed cyst-like UBF (*Asterisk*) filled with dense colloid-like substance in its lumen and predominantly associated with secondary derivatives of solid cellular masses (S) and small growing follicles. Haematoxylin and eosin stain. X40

Fig. 5: Thyroid gland of adult donkey showing ovoid duct-like UBF (*Asterisk*) presents in the vascular interlobar connective tissue and lined with typical single layer of cubical cells with central rounded nuclei and characteristic pseudostratified or stratified epithelium of UB follicle (*Arrowheads*). Notice the associating small growing follicles (*Double arrowheads*), epithelial outgrowths (*Arrows*) arising from the follicular wall, cellular streak (CS), and solid cellular masses with cystic formation (S). Haematoxylin and eosin stain. X100

Fig. 6: Thyroid gland of 6 months old donkey showing a group of small sized tubule and cystic-like UBFs of various shapes sometimes containing intraluminal colloid with various staining intensities (*Asterisks*), epithelial outgrowths from the follicular wall (*Arrows*), and the associated SCNs (S) with cystic formations (*Double arrowheads*). Some UBFs attached to the SCNs, forming mixed follicles (MF) lined by a single layer of cubical epithelial cells on one side, and cells of SCN on the other side. Haematoxylin and eosin stain. X100

Fig. 7: Thyroid gland of adult donkey showing main duct-like UB structure with wide lumen (*Asterisk*) giving rises to several tubule-like follicles (*Double Arrows*) into the surrounding connective tissue. Notice the associating secondary derivatives including the cellular outgrowths from the follicular epithelium (*Arrow*), differentiating small secondary UBFs (*Double arrowheads*) and solid cell masses (S) with cystic formation (CN) in some of them. Haematoxylin and eosin stain. X100

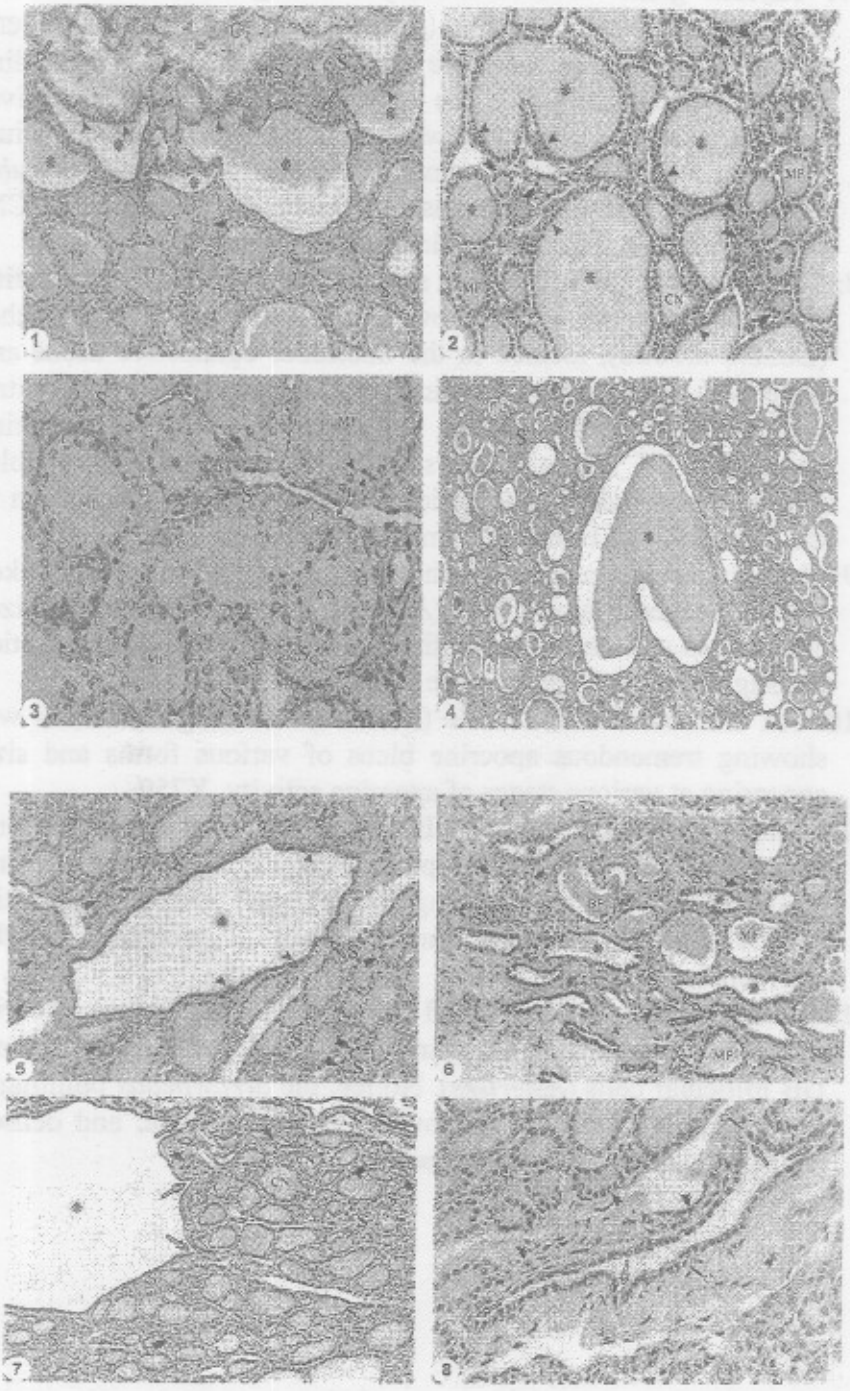
Fig. 8: Closer view of the upper right sector from Figure 7 demonstrating a tubule-like UBF with narrow lumen and finger like branches (*Double arrows*), located in the interlobar connective tissue and lined with single layer of typical cubical cells containing central dark rounded nuclei. Notice the clear cells (C) in the lining epithelium of UB tubule, small differentiating UB tubules (*Double arrowheads*) and solid cell masses (S) in association to the main follicle. Haematoxylin and eosin stain. X400

Fig. 9: Scanning electron micrograph of thyroid gland in adult donkey showing several large UBFs (*Asterisks*) possessing various sizes and shapes and associated with small secondary follicles. Notice the slightly folded wall of large UBFs. X200.

Fig. 10: An ultimobranchial follicle (*Asterisk*) with slightly folded wall showing tremendous apocrine blebs of various forms and sizes appearing at various stages of apocrine activity. X750.

Fig. 11: Inner surface of thyroid UB follicle showing flat polyhedral cells, each presents pleomorphic microvilli and single central cilium (*Arrowheads*) projecting over the cell surface. Notice the microvilli are numerous and densely aggregated at the intercellular borders. X3500.

Fig. 12: Inner surface of thyroid UB follicle showing various apocrine protrusions (aposomes) releasing from the apical surface of some UB follicular cells (*Asterisks*). Notice, the *arrowheads* pointing to a single cilium and the microvilli are pleomorphic, and densely aggregated at the intercellular borders. X3500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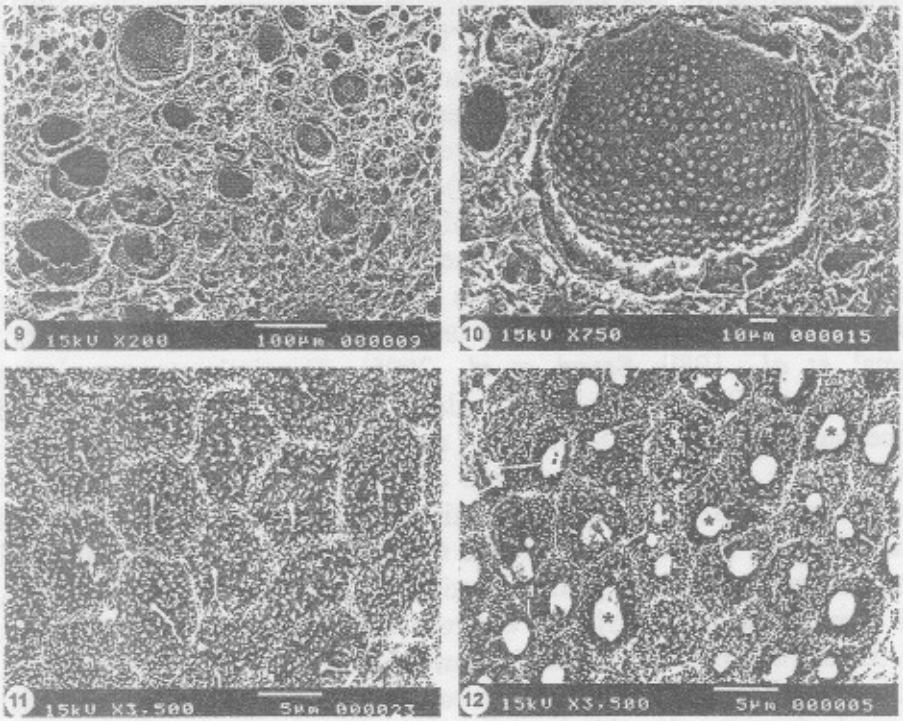

DISCUSSION

1. General remarks

The thyroid gland of mammals consists of two cell types, namely, follicular cells and C cells. These two cell types are of distinct embryonic origins. During development, the thyroid diverticulum, which is derived from the endodermal epithelium of the ventral pharyngeal floor, moves caudally down along the midline and forms two lateral lobes, giving rise to follicular cells. In contrast, the ultimobranchial body develops from the fourth pharyngeal pouch and migrates to its final place of residence, the thyroid gland, giving rise to the C-cells (Kameda *et al.*, 2007). The UBB is composed of cells and mesenchyme of neural crest origin that migrates caudally and attract the thyroid or fuses with the lateral lobes of the thyroid during embryonic life (Sugiyama, 1971; Sadler, 1990). Although minor in mass, UBB contribution to the thyroid is important because this structure has been cited as the source of calcitonin-producing cells in mammals including human (Pearse and Polak, 1971;

Polak, *et al.*, 1974). Calcitonin propably serves as an important regulator of fetal ossification and protect against hypercalcemia (DeLellis, 1981). In mammals the UBB is completely incorporated into the thyroid gland parenchyma and remains there as scattered endocrine cells (calcitonin or parafollicular cells) (Nunez and Gershon, 1978; Srivastav and Rani, 1988), whereas in non-mammalian vertebrates it is present as a discrete organ (Dacke, 1979; Robertson, 1986).

Therefore, the thyroid gland of the donkey in the present study exhibited as in most mammalian species, a secondary component; the ultimobranchial remnants of various forms and locations, which have been described alongside with the usual thyroid follicles (Sehe, 1966; Nagpal *et al.*, 1989; Sayed *et al.*, 2005). The intimate association between the UBB and the thyroid gland has been described as typical (Nunez and Gershon, 1978; Srivastav and Rani, 1988 and Harach, 1988). Although the same tendency which definitely relate the possibility of association of UBB with parathyroid tissue in goat (Roy *et al.*, 1978) and in rat (Calvert, 1975) or with thymic tissue (Roy *et al.*, 1978). Since all these tissues were pharyngeal derivatives as described by Arey (1966), their close association intricated and resulted into occurrence of UBFs in one tissue or other. While in the lower vertebrates (fish, amphibians, reptiles and birds), the UB bodies, secreting hormone calcitonin, exist separately from the thyroid gland (Irmak and Kirici, 2004). Defective or absent development of the fourth pharyngeal arch and therefore the UBB, results in DiGorge anomaly (DGA), principally affects derivatives of the third and fourth branchial pouches, including the UB body (Lammer and Opitz, 1986). Williams *et al.* (1989) reported that the thyroidal malformation owing to maldescent of the central thyroid, showed UBB derived thyroid follicular cells, not of endodermal origin in man.

The UBBRs in donkey thyroid gland of present investigation were described in form of ectopic UB follicles, cysts and tubules of various sizes and shapes, in association to solid and/or cystic cellular masses and mixed follicles (Harach 1988; Beckner, *et al.*, 1990). This observation is also indicated by Roy *et al.* (1978) in goats, Kirkeby and Zelander (1979) in guinea pig and Sayed *et al.* (2005) in buffaloes.

2- Occurrence, morphological features and structure of UBFs

In consistency with the previous literatures of Clark (1971) and Takagi and Yamada (1982), various shapes and sizes of ectopic UB follicles; wide and narrow tubular UB follicles; long branched and short small tubular like follicles, wide duct-like follicles; irregular or labyrinth-like cystic follicles; bi-lobed and/or mulilobulated cystic-like ones were

detected in the thyroid gland of donkey. These follicles were present singly or in groups and located peripherally under the capsule or situated deeply in the interlobar connective tissues surrounded by thyroid follicles inside the thyroid gland. Their lumen was empty or filled with light or dense colloid-like substances. This finding is also in agreement with that described in goat thyroid (Roy *et al.,* 1978) and in buffalo thyroid (Sayed *et al.,* 2005).

3. Lining epithelium and cell populations of UBFs

The epithelial lining of UB follicular wall varied from simple squamous to stratified in addition to the usual cuboidal follicular epithelium (Wetzel and Wollman, 1969 and Neve and Wollman, 1971; Takagi and Yamada, 1982). Stratification of the epithelial cells has been considered as criterion to distinguish UB follicles (Roy *et al.,* 1978). In the present study the UBFs were lined mostly with single layer of normal simple follicular epithelium in addition to stratified and pseudostratified epithelium in some places of the follicular wall, while other follicles showed attenuated squamous epithelial lining. The cellular components of UB epithelium comprised of usual follicular cuboidal cells and the clear C-cells. Other UB follicles showed the cells of SCNs merged with their follicular wall lining epithelium. It can be deduced that the UB follicles act as a source of follicular cells, needed to form small new thyroid follicles (Calvert, 1972, 1975). Epithelial lining of most UBFs in the present surface ultrastructural study revealed the presence of apical microvillus border, apical cytoplasmic blebs, as well as single cilium projecting over most of UB follicular cells. This finding is in accordance with that presented by Atoji *et al.* (1999) in one-humped camel and Sayed *et al.* (2005) in buffalo. While studying the ultrastructure of UBG of *Pseudemys scripta* and *Chrysemys picta* (Khairallah and Clark, 1971) reported a single cell type in the follicular epithelium. These cells are well equipped for protein and polysaccharide synthesis, having well developed Golgi regions, enlarged endoplasmic reticulum cisternae and a large number of free ribosomes. A distinctive characteristic of these cells is the presence of numerous small electron dense granules (150- 250 nm), which are bounded by a smooth surfaced membrane. There is evidence of release of these granules from the distal areas of the cells into the surrounding extracellular space. Another unusual feature is the presence of numerous large cytoplasmie bodies or granules (800-1000 *nm* in diameter) found in the luminal region of the cells in close association with apical plasmalemma.

In human branchial-like cystic remnants in the thyroid gland, the epithelial lining of the cysts was usually stratified squamous epithelium but may be focally respiratory- type epithelium (Carter and Ulusarac, 2003) or lined by few layers of squamous cells filled with mucinous material (Michal *et al.*, 2005).

4. Structure, morphological types and cell populations of SCNs or C-cell complexes

Several histological and immunohistochemical studies have been made on the occurrence of solid cell nests, as an UB remnants in the thyroid gland of mammals and human (Harach, 1985, 1988; Cameselle-Teijeiro *et al.*, 1994). This current study is in line with the previous studies which emphasized that these structures (SCNs) display 3 morphological types; solid and cystic clusters or mixed follicles that may be made of follicular cells admixed with SCNs and contained colloid substance in their lumens (Harach and Wasenius, 1987; Mizukami *et al.*, 1994). The present observation is also emphasized in study of Harach (1988) who concluded that the solid and cystic cell clusters as well as the mixed follicles were made of follicular epithelium and that these structures are considered to be normal components of the thyroid gland and of UB origin. The cell clusters of present study (SCNs) is in agree with that described in the previous literatures which were found to be composed of main polygonal basophilic cells with round to oval nuclei and larger size C-cells with pale staining cytoplasm and formed a small proportion of the SCNs (Cameselle-Teijeiro *et al.*, 1994; Martin *et al.*, 2000). Some authors found that SCNs in human were electron microscopally comparable to mammalian UB tissue (Harach and Wasenius, 1987). Kuhn and Malvi (2006) observed solid and cystic cell nests, as UB body remnants in human thyroid gland. Other authors did not observe C-cells in SCNs of human thyroid (Ozaki *et al.*, 1991), whereas others reported them inside SCNs (Mizukami *et al.*, 1994; Reis-Filho *et al.*, 2003). Harach (1985) and Martin *et al.* (2000) demonstrated that C-cells occurred in 29-50% of SCNs in human thyroid and were mainly located in the periphery of the nests (Mizukami *et al.*, 1994). Mixed UB follicles, a constituent of UBBRs in human were comprised of cells resembling main cells of SCNs and follicular cells, forming lumen–like pattern which contains colloid–like material and cell debris (Harach and Wasenius, 1987; Cameselle-Teijeiro *et al.*, 1994).

According to Leblanc *et al.* (1990), the SCNs or C-cell complexes were found to be contained four cell types in various proportions: 1) follicular cells staining for thyroglobulin; 2) C-cells staining for

calcitonin, calcitonin gene-related peptide, and neuron specific enolase; 3) cuboidal and stellate cells in follicle-like structures are staining positively for somatostatin; and 4) undifferentiated cells staining negatively for all of above mentioned antigens. Immunohistochemical evaluation of SCNs has revealed that main cells and C-cells express high and low molecular weight cytokeratins, as well as carcinoembryonic antigen, but show a differential expression of neuroendocrine markers. The main cells are usually positive for somatostatin and neurotensin, while C-cells are usually immunoreactive for calcitonin and calcitonin gene-related peptide (Reis-Filho *et al.,* 2003). Moreover, it is tempting to speculate that C-cells of SCNs have already triggered their pathways towards a parafollicular differentiation (Reis-Filho *et al.,* 2003). However, it is found that the cuboidal cells in the mixed follicles were negative for p63 and cytokeratins, while were positive for thyroglobulin, so those cells arranged in follicular–like pattern and have also triggered their differential pathways towards a follicular differentiation (Reis-Filho *et al.,* 2003). SCNs may pose some difficulties in routine thyroid pathology practice. Harach (1988) and Cameselle–Teijeiro *et al.* (1994) stressed that SCNs may be confused with squamous metaplasia and carcinoma, mucoepidermoid carcinoma of the thyroid gland and C-cell hyperplasia. The presence of different cell populations in SCNs, suggests the existence of a stem cell for C-cells or more cellular types in the UB nests (Wollman and Hilfer, 1978).

5. Presence of apocrine activity in the UB follicular epithelial cells

The present study demonstrated the presence of apocrine activity in the UB follicular epithelial cells in the thyroid gland of donkey. A similar mode of secretion has been revealed by both scanning and electron microscopy in the thyroid gland of one-humped camel (Sayed *et al.,* 1998; Atoji *et al.,* 1999) and buffalo (Sayed *et al.,* 2005). No apocrine secretion of UB follicular epithelial cells has been found in the thyroid glands of the other mammalian species studied so far (Fujita, 1975). Apocrine secretion is morphologically characterized by the presence of apocrine protrusions over the level of tight junction, on the apical or free surface of secretory cells (Kurosumi *et al.,* 1984). Dome or balloon-shaped apocrine protrusions with a smooth surface and contain homogeneous or fine granular materials with few organelles, which are large enough to be easily detectable by either light or electron microscopy (Atoji *et al.,* 1999). However, no description of apocrine secretion in the thyroid gland of donkey is found in the literature. The surface ultrastructural features of apocrine protrusions in the UB

follicular cells of present investigation are very similar to those described in the thyroid gland of one-humped camel (Atoji *et al.*, 1999) and buffalo (Sayed *et al.*, 2005). In conclusion, apocrine release in the UB follicular cells in the thyroid gland of donkey is not an artifact but rather an extrusion mechanism of soluble or membrane-associated proteins.

6. Presence of well developed apical microvillus border in the UB follicular epithelial cells

The present investigation of donkey's thyroid gland showed a well developed apical microvillus border of UB follicular epithelial cells. This finding is in line with that revealed in the one-humped camel (Sayed *et al.*, 1998; Atoji *et al.*, 1999) and buffalo (Sayed *et al.*, 2005). De Groot and Stanbury (1975) referred these microvilli as to serve for increasing the cell secretory surface. Banks (1981) postulated that the microvilli move in and out of the colloid along the apical border of follicular cells. However, administration of TSH induces the formation of multiple pseudopods or microvilli in the thyroid follicular cells (Ekholm and Wollman, 1975). The present findings together with that of previous investigations suggested that the presence of more or less developed microvillus border is closely associated with the activity of thyroid gland.

7. Presence of single cilium projecting over the apical free surface of UB follicular epithelial cells

The presence of ciliated cells has already been reported in human cystic and solid remnants in fetal thyroid (Beckner *et al.*, 1990), in infant thyroid (Carpenter and Emery, 1976), and in the thyroid of several mammals, such as the guinea pigs (Zelander and Kirkeby, 1977). In the present investigation a single cilium is protruding from the apical surface of most UB follicular cells in thyroid gland of donkey. A similar observation has been reported in the one-humped camel (Sayed *et al.*, 1998; Atoji *et al.*, 1999) and buffalo (Sayed *et al.*, 2005). Gould *et al.* (1981) observed one to four cilia are protruding from the apical surface of each follicular epithelial cell in human thyroid. In this respect, however, cilia have been also observed in the cells of some tissues or organs among which is the thyroid gland of chickens and dogs (Ghadially, 1988). The significance of such cilia remains unclear; however it may possess a chemoreceptor or mechanoreceptor function (Ghadially, 1988).

8. Anatomical and functional significance of UBBRs in mammalian thyroid gland

The UBBRs contributes to the embryological development of the thyroid gland in mammals (Apel *et al.*, 1994), and are believed to be

involved in the development of calcitonin cells (Patey *et al.*, 1996). SCNs and mixed thyroid follicles, constituents of UBBRs, contributes both C-cells and follicular cells to the thyroid gland in mammals including human (Williams *et al.*, 1989; Conde *et al.*, 1992). Calcitonin produced by C-cells, in this setting, probably serves as an important regulator of fetal ossification and may protect against hypercalcemia during the prenatal period when there is high calcium absorption (DeLellis, 1981). Additional substances produced by C-cells include somatostatin, bombesin, substance P, gastrin-releasing peptide, histaminase, chromogranin and neuron-specific enolase (Santa *et al.*, 1988). In addition, these cells exhibit the characteristics of amine precursor uptake and decarboxylation (APUD) cells such as argyrophilia and cytoplasmic electron dense-cored vesicles (Norris, 1985). Therefore, the thyroid C-cells are establishing as members of the diffuse neuroendocrine system.

On other hand, the UB tissue is suggested to be implicated in the genesis of mixed thyroid tumors (Bykov, 1993) such as mucoepidermoid carcinomas (Harach *et al.*, 1993; Ando *et al.*, 2008), primary sclerosing mucoepidermoid carcinomas (Harach *et al.*, 1993; Hunt *et al.*, 2004), branchial cleft-like cysts (Apel *et al.*, 1994; Michal *et al.*, 2006), as well as lymphoepithelial cysts (Carter and Ulusarac, 2003). In addition, the piriform sinus fistulae (remnants related to the UBB) trace the migration route of the UBB to the thyroid gland (Miyauchi *et al.*, 1992).

9. Conclusions

In conclusion, the UBB contribution to the thyroid gland is important because this structure has been cited as the source of both C- and follicular cells in mammalian thyroid including human. In addition, the UBBRs contribute to the embryological development and normal growth of thyroid gland in mammals, and are believed to be involved in the development and differentiation of thyrocalcitonin cells. Despite the anatomical and functional significance of UBBRs remains disputable, the heterogenous expression of antigens in SCNs has indicated that the cells comprising these structures have different biological roles; at least some C-cells and/or follicular cells would originate in SCNs. Furthermore, it has been suggested that these remnants of UBB might constitute the origin of some structures described in the thyroid gland as well as of some types of thyroid neoplasms. Future studies should center on the immunohistochemical and ultrastructural features of UBBRs in the thyroid gland of donkeys.

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