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Histomorphological Structure of the Ultimobranchial gland in Male Mulard Ducks (Cairina moschata × Anas platyrhynchos)

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ABSTRACT: The ultimobranchial gland of the mulard duck is a bilateral endocrine organ that is located in the thorax. The current study aimed to describe the topographical, anatomical and histological picture of the ultimobranchial gland in mulard ducks. Eleven healthy adult males of ducks were selected. Gross morphology and histological analysis of ultimobranchial tissue was performed. Grossly, ultimobranchial glands appeared yellow spherical bodies on both sides of the trachea near the syrinx, parallel to the common carotid arteries and cranio-lateral to the junction of the subclavian and common carotid arteries. Each ultimobranchial gland composed of two lobes adhered to each other and appeared as a single gland. Histologically, Ultimobrachial gland had a delicate connective tissue capsule around the gland that composed mainly of Collagenous fibers. The main cells of the glands were parafollicular or C cells which supported by connective tissue stroma. C cells having columnar shapes with centrally located oval nucleus. They were organize in many clusters of various sizes and also single C-cells were present scattered throughout the stroma of the gland. The C cells showed on reaction with PAS stain, whereas the connective tissue stroma and capsule were reacted positively with PAS stain. This study characterized ultimobranchial gland location, gross anatomy and microscopic features in mulard ducks

KEYWORDS: Ultimobranchial gland, Histomorphology, Ducks.

1. Introduction

Calcium is necessary for normal biological processes in birds, including bone and egg production, nerve transmission, muscle contraction as well as blood coagulation. The ultimobranchial glands are a source of the hybocalcemic peptide hormone or calcitonin hormone that regulates the balance of serum calcium and blocks the transfer of calcium from bone to blood [1, 2, 3, 4]. Most important hormonal systems involved in the regulation of calcium levels in the body; parathyroid hormone from parathyroid glands, calcitonin from ultimobranchial glands, vitamin D and the reproductive hermones especially estrogens [5, 6, 7, 6]. The ultimobranchial gland of lower vertebrates (birds, fish amphibian and reptiles) remains as separated organs during embryonic and adult life. Unlike the ultimobranchial glands of mammals, it is normally fused with thyroid gland shortly before birth and disappear in adult life [8, 9, 10, 11, 12].The ultimobranchial glands in birds are usually a pair of small bodies, each located on either side of the trachea caudal to the thyroid and parathyroid endocrine glands, generally close to the origin of the subclavian and common carotid arteries [13, 14, 15]. Much variation regarding the location of ultimobrachial has been reported by different workers [16, 14]. There is a little available information concerning the anatomical and histological aspects of ultimobranchial glands in birds. So, the aim of the present study is to give more information about the normal topographical, anatomical and histological picture of ultimobranchial glands in mulard ducks.

2. Materials and methods

2.1. Birds

The present work was performed on eleven adult males of healthy Egyptian moulard duck (or "mulard") which belong to the order Anseriformes. Mulards are one of the most popular duck types. These ducks are hybrids between male of Muscovy ducks (Cairina moschata) and female of White Pekins (Anas platyrhynchos). Like many hybrids, they are sterile, reared for meat which have a rich and

bold flavor. All specimens were obtained from the Assiut governorate. The mean body weight of the molar ducks were about 3000 - 3500 gm respectively. All of birds were slaughtered by using Halal method of slaughtering.

2.2. Ethical approval

The Ethics committee of Assiut University and Veterinary authority, Egypt has approved this study.

2.3. Gross Examination

Four birds were used for gross anatomical studies. The birds were incised from the cervical region to the thorax and the ultimobrancial glands and the surrounding tissues including the trachea, esophagus and blood vessels were exposed. The shape, colour, location and relative topographic in-situ position of ultimobranchial glands were recorded.

2.4. Histological examination

Five birds were used for the light microscopical analysis. Ultimobranchial glands were dissected carefully using a scalpel immediately after incision and they were fixed in Bouin's solution. The fixed specimens were dehydrated in ascending grades of ethanol, cleared in methyl benzoate, embedded in paraffin wax. Finally, paraffin blocks of the processed samples were prepared. Thin sections (5-6 µm thick) were cut, dried in an electrical incubator and stained with Harris's hematoxylin and eosin (H&E) for detection of general structure of the gland, Periodic Acid-Schiff (PAS) for detection of natural mucopolysaccharides, Masson's trichrome for detection of collagenous fibers[17].

2.5. Semi-thin and transmission electron microscopy

Two birds were used for semi-thin and transmission electron microscopy. Small specimens from ultimobranchial gland of duck were preserved in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.3 for 4 hours at 4 °C They were washed in the same buffer used and then post-fixed in 1% osmic acid in 0.1M Na-cacodylate buffer for further 2 hours at room temperature. The samples were then dehydrated in ethanol and embedded in Araldite-Epon mixture. Semi-thin sections (1µm in thickness) were cut and stained with Toluidine blue and examined under light microscope [18].

3. Results

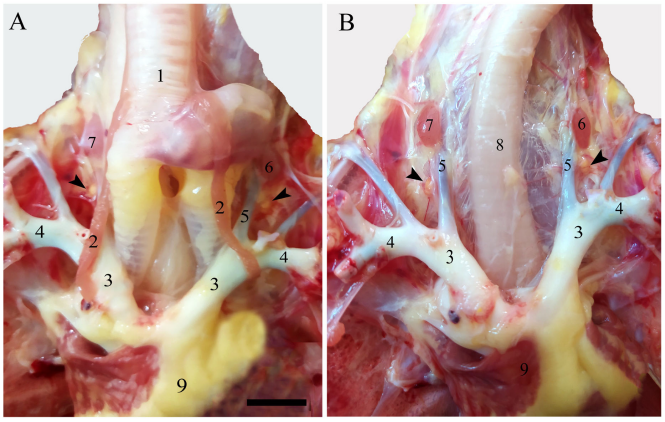

Figure 1: In situ ventral views of the thyroid glands in male mulard duck; (A) in presence the syrin and (B) after removal the syrinx showing: 1. Syrinx; 2. Sternotrachealis muscles; 3. Innominate artery; 4. Subclavian artery; 5. Common carotid artery; 6. Left thyroid gland; 7. Right thyroid gland; 8. Esophagus; 9. Heart; Ultimobranchial glands (black arrowheads).

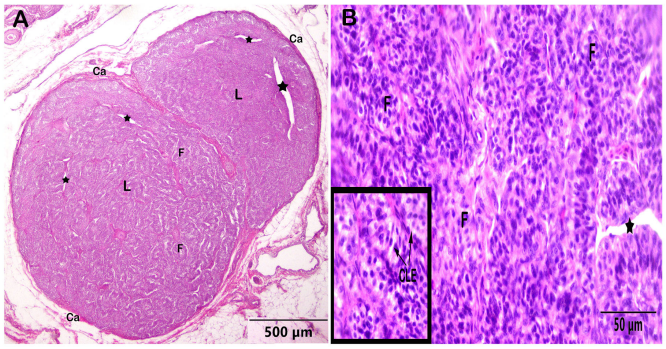

Figure 2: (A-B): Longitudinal paraffin sections stained with H&E in ultimobranchial gland of male mulard duck showing: The ultimobranchial gland composed of two lobes (L) which surrounded by a thin connective tissue capsule (Ca). The clusters of C-cells in the form of follicle like structure (F) consists of columnar or pseudostratified columnar epithelium (CLE). Blood vessels distributed within ultimobranchial gland (black stars).

3.1. Gross morphology and topographic location

The mulard duck has a pair of spherical-shaped ultimobranchial glands in yellow color on both sides of the trachea near the syrinx Fig. 1A. The glands were easily detected, not covered by adipose tissue. Each ultimobranchial gland composed of two lobes adhered to each

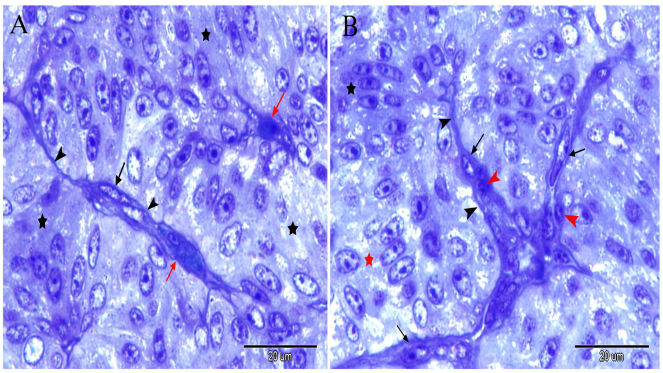

Figure 3: Semithin sections stained with toluidine blue in ultimobranchial gland of male mulard duck showing: Some C cells have dark cytoplasm (black stars) and others have light cytoplasm (red stars). The connective tissue stroma contains numerous telocytes (black arrows), fibroblasts (red arrows) and immune cells (red arrowheads).

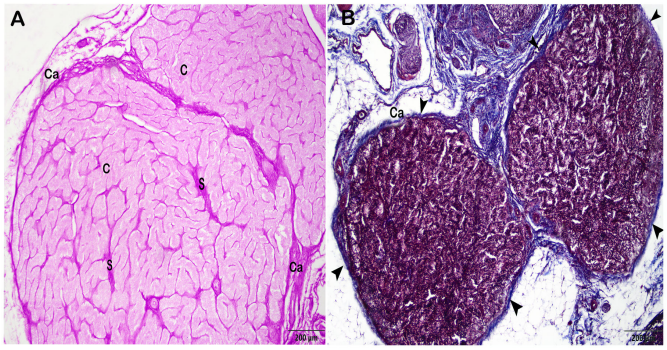

Figure 4: Paraffin sections in ultimobranchial gland of male mallard duck stained with PAS stain (A) and Masson's Trichrome stain (B) showing: A: The connective tissue stroma (S) and capsule (Ca) reacted positively with PAS stain. The C-cells (C) showed no reaction with PAS stain. B: The connective tissue capsule (Ca) which surrounded the ultimobranchial gland composed mainly of collagenous fibers (black arrowheads).

other and appeared as a single gland Fig. 1B). The ultimobranchial glands were located parallel to the common carotid arteries and cranio-lateral to the junction of the subclavian and common carotid arteries(Fig. 1) . The bilateral ultimobranchial glands were observed as considerably small in size and a symmetrically located on both sides. The left gland usually near to the caudal pole of the thyroid gland, while the right gland far away from the caudal pole of the thyroid gland, in a midway between the thyroid and junction of the subclavian and common carotid arteries (Fig. 1B).

3.2. Histological observation

Ultimobrachial gland had a delicate connective tissue capsule around the gland that composed mainly of Collagenous fibers (Fig. 4B). The parenchyma of the gland composed of parafollicular or C cells which supported by connective tissue stroma. The C cells having columnar shapes with centrally located oval nucleus. The cytoplasm of C cell was stained with a reddish color and the nucleus appeared light (Fig. 2 B). C cells organize in many clusters and also single C-cells were present scattered throughout the stroma of the gland. The clusters of C cells were placed as cords or duct-like follicles of various sizes. These follicles formed by single-layered follicular cells; unistratified or pseudostratified columnar epithelium (Fig. 2). Some follicles had C cells with clear cytoplasm and some follicles had C cells with dark cytoplasm (Fig. 2B). The C cells showed on reaction with PAS stain, whereas the connective tissue stroma and capsule were reacted positively with PAS stain (Fig. 4A). The blood vessels were distributed within the ultimobranchial glands, so they were highly vascularized glands (Fig. 4A). Semithin sections stained with toluidine blue revealed that; the parenchyma of ultimobranchial gland consists of cells apparently all of the same type, known as C cells which arranged in follicles and supported by connective tissue stroma. The C cells having columnar shapes and oval nucleus contains a mass of chromatin and one - two nucleolus. Some C cells have dark cytoplasm and others have light cytoplasm (Fig. 3). The connective tissue stroma contains numerous telocytes, fibroblasts and immune cells. Telocytes shows a relatively small fusiform cell body and two long cytoplasmic processes that called telopodes (Fig. 3). Fibroplast possessing spindle shaped cell body and actively secreating matrix with an oval euchromatic nucleus (Fig. 3A).

Discussion

The ultimobranchial glands are paired in birds, some amphibians, single in some salamanders and lizards as well as absent in some frogs and in caecilians [19, 20]. The ultimobranchial glands are paired spherical organs in mulard ducks in the current investigation as described earlier

by Moghanlo and Mohammadpour[21] in guinea fowl, Mohammed and Al-Badri[22] in geese and Hanaa and Shakir [23] in Iraqi turkey. In the present study, the ultimobranchial glands were small in size and yellowish in color. Dissimilar from the result of Hanaa and Shakir [23] in Iraqi turkey, Mohammed and Al-Badri[22] in geese and Moghanlo and Mohammadpour [21].in guinea fowls who reported that each of ultimobranchial glands was small in size and pink to dark red in color. Gross investigation on the ultimobranchial glands in mulard ducks appeared that the glands were not surrounded by adipose tissue and easily detected, which was in agreement with Mohammed and Al-Badri [22] in young geese and disagreement with Hodges[24] and King and McLelland[25] in domestic fowl, Hanaa and Shakir [23] in Iraqi turkey, Moghanlo and Mohammadpour [21] in guinea fowl and Mohammed and Al-Badri [22] in adult geese who revealed that the ultimobranchial glands were covered by a mass of adipose tissue which hide the boundaries of the glands. In the current study, the ultimobranchial glands in mulard ducks showed that each gland composed of two lobes adhered to each other and appeared as a single gland. This result was in line with the previous findings in pigeons by Bose and Das [26] who stated that each ultimobranchial gland consisted of two lobes, one lobe was located caudal to thyroid as diffused mass while the other lobe was situated caudal to parathyroid gland. The topographical findings of the ultimobranchial glands of mulard ducks in this study were almost the same as the previous findings mentioned by King and McLelland [25, 27], Moghanlo and Mohammadpour [21] in guinea fowl, Hanaa and Shakir [23]in Iraqi turkey, Mohammed and Al-Badri [22] in geese and Takagi and Yamada [28] in grass parakeet. Swarup and Das [9] stated example of move out of ultimobranchial gland towards thyroid leaving behind the parathyroid in Pied myna. The anatomical results in this investigation showed that, the pairs of ultimobranchial gland of mulard ducks were asymmetrically located within the thorax. These results are similar to those of guinea fowls [21]. Iraqi turkeys [23], young geese[22] and long-legged buzzards[29]. The Histological examination of the present work showed that the ultimobrachial gland enclosed by a thin connective tissue capsule that composed mainly of collagen fibers. These results were similar to those found in chickens [30, 25], guinea fowls [21], Iraqi turkeys [23], geese [22] and in grass parakeet [28]. The chief cells present in this study were the calcitonin cells or C cells as mentioned by Nike et al[31] in many domestic birds and Sasayaman et al. [3] in teleost fishs. The shapes of these cells were either rounded, oval or polygonal in shape, which was in agreement with Ito et al. [32] in chicken, Takagi and Yamada [28]) in grass parakeet, Mohammed and Al-Badri [22]in geese, Moghanlo and Mohammadpour [21] in guinea fowl, Hanaa and Shakir [23] in Iraqi turkey and Yadav and Srivastav [33] in pigeon. The present study supported the previous findings recorded by Mohammed and Al-Badri[22]in geese and Hanaa and Shakir [23]) in Iraqi turkey who revealed that, some groups of C cells having pale cytoplasm and other groups having dark cytoplasm. In the present investigation the ultimobranchial glands were highly vascularized glands, this result was analog with that stated by Mohammed and Al-Badri[22] in geese, Hanaa and Shakir [23] in Iraqi turkey, Takagi and Yamada [28] in grass parakeet, Yadav and Srivastav [34] and Bose and Das [26] in pigeon, Kameda et al. [35] and Ali et al. [36] in chiken.

Conclusion

The anatomical study on the ultimobranchial gland of ducks, showed that the glands asymmetrical located in the thorax on both sides of trachea, just cranio-lateral to the junction of the subclavian and common carotid arteries. Histologically, the main cells formed the ultimobranchial glands were calcitonin cells or C cells.

Conflict of interest

The authors declare no conflict of interest

4. Results and discussion

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