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Thyroid fibrous band and levator glandulae thyroideae muscle: two different structures associated with the pyramidal lobe of the thyroid gland

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Abstract: We investigated two structures that are in close association with the pyramidal lobe of the thyroid gland. Our investigation was performed using microdissection and histological examination in 106 human postmortem specimens. The first investigated structure was identified as the thyroid fibrous band that was present in 28.3% of cases. This band was always associated with the pyramidal lobe (which was significantly longer and thicker when associated with this band) and it had a constant hyo-pyramidal extension; it was located close to the midsagittal plane and predominantly composed of dense irregular connective tissue. The second investigated structure was the levator glandulae thyroideae muscle, which was associated with the pyramidal lobe in only 13.6% of cases. This muscle had a double extension, hyo-pyramidal and laryngo-pyramidal, located farther from the midsagittal plane, it was longer and thinner than the thyroid fibrous band and predominantly composed of striated muscle fibers. We confirmed our hypothesis that the thyroid fibrous band, which may be considered as the partial fibrous remnant of the thyroglossal duct and levator glandulae thyroideae, and which may be considered as infrahyoid or laryngeal muscle, are two different structures of the thyroid gland.

Keywords: anatomy; levator glandulae thyroideae muscle; pyramidal lobe; thyroid fibrous band; thyroid gland

INTRODUCTION

In our previous study, we reported the presence of a fibrous or muscular band found in close association with the pyramidal lobe of the thyroid gland [1]. This coexistence was present in 59.4% of cases and was found to be very important because the size of the pyramidal lobe was highly significantly dependent on the presence of this band. The pyramidal lobe was four times longer and its base was 2.5 times wider and three times thicker when associated with the presence of this band, defined by visual inspection of its structure using a dissecting magnifier. Also, this band has a dual origin because it might be a fibrous remnant of the thyroglossal duct or the levator glandulae thyroideae muscle.

The thyroglossal duct is a transient and epithelialized duct that develops along the migratory path of the median thyroid primordium close to the midline of the neck. This duct connects the primitive pharynx and descending median thyroid primordium and it becomes visible during the fifth week of gestation [2]. Descent of the thyroid gland is completed by the end of the 7th week of gestation [3], and the thyroglossal duct usually disappears by the end of the 10th week of gestation [4]. In some cases, the remnants of the thyroglossal duct may persist after the 10th week of gestation in the form of strip composed of fibrous or muscular tissue [5].

The levator glandulae thyroideae muscle is usually described as a unilateral muscle originating on either the hyoid bone or thyroid cartilage, and inserted on the pyramidal lobe, isthmus or lateral lobe of the thyroid gland [6-8]. This accessory muscle is classified into five types according to its origin and insertion: type I or hyopyramidalis, type II or thyreopyramidalis, type III or thyreoglandularis, type IV or hyoglandularis and type V or tracheoglandularis [9]. The first two

types of the muscle are associated with the pyramidal lobe of the thyroid gland. The incidence of the levator glandulae thyroideae muscle as reported in the literature ranges widely from 0.5% to 49.5% of cases [10-13], and the structure of this muscle is described as fibrous [13,14], fibroglandular [15], fibromuscular [13], fibromusculoglandular [16] or muscular [7,17].

In the present study, our aim was to resolve the dilemma regarding the origin and structure of the fibrous or muscular band found in close association with the pyramidal lobe of the thyroid gland, by combining microdissection and histological examination.

MATERIALS AND METHODS

Specimens and ethics statement

Investigation of the thyroid fibrous or muscular band (FMB) was performed in 106 human postmortem specimens without macroscopically visible pathological changes or thyroid disease history (61 male and 45 female, mean age 58.1 years, range from 15 to 85 years). The postmortem specimens were blocks of tissues including the tongue, hyoid bone with related muscles, larynx, trachea, thyroid gland, the distal portion of the pharynx and proximal portion of the esophagus. All postmortem specimens used in this study were obtained in accordance with the ethical standards and legal recommendations of the institutional research committee (Ethics Committee of the Faculty of Medicine, University of Belgrade, No.29/X-11) and with the 1964 Helsinki Declaration and its later amendments. All postmortem specimens were fixed in buffered formalin for at least two months.

Microdissection

After fixation, the postmortem specimens were carefully dissected under a dissecting magnifier in order to expose the thyroid gland and related structures. During microdissection of the thyroid gland, we clearly identified the FMB, but we noted that it was present either as a fibrous band or a muscular band according to visual inspection only. When the FMB was presented in the form of a fibrous band, it was a more rounded structure, whereas when it appeared in the form of a muscular band, it had a more slip-like structure.

Measurements and statistical analyses

Measurements were performed directly, using a digital electronic caliper (Womax, Germany) sensitive to 0.01 mm. Statistical analyses were performed using an inhouse program (Institute of Medical Statistics and Informatics, Faculty of Medicine, University of Belgrade). $P<0.05$ was considered significant.

Histological examination

In order to prove the real nature of the FMB, we decided to perform histological examination of this probable dual structure. The histological examination was performed in 13 specimens of the FMB following microdissection (9 specimens of the FMB in the form of a fibrous band and 4 specimens in the form of a muscular slip). The specimens of the FMB were embedded in paraffin, cut into 3-μm-thick transverse sections mounted onto adhesive-coated glass slides (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany), and dehydrated with a graded ethanol series. Sections were stained with hematoxylin and eosin (H&E), and according to Masson's trichrome staining for collagen of tissue sections. In addition, a basic immunohistochemical protocol was performed. In the first step of this protocol, the endogenous peroxidase activity was blocked in specimens during the incubation step with hydrogen peroxide (H_2O_2, 3%) for 5 min at room temperature. The slides with sections were placed into antigen retrieval solutions (0.01 M citrate buffer, pH 6.0 and epitope retrieval solution, pH 9.0) and heated in a microwave unit operating at 780 W for 20 min. Afterwards, the slides with sections were cooled for 20 min at room temperature, rinsed with distilled water for 5 min and exposed to Tris-buffered saline (TBS), pH 7.6. In the next step, the primary antibodies against collagen IV and striated and smooth muscle actin were used (Anti-Human Collagen IV, Clone CIV 22, 1:50; Anti-Sarcomeric Actin, Clone-Alpha-Sr-1, 1:100; Anti-Human Smooth Muscle Actin, Clone 1A4, 1:100; DAKO, Glostrup, Denmark) in order to visualize and prove the nature of the tissues in the FMB sections. The staining signals were developed and detected with a sensitive detection system following the manufacturer's instructions (Thermo Fisher Scientific, Lab Vision Ultra Vision LP Detection System: HRP PolymerDAB Plus Chromogen, Waltham, Massachusetts, USA). The glass slides with sections were covered with cover glass (Thermo

Scientific, Menzel-Gläser, Braunschweig, Germany) in a mounting medium (Permount, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Higher magnification images were captured with a Leica DFC295 digital color camera, mounted on a Leica DMLS research microscope. Histological examination confirmed the dual nature of the FMB, which was predominantly found as a fibrous band or a muscular slip.

Terminology

We used the term thyroid fibrous band when the FMB was predominantly in the form of a fibrous band. When the FMB was predominantly in the form of a muscular slip, it was identified as the levator glandulae thyroideae muscle. Both terms indicate the main histological nature of these two different structures and their association with the thyroid gland.

RESULTS

The thyroid fibrous band (TFB) is always associated with the pyramidal lobe

The TFB was found in 28.3% of cases (30 out of 106 specimens). When it was present, the TFB was always associated with the pyramidal lobe of the thyroid gland, which was found in 55.7% of cases (59 out of 106 specimens).

The size of the pyramidal lobe is influenced by the presence of the TFB

The size of the pyramidal lobe was influenced by the presence of the TFB as the pyramidal lobe was highly significantly longer (Z=2.725, P=0.008) and significantly thicker (Z=2.000, P=0.048) when associated with the TFB, compared to cases in which the pyramidal lobe was not associated with the TFB. We used the Mann-Whitney (sum rank) test to compare the measured parameters of these two groups when the distribution of these variables tested using the Kolmogorov-Smirnov test was not normal.

Location and size of the TFB

The TFB extended from the hyoid bone inferiorly to the apex of the pyramidal lobe with peripheral and circular insertion along its fibrous capsule and a central insertion with fibers that penetrated radially through the apex of the pyramidal lobe (Fig. 1). The TFB was always found in front of the thyrohyoid membrane and close to the midsagittal plane. The location of the TFB was compared as regards which side, and was found to be identical; in 15 specimens it was located on the left side and in 15 specimens it was located on the right side. The mean length of the TFB was 14.1 mm while the mean thickness of this structure was 3.0 mm (Table 1).

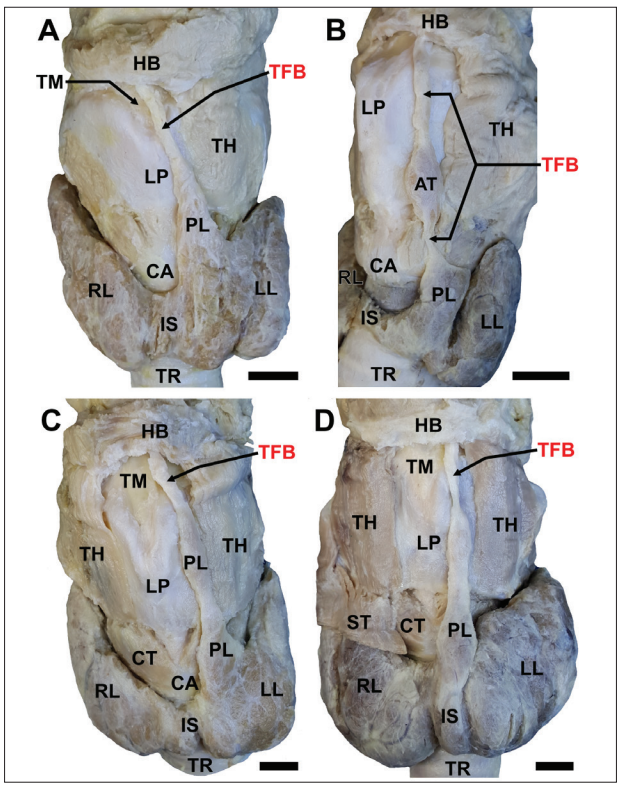

Fig. 1. Thyroid fibrous band (TFB) associated with the pyramidal lobe of the thyroid gland (PL). **A** – Specimen 82. **B** – Specimen 91. **C** – Specimen 96. **D** – Specimen 108. RL – right thyroid lobe; LL – left thyroid lobe; IS – thyroid isthmus; AT – accessory thyroid tissue ; LP – laryngeal prominence; HB – hyoid bone; TM – thyrohyoid membrane; CA – cricoid arch; CT – cricothyroid muscle; TH – thyrohyoid muscle; ST – sternothyroid muscle (reflected); TR – trachea. Scale bar – 1 cm.

Table 1. The size of the thyroid fibrous band (TFB) in 30 postmortem specimens, demonstrated by two measured parameters (length and thickness) and standard deviations (SD). Means are presented with the range (minimum-maximum) given in parentheses.

TFB	Mean (mm)	SD
Length	14.1 (2.9-49.5)	10.7
Thickness	3.0 (1.0-5.4)	1.1

The TFB is predominantly composed of dense irregular connective tissue

Histological examination confirmed a predominant fibrous nature of the TFB; it is predominantly composed of dense irregular connective tissue. The fibers in this tissue did not show a clear orientation and they were rather interwoven into a mesh-like network. These fibers were mainly collagen fibers with their typically wavy appearance, and fibroblasts were observed as the predominant cell type scattered sparsely across the tissue (Fig. 2A, B). The density of these collagen fibers was higher inside the most superior part of the TFB, immediately below its attachment to the hyoid bone. We did not identify any lumen-like spaces within the TFB in the specimens used for histological examination.

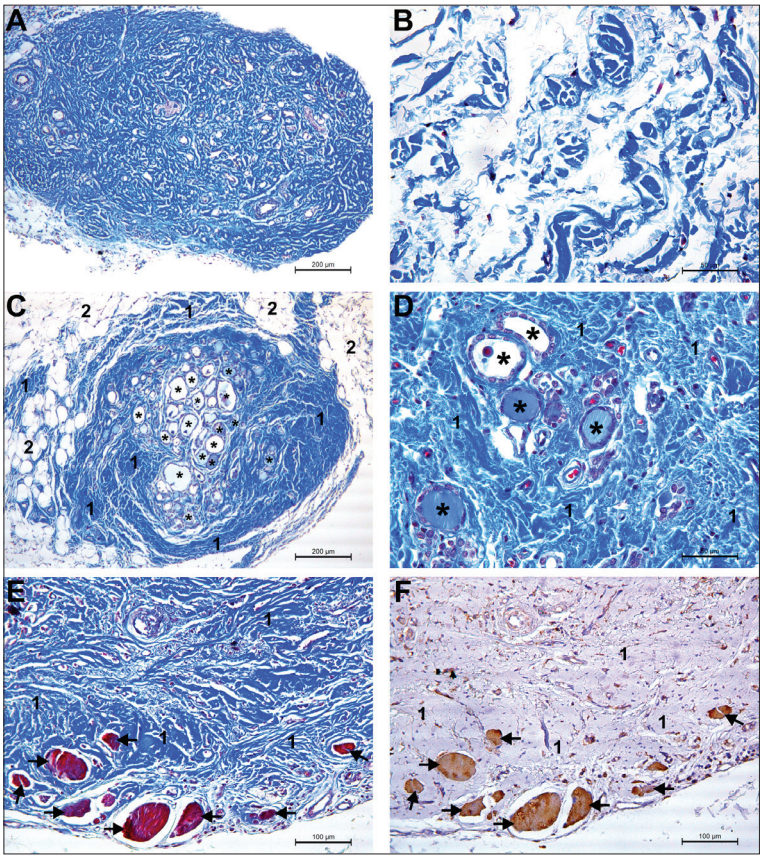

Fig. 2. Thyroid fibrous band (TFB) and histological findings. **A** – Specimen 77, Masson's trichrome staining indicating the predominantly fibrous nature of the TFB with a dense network of collagen fibers (blue-stained). **B** – Specimen 108, Masson's trichrome staining showing collagen fibers with their typically wavy presentation. **C** – Specimen 78, Masson's trichrome staining indicating thyroid tissue in the central portion of the TFB with numerous and densely packed follicles (black asterisks). **D** – Specimen 96, Masson's trichrome staining showing multiple thyroid follicles (black asterisks) which are filled with a colloid-like substance in part (blue-stained content) and surrounded by well-developed connective tissue of the TFB. **E** – Specimen 82, Masson's trichrome staining indicating rare striated muscle fibers inside the TFB (black arrows). **F** – Specimen 82, α-sarcomeric actin immunostaining showing a rare striated muscle fibers inside the TFB (black arrows). 1 – dense irregular connective tissue with a dominant network of collagen fibers; 2 – adipose tissue with adipocytes appear as empty profiles.

Ectopic thyroid tissue is frequently present along with the TFB

Accessory thyroid tissue was found along the TFB (Fig. 1B) as well as normal thyroid follicles trapped in the TFB (Fig. 2C, D). The thyroid tissue associated with the TFB was found in 77.8% of cases (in 7 out of 9 histological specimens of the TFB). The presence of thyroid follicles was more frequent in the upper parts of the TFB, close to the hyoid bone. These thyroid follicles were interposed among the fibers in the form of islands, lined by the cuboidal epithelium and often filled with a colloid-like substance (Fig. 2C, D). The presence of a colloid-like substance inside the follicles indicated their functional activity. In addition to the thyroid tissue, adipose tissue was also found along the TFB (in 44.4% of cases, 4 out of 9 histological specimens of the TFB). The presence of the striated muscle fibers was rare inside the TFB. In only one of our histological specimens (i.e. 11.1% of cases, 1 out of 9 specimens), we found grouped striated muscle fibers in two spots (Fig. 2E,F); these muscle fibers were surrounded by dense irregular connective tissue.

The levator glandulae thyroideae (LGT) is the infrahyoid or laryngeal muscle

The LGT muscle was found to be associated with the pyramidal lobe in 13.6% of cases (in 8 out of 59 specimens). In 4 out of these 8 specimens, it was in the form of a single muscular slip extending inferiorly from the hyoid bone to the apex of the pyramidal lobe (Fig. 3). When it was present in the form of a single muscular slip, the LGT was located farther from the midsagittal

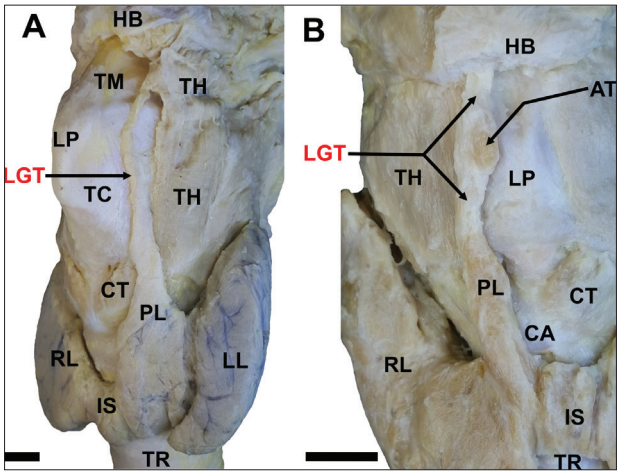

Fig. 3. Levator glandulae thyroideae muscle (LGT) associated with the pyramidal lobe of the thyroid gland (PL). **A** – Specimen 39. **B** – Specimen 92. RL – right thyroid lobe; LL – left thyroid lobe; IS – thyroid isthmus; AT – accessory thyroid tissue ; TC – thyroid cartilage; LP – laryngeal prominence; HB – hyoid bone; TM – thyrohyoid membrane; CA – cricoid arch; CT – cricothyroid muscle; TH – thyrohyoid muscle; TR – trachea. Scale bar – 1 cm.

plane compared to the location of the TFB. The LGT in the form of a single muscular slip was more frequently located on the left side of the midsagittal plane (in 3 out of 4 specimens). According to the location of this single muscular slip and the origin of its superior attachment, it can be considered as the infrahyoid muscle. The mean size of this single muscular slip was 29.9 mm (length), 4.65 mm (width) and 1.03 mm (thickness).

In the remaining 4 of these 8 specimens, the LGT was in the form of muscular slips extending from the thyroid cartilage of the larynx to the pyramidal lobe. When found in this form, the LGT was also located farther from the midsagittal plane and more frequently on the left side of the midsagittal plane (in 3 out of 4 specimens). According to their attachments, these muscular slips may be considered as laryngeal muscles in association with the pyramidal lobe.

The LGT is a striated muscle

Histological examination showed that the LGT was a striated muscle composed of muscle fibers that appeared with roughly circular or polygonal outlines in the thin transverse sections used (Fig. 4A, B). These muscle fibers were arranged in small and irregular groups (bundles or fascicles), visible within the muscular slips. The surface of the LGT was covered by the visible fascia in the form of a thin sheet of connective tissue (Fig. 4C). Immunohistochemical expression of α-smooth muscle actin was detected only in the walls of blood vessels.

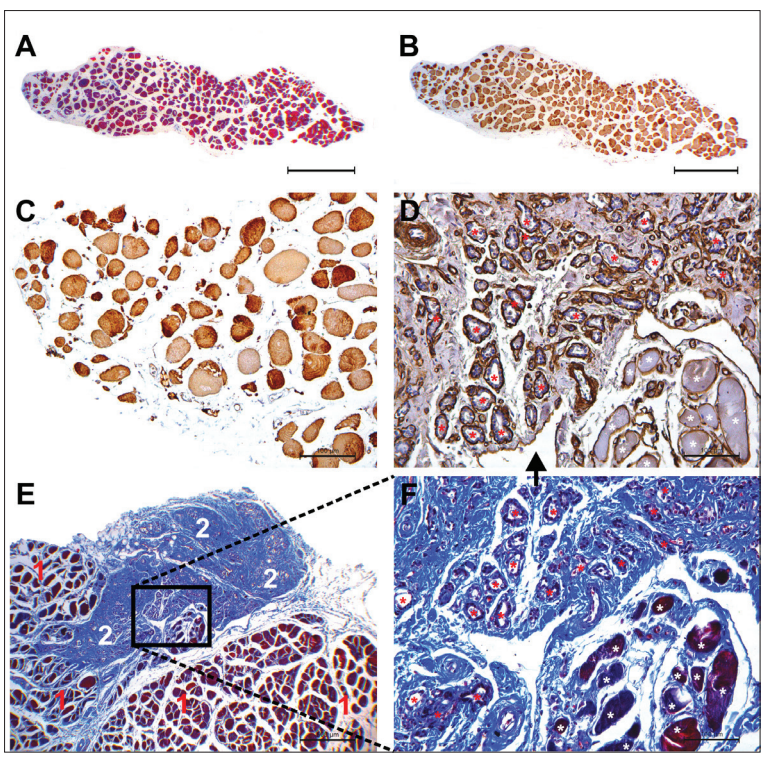

Fig. 4. Levator glandulae thyroideae muscle (LGT) and histological findings. **A** – Specimen 92, Masson's trichrome staining indicates the striated muscle fibers (circular or polygonal red-stained outlines) with sparse perimysium (blue-stained). **B** – Specimen 92, α-sarcomeric actin immunostaining showing expression in muscle fibers. **C** – Specimen 92, α-sarcomeric actin immunostaining indicating positive muscle fibers. **D** – Specimen 94, collagen IV immunostaining showing expression in the basement membranes of thyroid follicles (red asterisks); muscle fibers are marked by white asterisk. **E** – Specimen 94, Masson's trichrome staining indicates normal thyroid tissue surrounded by well-developed connective tissue stroma (2) hidden under the deep surface of the LGT (1). **F** – Specimen 94, Masson's trichrome staining showing multiple thyroid follicles (red asterisks) adjacent to the striated muscle fibers (white asterisks). Scale bar – 500 μm (4A, B).

Table 2. The main differences between the thyroid fibrous band (TFB) and the levator glandulae thyroideae muscle (LGT) as structures in close association with the thyroid gland.

	TFB	LGT
Structure	Predominantly fibrous	Predominantly muscular
Extent	Constant hyo-pyramidal	Variable hyo-pyramidal
Location	Close to the midsagittal plane	Farther from the midsagittal plane
Length	Shorter	Longer
Thickness	Thicker	Thinner
Thyroid follicles	Very frequent	Less frequent

Ectopic thyroid tissue is frequently present along with the LGT

Accessory thyroid tissue was detected along the LGT (Fig. 3B) as well as normal thyroid tissue (Fig. 4D-F). The thyroid tissue associated with the LGT was found in 50% of cases (in 2 out of 4 histological specimens of the LGT). This thyroid tissue was found either inside the LGT, where it was surrounded by muscle fascicles, or hidden under the deep surface of the LGT (Fig. 4E). Numerous and densely packed thyroid follicles were surrounded by well-developed connective tissue stroma (Fig. 4D, F).

DISCUSSION

We found that the TFB and LGT are two different structures of the thyroid gland and we confirmed our previous hypothesis about the dual nature of the fibrous or muscular band (FMB) which was found in close association with the pyramidal lobe of the thyroid gland [1]. The thyroid fibrous band was identified in 28.3% of cases as the fibrous band composed of dense irregular connective tissue with a constant hyo-pyramidal extension. It is located close to the midsagittal plane along the developmental migratory path of the median thyroid primordium, indicated by the thyroglossal duct, which is usually obliterated during the period between the 7th and 10th weeks of gestation [18]. The pyramidal lobe of the thyroid gland develops around the inferior portion of the thyroglossal duct and it may be considered as the most caudal remnant of the thyroglossal duct [13,19]. Accessory thyroid tissue was found along the TFB as well as normal thyroid follicles trapped in the TFB in the form of islands. This accessory thyroid tissue found above the thyroid gland is described as the tissue derived from the thyroglossal duct [20]. These findings lend support to our previous hypothesis that the TFB represents a fibrous remnant of the thyroglossal duct [1]. Actually, it might represent the partial fibrous remnant of the thyroglossal duct that stretched between the body of the hyoid bone and the apex of the pyramidal lobe. Also, if the pyramidal lobe of the thyroid gland and the TFB represent the remnants of the same structure as that of the thyroglossal duct, we should expect a certain type of stronger correlation between these two structures. Our two findings support this correlation between the pyramidal lobe and the TFB. The first finding was of a constant coexistence between these two structures that was found in 28.3% of cases. The second was the strong influence of the TFB on the size of the pyramidal lobe since the pyramidal lobe is highly significantly longer and significantly thicker when associated with the TFB.

The question concerning the possible TFB development process needs to be discussed. The structure comparable to the TFB as a remnant of the thyroglossal duct is not visible in human embryos and fetuses during 6-15 weeks of gestation in the area between the hyoid bone and thyroid cartilage [3]. During this period of gestation, the hyoid body is not located anterior to the epiglottis and is clearly separated from the thyroid cartilage, as it is in adults. It is located anterior and close to the thyroid cartilage so that the hyoid body and the thyroid cartilage are located at the same superior-inferior level because the neck is still not completely extended [3]. At the same time, the pyramidal lobe of the thyroid gland extends upward to a site near the hyoid body, or to the restricted area between the hyoid body and the thyroid cartilage. The descent of the thyroid gland is already completed at the end of the 7th week of gestation when it occupies its location at the level of the 3rd to the 6th cervical vertebrae [3]. The separation between the hyoid bone and thyroid cartilage occurs much later, as the neck extends in combination with laryngeal descent [3,21]. During this period of separation, a remnant of the thyroglossal duct or the connective tissue in association to the pyramidal lobe of the thyroid gland might also be extended in order to form the TFB, as was recorded in our specimens. Further studies on the embryology of

the connective tissue in association to the pyramidal lobe might resolve the dilemma about the origin of the TFB, but they are extremely demanding.

An early description of the TFB was given by Gruber [22] and mentioned later [24]. This structure was described as the "ligamentum suspensorium lobi pyramidalis", which extends from the apex of the pyramidal lobe to the hyoid bone. The same structure was also noted as the "fibrous tissue" that connects the pyramidal lobe with the hyoid bone, found in 11.5% of cases (in 12 out of 104 specimens in which the pyramidal lobe was present) [20]. Organ and Organ [24] stated that the thyroglossal tract may persist as a fibrous cord, while Ozgur et al. [13] described a band of connective tissue that continues from the apex of the pyramidal lobe to the hyoid bone containing the accessory thyroid tissue. The abovementioned band of connective tissue, which is comparable to the TFB, was described as the structure that develops from the connective tissue remnants of the thyroglossal duct [13] or is clearly distinguished as the "connective tissue remnant of the thyroglossal duct", as per the translation of the German term "Bindegewebiger Űberrest des Ductus thyreoglossus" [25].

The TFB may be the origin of thyroglossal duct-associated lesions. One of these rare lesions is the thyroglossal duct cyst in cases when the thyroglossal duct persists [26], and 76% of these cysts (in 520 out of 685 cases) are infrahyoid in their location [27], precisely in the region where the TFB was found. Another of these rare lesions is papillary thyroid carcinoma, which was identified in 7.4% of cases (in 18 out of 242 thyroglossal duct-associated lesions) according to the study of Wei et al. [26]. The origin of this malignancy may be accessory thyroid tissue found along the TFB or normal thyroid follicles trapped in the TFB in the form of islands. These findings may define the TFB as a structure of potential risk for the development of malignant thyroid disease.

Malignant transformation of ectopic thyroid tissue is relatively uncommon (less than 1%), and ectopic thyroid tissue was observed in the midline of the neck, along the thyroglossal duct in 21% of cases (in 6 out 28 ectopic thyroid tissues with different locations) [28]. Also, the frequent presence of ectopic thyroid tissue along with the TFB (78%) and LGT (50%) should be considered during extensive surgical treatment of malignant thyroid disease. Surgical excision of these two structures associated with the pyramidal lobe during total thyroidectomy may reduce the risk of thyroid recurrence. Thyroid recurrence after thyroid lobectomy or total thyroidectomy in patients with papillary thyroid carcinoma was observed in 2.9% of cases (in 5 out of 173 patients) [29]. The LGT muscle is associated with the pyramidal lobe in 13.6% of cases in the form of single or multiple muscular slips composed of striated muscular tissue. The LGT associated with the pyramidal lobe is located on the left side of the mid-sagittal plane (75% of cases) and farther from it when compared with the location of the TFB. The extent of this muscle is variable and it may be hyo-pyramidal when it extends from the hyoid bone to the pyramidal lobe, or laryngo-pyramidal when it extends from the thyroid cartilage of the larynx to the pyramidal lobe.

The LGT with hyo-pyramidal extension is typically in the form of a single muscular slip, and according to these two characteristics, it is very similar to the TFB. The macromorphological similarity of these two structures could be the cause of their incorrect identification. The false identification of these two structures may also stem from a very rare finding of a few scattered striated muscle fibers among the connective tissue of the TFB in our histological specimens. The presence of these segregated striated muscle fibers is enigmatic, but indicates the possible developmental interference of the pyramidal lobe and TFB as the possible thyroglossal duct remnants on one side, and anterior cervical and lingual muscles on the other side during extension of the neck in late-term human fetuses [3,21]. The unexpected finding of a normal thyroid tissue associated with the LGT in our histological specimens supports this explanation.

The LGT with the laryngo-pyramidal extension may be considered as the laryngeal muscle in association with the pyramidal lobe. These two types of LGT in association with the pyramidal lobe are combined and described as "the musculus levator glandulae thyroideae anterior" according to Sobotta [23]. Also, these two types of LGT (hyo-pyramidal and laryngo-pyramidal) are classified as hyo-pyramidal and thyroid-pyramidal types according to the classification of Mori [9] and described by several authors [17,20]. Further investigations may indicate the presence of the

LGT with a combined extension, hyo-pyramidal and laryngo-pyramidal, in cases when it is composed of multiple muscular slips with their double attachments to the hyoid bone and thyroid cartilage of the larynx.

CONCLUSIONS

The pyramidal lobe of the thyroid gland is associated with two different structures, the thyroid fibrous band and the levator glandulae thyroideae muscle. The pyramidal lobe of the thyroid gland is much bigger when associated with the thyroid fibrous band, which may be a useful anatomical landmark for its identification. Ectopic thyroid tissue is frequently present along with the thyroid fibrous band (78%) and levator glandulae thyroideae muscle (50%), and clinicians should take into account that this thyroid tissue might be the potential origin of thyroid cancer.

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Conflicts of interest disclosure: The authors declare that they have no conflict of interest.

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