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Muscular Anatomy of the Human Ventricular Folds

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Abstract

Objective—The purpose of this study was to better understand the muscular anatomy of the ventricular folds (VF) to help improve biomechanical modeling of phonation and to better understand the role of these muscles during phonatory and non-phonatory tasks.

Method—Four human larynges were decalcified and sectioned coronally from the posterior to anterior using a CryoJane tape transfer system, and stained using Massons trichrome. The total and relative area of muscles observed in each section were calculated and used for characterizing muscle distribution within the ventricular folds.

Results—The ventricular folds of the larynges contained anteriorly coursing thyroarytenoid and ventricularis muscle fibers lying in the lower half of the VF posteriorly, with some ventricularis muscle evident in the upper and lateral portion of the fold more anteriorly. Very little muscle tissue was observed in the medial half of the fold, and the anterior half of the VF was largely devoid of any muscle tissue. All four VF's contained muscle bundles coursing superiorly and medially through the upper half of the fold toward the lateral margin of the epiglottis.

Conclusions—While variability in expression was evident, the well-defined thyroarytenoid muscle was readily apparent lateral to the arytenoid cartilage in all specimens.

Keywords

Human larynx; ventricular folds; histological study; ventricular muscle

Introduction

The ventricular folds, also known as the vestibular or false vocal folds are located above the true vocal folds and separated from them by the laryngeal ventricle. They are commonly referred as “false” vocal folds as they historically have been thought not to be directly involved in the production of “normal” voice. However, they are part of the vocal articulation during glottal stops and pressed phonation¹ and actively involved during Mongolian throat singing². They have been linked to closure of the laryngeal lumen during swallowing and other primitive reflexes such as coughing, gagging, etc³. They lubricate the true vocal folds by secreting mucosa through their glands and provide antimicrobial defense protection⁴.

During phonation, the ventricular gap is kept large enough by the laryngeal or ventricular muscles to prevent unwanted ventricular fold vibration. This gap is usually larger in males than in females⁵. When the VF controlling muscles fail to keep them apart or the VFs become larger than usual, the increased glottal resistance creates a condition favorable for

unwanted vibrations and voice disorders. Such a decreased VF gap was shown to have major aerodynamic and acoustic effects during the phonation of excised canine larynges^{6,7}. For example, Alipour et al.⁶ and also Finnegan and Alipour⁷ determined that medial compression of the VF increased glottal flow resistance, as well as sound intensity.

The ventricular fold is usually composed of muscular, glandular, adipose and connective tissues that contribute to its phonatory and non phonatory maneuvers. Due to the lack of a complete understanding of the complex VF histological structure, its muscular and physiological functions have been the subject of many recent studies. Recent morphological studies of VF have confirmed the existence of various distinct muscle bundles^{8–10}. Further, the activity of the thyroarytenoid muscle bundle during phonation has been confirmed with electromyography¹¹.

In an extensive morphological study of the VF musculature of 11 larynges of human fetuses and 6 adult human larynges, Motta¹² found three groups of compact fascia in the superior-lateral portion of the false vocal folds and identified their origin and directions. According to Motta, these muscular fibers originate from the anterior-lateral border of the arytenoid cartilage and terminate in the sub mucosa of the ventricular fold. The main fascia of the ventricular muscle splits into two bundles. One bundle courses towards the medial aspect of the VF and the other courses towards the lateral aspect. He also suggested that elastic fibers in the VF might be responsible for the return of the VF to its initial shape after each muscular contraction.

Kotby et al.⁸ studied the microstructure of the human laryngeal ventricle and VF by making coronal sections from 17 normal adult human larynges (8 male and 9 female) to describe the epithelial, glandular and muscular structures of the VF. They reported that the ventricle was comprised of mucosa and muscle, where the mucosa included epithelium, and a lamina propria with a loose layer of elastic and collagenous fibers. They observed that the thyroarytenoid muscle formed the lateral boundaries of the ventricle and that the ventricularis muscle was bilaterally present in most cases, with across subject variations.

Another extensive morphological study of the ventricular folds was conducted by Aoyagi¹³. In this study, the microstructure of ventricular folds was acquired from 54 human larynges obtained from autopsy (27 male and 27 female ranging in age from 20 to 79 years). In addition to an examination of the epithelial region for the effects of aging and smoking, they investigated the lamina propria for the distribution density of glandular, adipose, and connective tissues. They found that glandular tissue gradually disappeared with age with more noticeable changes observed in females. On the contrary, adipose tissue increased with age with significant increases observed in females. They also found a large quantity of collagen and elastin in the ventricular sub-epithelium. They noticed that the distribution of collagen decreases in the elderly yet the distribution of elastin was similar in all ages.

The microstructure of the ventricular fold contributes to its mechanical properties and behavior during articulation and phonation. However, it is not clear how this structure contributes to adduction/abduction before, during, and after phonation. A biomechanical model of phonation with provision for ventricular control such as Alipour et al.¹⁴ could help us better understand this process. However, such a model requires not only information on the viscoelastic properties of ventricular folds, but also origin and insertion points of individual ventricular muscle, its cross sectional area, and fiber orientation within the VF for a successful modeling. For example, the percentage of muscle tissue in the VF cross sections defines the active tension contribution of the VF to its stiffness and maneuverability. It may also affect the ventricular pressure that has major influence on the dynamics of true vocal folds. The purpose of this study was to complement the existing data on these muscles by

determining their cross-sectional distribution along the anterior-posterior length of the ventricular folds.

Methods

Sectioning and Staining

Four formalin-fixed elderly adult female human larynges (hereafter referred to as L1, L2, L3, L4) obtained from the Deeded Body Program at the University of Iowa were studied. The samples were obtained without PI control over gender and age and no information about the donors were provided due to privacy policies except that they were elderly females. The right half of each larynx was trimmed to include the upper half of the true vocal fold and the entirety of the ventricular fold. Each hemilarynx was then decalcified, embedded in tissue freezing medium (TFM, Trinagle Biomedical Services, Durham, NC), and stored in a -80°C freezer. The CryoJane Tape Transfer System (Instrumedics, Inc., St. Louis, MO) was used for tissue sectioning. The frozen tissue block, CryoJane tissue tape and ultraviolet light activated adhesive coated slides (Leica Microsystems, Buffalo Grove, IL) were first placed in the cryostat (Microm HM505E, Richard-Allen Scientific, Kalamazoo, MI) and allowed to reach -35°C . Once mounted in the cryostat, ten micron thick coronal sections were cut proceeding from posterior to anterior throughout each hemilarynx. Beginning approximately at the middle of the arytenoid cartilage and continuing anteriorly as far as possible, sections spaced 1 mm apart were transferred to slides for subsequent staining. The anterior-posterior location of each saved section was recorded on a lateral view photograph of the hemilarynx for future reference. Saved slides were stored at -20°C , and subsequently stained using Masson trichrome to provide contrast among all of the major tissue types of interest. Specifically, the Massons trichrome stain is a three color staining protocol well suited to differentiating muscle and collagen from the surrounding tissue.

Analysis

Stained sections were digitally scanned using an Olympus BX61 light microscope (Olympus America, Center Valley, PA) with a resolution of 4800 pixels per inch and saved as TIFF image files. Consistent color balance across sections was attempted by identifying an area towards the boundary of each slide devoid of tissue and white balancing to that area. Post processing was applied to scanned images within Adobe Photoshop to achieve as consistent as possible brightness across scanned images. No other alterations in image color were employed. For each image file, using Adobe Photoshop (CS5, Adobe Systems, Inc., San Jose, CA), the margins of the false vocal fold were outlined. The inferior border extended from the most inferior edge of the VF laterally to the medial edge of the thyroid cartilage lamina. The lateral border extended vertically along the medial edge of the thyroid lamina. The superior border extended from the medial edge of the epiglottis laterally to the medial edge of the thyroid lamina, or to a line extended vertically from that edge. The medial border was the medial edge of the VF. Using these borders, the VF was divided into four quadrants (Figure 1, **lower right panel**), with the intersection of the four quadrants determined using the maximum width and height of the VF. This arbitrary definition helps us to quantify the location of muscle fibers within the VF cross section for comparison.

Sections lying posterior to the posterior margin of the laryngeal ventricle were visually inspected for the presence and position of muscle tissue in the vicinity of the arytenoid cartilage. For each remaining section, beginning with the first section where the ventricle became visible (labeled as Section 1), ImageJ image processing and analysis software (<http://rsb.info.nih.gov/ij/>) was used to record the cross-sectional area of each quadrant. Within each quadrant, ImageJ was used to outline and manually trace the cross-sectional area of all visible muscle tissue. From these data, the total amount of muscle tissue within

each quadrant was recorded in absolute terms (mm^2), and as a proportion of the cross-sectional area of that quadrant.

Results

Figure 1 shows four selected coronal sections of larynx L1 with each location identified on the upper photos with a vertical line, with section numbers increasing from posterior to anterior. In the region far enough posterior to the laryngeal ventricle that the quadrate lamina of the cricoid is still visible in the section, all four larynges revealed a considerable concentration of anteriorly coursing muscle fibers just lateral to the arytenoid cartilage identified previously by various authors as the thyroarytenoid muscle (most visible in section 9, quadrant 4). These fibers were, however, not limited to the area inferior to where the laryngeal ventricle appears in more anterior sections, instead extending superiorly into the space that will develop into the lower lateral quadrant (quadrant 4) of the VF.

Figure 2 includes the measured FVF area in four quadrants in all human larynges, as well as total FVF area (left y-axis) for each coronal section beginning with the first section where the ventricle became visible (posteriorly) and extending anteriorly as far as possible. Muscle tissue area relative to FVF area is also graphed as a function of section number (right y-axis). In the region at and just anterior to the posterior border of the laryngeal ventricle, three of the four larynges (L1, L2, L4) presented with a anteriorly coursing thyroarytenoid and ventricularis fibers in quadrant 4. In the other specimen (L3), a large laterally positioned adipose tissue mass resulted in a more medial positioning of these fibers. In all four larynges, isolated muscle fiber bundles were observed scattered primarily throughout quadrants 3 and 4, separated by adipose and other tissue. Some examples of these may be observed in Figure 2.

A comparative chart (Figure 3) depicts muscle cross sectional area relative to total VF area across all 4 larynges at three different points along the length of the VF; near the vocal process of the arytenoid (VP), at a point one third of the way anteriorly from the vocal process (Third), and at the midpoint of the length of the VF (Half). In larynges L1, L2, and L3, muscle area is densest at the one third point, decreasing in cross sectional area from there to the midpoint of VF length. This is partly due to a decrease in TA fiber quantity, and partly because of the spread of ventricularis towards thyroid cartilage.

Moving anteriorly, scattered bundles of thyroarytenoid fibers continued to be observed in the lower lateral (fourth) quadrant of all four larynges (Figure 4). In two larynges (L1, L2), small anteriorly coursing ventricularis muscle bundles were also observed in quadrant 3, with very little muscle tissue observed in quadrant 1. In larynges 3 and 4, a few bundles were observed in the upper half of the VF, with the fibers that were apparent located in quadrant 1 for L3 and at the junction of quadrants 1 and 3 for L4. Overall, L4 was observed to have less muscle mass throughout the VF compared to the other three. Of all four larynges, L3 was the only one with appreciable amounts of muscle tissue in quadrant 2, due to the presence of the large laterally positioned adipose tissue mass mentioned previously.

At approximately the midpoint of the anterior-posterior extent of the ventricle, its connection to the laryngeal saccule became evident in all four larynges. The saccule is a membranous sac lying between the false vocal fold and the inner surface of the thyroid cartilage. Anterior to this point, very few if any muscle fiber was observed in any of the four larynges (Figure 2). However, just posterior to this region in larynges 1, 2, and 4, separate aggregates of the ventricularis were observed medial and lateral to the emerging saccule. These bundles may correspond to the anteromedial and anterolateral muscle systems referred to by Reidenbach⁹.

While the majority of muscle tissue observed in the FVF coursed in an anterior direction, all four FVF's contained muscle bundles coursing superiorly and medially toward the lateral margin of the epiglottis (Figure 4). This fiber course typically emerged in the upper half of the VF at a point where its cross sectional area was greatest, and as was the case with all muscle tissue, was not observed in the anterior half of the VF.

All four larynges were dominated in quadrants 1 and 2 by connective, glandular, and adipose tissues. With the exception of a very few scattered muscle bundles, all of the muscle observed in the VF was located lateral to these tissues. Of additional interest, VF cross-sectional area was greatest in the posterior 1/3 of the fold. Muscle cross-sectional area was similarly greatest in that same region.

Discussion

As observed by many previous authors, the well-defined thyroarytenoid muscle was readily apparent lateral to the arytenoid cartilage in our four specimens. As the posterior border of the laryngeal ventricle was approached, anterior coursing fibers of the ventricularis muscle, as observed by Kotby et al.⁸ appeared separate from the thyroarytenoid and placed higher in the VF. These fiber bundles were typically irregular in cross-section and somewhat scattered within the VF tissue but lateral to the main mass of glandular tissue. As observed by Kotby et al.⁸, little to none of the ventricularis muscle was observed in the anterior half of the VF. Interestingly, the heaviest concentration of ventricularis fibers appeared in the region anterior to the posterior border of the ventricle coinciding with the highest cross-sectional VF area. Further, with the exception of larynx 4, which had relatively little muscle mass overall, the highest concentrations of ventricular muscle fibers tended to appear at a level that corresponded vertically with the thickest portion of the VF. Further, as evident in Figure 4, with the exception of very few isolated fibers, all of the ventricularis muscle mass lies lateral to the elastic, glandular, and adipose tissues that comprise the main bulge of the VF. These observations are consistent with findings of Reidenbach⁹ that contraction of these anteriorly coursing fibers deforms the tissue mass of the VF medially.

It is also interesting to note that anterior to the midpoint of the laryngeal ventricle, the absence of muscle tissue occurs coincident with the presence of the laryngeal saccule and the large decrease in VF cross-sectional area. One might postulate that the efficiency of VF medialization is optimized by concentrating muscle mass where shortening can have the greatest functional impact. That is, contraction of the high concentration of muscle tissue immediately lateral to the area of the false vocal fold having the greatest mass and resting state medial position might be expected to achieve the greatest degree of medially directed narrowing of the supraglottic space.

All four larynges contained obliquely oriented fibers identified in the region just anterior to highest VF cross-sectional area, albeit weakly represented in larynx 4. The position of these fibers roughly corresponds to fibers identified as thyroepiglotticus by Kotby et al.⁸ who reported their "midlarynx" existence (i.e. at midpoint anteriorly). However, Kotby et al.⁸ described these fibers as forming the lateral boundary of the ventricle and saccule. The obliquely oriented fibers observed in this study were located medial to the saccule position and emerged posterior to where the saccule first appears. Further, these oblique fibers were not observed to extend laterally to the thyroid cartilage. These fibers were typically observed to lie superior and medial to the bulk of ventricularis muscle fibers, and hence extending superior to the primary medial bulge of VF tissue. From a functional standpoint, it could be postulated that contraction of these oblique fibers concentrates deformation of VF tissue mediated by ventricularis muscle contraction in a medial direction by deforming the upper medial quadrant of the VF in a medial and inferior direction. It is, of course, important to

point out that these obliquely oriented fibers were not extensively represented and might possibly not be all that significant functionally.

A final observation is that no fibers corresponding to the superior thyroarytenoid muscle were readily identifiable in these four larynges. This was somewhat surprising; although Zemlin¹⁵ stated that this muscle is variably expressed, occurring in about 50% of laryngeal specimens.

While some consistencies in muscle expression, location, and course were observed in the present study, individual variability was also clearly present. This study is not the first to observe variations across specimens. The primary muscle of interest in attempting to understand the functional anatomy of the VF in the present and numerous previous studies is the ventricularis muscle. Kotby et al.⁸ have suggested that the variability in its expression renders the ventricularis muscles role in VF medialization “rudimentary”. We have postulated here based on its orientation when present, and in agreement with Reidenbach⁹, that ventricularis muscle contraction could result in FVF medialization. The fact that the VF’s are capable of such movement is not open to question. The mechanism responsible remains to be elucidated, and will be the subject of our continuing histologic and biomechanical modeling efforts.

A finite-element computational model of the true and ventricular folds requires not only the mechanical properties of individual tissue components; it requires the anterior-posterior cross-sectional areas of these structures that are provided in this study. This data indicated the three-dimensional positioning of this structure that should be considered in the modeling. Also, any modeling of the ventricular folds adduction and articulatory movements should benefit from the knowledge of muscular tissues distributions in these quadrants of VF.

A potential limitation of this study lies in the fact that the data presented was acquired from 4 elderly female larynges. Still, information of significance to our ongoing finite modeling efforts has been acquired through this quantification of muscle fiber distribution throughout the anterior-posterior extent of the VFs. Our future studies will expand on this work by conducted detailed and quantitative analyses of both muscular and non-muscular (especially elastin and collagen) tissues within the VFs of both younger and elderly male and female larynges. The information gleaned from these studies will be used to advance a model of the control of VF adduction and compression during articulation and non-speech tasks.

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Figure 1.

Four coronal sections of L1 with their location indicated by a vertical black line in the upper hemilarynx images, and section number indicated below the histology images. The division of ventricular fold into four quadrants is shown in section 2. The presence of the thyroarytenoid muscle in the sections 5 and 7 (fourth quadrant) is marked with label (TA).

Figure 2.

Total FVF cross-sectional area (left y-axis) and muscle cross-sectional area within each quadrant and all quadrants combined expressed as a percentage of total FVF area (right y-axis) for four larynges. Solid black vertical line represents anterior margin of vocal process of arytenoid. Dashed vertical line represents section first showing communication between laryngeal ventricle and laryngeal saccule.

Figure 3.

Muscle cross sectional area relative to total VF area across all 4 larynges at three different points along the length of the VF; near the vocal process of the arytenoid (VP), at a point one third of the way anteriorly from the vocal process (Third), and at the midpoint of the length of the VF (Half).

Figure 4. VF cross sections of L1 (upper left, section 5), L2 (upper right, section 4), L3 (lower left, section 3), and L4 (lower right, section 2). White arrows – aryepiglottic muscle fibers, Black arrows – ventricularis muscle fibers, Black arrowheads – thyroarytenoid muscle fibers.