Histology of the Cricothyroid Membrane: A Clinical Perspective

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Abstract Puncture of the cricothyroid membrane is a component of several clinical procedures. Among the several complications are the immediate risk of bleeding, long-term risk of subglottic stenosis and hoarseness of voice. Presence of blood vessels in the cricothyroid membrane has also been implicated in the extra-laryngeal spread of laryngeal cancers. Though various authors have described the attachments of the cricothyroid membrane, very few have studied its histology. We studied the histology of the cricothyroid membrane using hematoxylin and eosin, Mallory's trichrome and Verhoeff's special stain. The cricothyroid membrane was found to be continuous on its deeper aspect with the mucosa of the subglottic larynx and lined by pseudostratified ciliated columnar epithelium. The membrane was observed to be fibroelastic, containing equal proportions of collagen and elastic fibers and numerous fibroblast nuclei. Numerous blood vessels are seen traversing through the membrane, which probably connect intralaryngeal with other extralaryngeal vessels. These histological findings help to further understand the complications of cricothyroidotomy and spread of laryngeal cancer.

Keywords Cricothyroid membrane · Histology · Subglottic stenosis

Introduction

Puncture of the cricothyroid membrane is a component of several important clinical procedures. These include cricothyroidotomy—for emergency airway access, botox injections into the vocal cord for patients with adductor spasmodic dysphonia, scintigraphic measurement of tracheal mucus velocity in patients with muco-ciliary dyskinesia, retrograde intubation of larynx, intra operative neuro-monitoring of the recurrent laryngeal nerve during thyroid surgery, minitracheotomy for clearance of excess tracheobronchial secretions and percutaneous collagen augmentation for treatment of parkinsonian hypophonia. Among the several complications of the procedure, are the immediate risk of bleeding [1–7], the long-term risk of subglottic stenosis [8–10], dysphonia and hoarseness of voice [5, 8, 11, 12]. Tissue injury is known to cause varying degrees of scar formation during wound repair. Scarring occurs due to fibroblast proliferation and cross linkage of collagen proteins. Carcinoma of the larynx spreads extralaryngeally by invading the cricothyroid membrane [13]. Presence of blood vessels in the cricothyroid membrane has been implicated in the extra-laryngeal spread of laryngeal cancer [14, 15].

Though various authors have described the attachments of the cricothyroid membrane, very few have studied its histology. We studied the histology of the membrane using special stains to further understand the complications of the procedure and spread of laryngeal cancer.

Materials and Methods

The cricothyroid membrane in the region between the anterior arch of cricoid and lower border of thyroid cartilage was collected from 10 adult human cadavers donated

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to the Institute of anatomy, Madras Medical College and Research Institute, Chennai under the body donation program for teaching and research purposes. This is the region commonly punctured in any of the above procedures. The cricothyroid membrane was fixed in 5% formalin saline and embedded in paraffin wax. Ribbon sections were taken using a rotary microtome at 6 micron thickness. Staining was done using 3 methods:

- Haematoxylin and eosin stain
- Mallory's trichrome stain—specific for connective tissue
- Verhoeff's specific stain for elastic and collagen fibres

Photomicrography was done at 4×, 10× and 40× magnifications.

Results of Histology

The cricothyroid membrane was found to be continuous on its deeper aspect with the mucosa of the subglottic larynx (Figs. 1 and 2). The laryngeal surface is lined by pseudostratified ciliated columnar epithelium. The membrane is observed to be fibroelastic, containing collagen and elastic fibres in almost equal proportion. A ventral dense band of fibroelastic tissue is seen representing the median cricothyroid ligament followed by a deeper layer of loose connective tissue just beneath the laryngeal mucosa (Fig. 1a). Numerous blood vessels are seen traversing through the membrane (Fig. 1a and b). Numerous glandular acini are seen in the lamina propria. Haematoxylin-eosin staining shows bundles of parallel elastic fibres interspersed with the collagen fibres (Fig. 1a and b). In Verhoeff's special stain for elastin (Fig. 2a and b), elastin fibres take up a black stain and collagen fibres have a red appearance. The collagen and elastic fibres are seen interspersed in almost equal proportions. Mallory's trichrome stain (Fig. 2c and d) shows blue collagen fibers interspersed among pink elastin fibers.

Discussion

The cricothyroid ligament has several alternative names including cricovocal membrane, conus elasticus and cricothyroid membrane [16]. Lanz and Wachsmuth [17] and Hollinshead [18] describe the conus elasticus to be a strongly developed layer of elastic tissue. Conversely, Reidenbach, on studying the histology of plastinated sections of larynx states that the cricothyroid membrane is composed of densely arranged collagen fibres that are pierced by small blood vessels [14]. Though our study agrees with Reidenbach on the presence of numerous blood

Fig. 1 a and b Shows H/E stain of cricothyroid membrane. Note the ventral dense arrangement of fibers. Common for all figures (A pseudostratified columnar epithelium, B glandular tissue, C Blood vessels, D ventral dense connective tissue)

vessels, we however found an equal predominance of collagen and elastic fibers. The abundance of collagen fibers and numerous fibroblast nuclei may account for the stenosis following puncture of the cricothyroid membrane.

Acute, brisk, hemorrhage following cricothyroidotomy is attributed to laceration of vessels in close relation to the cricothyroid membrane. The endolaryngeal arteries in the submucosa of larynx are thought to anastomose with the transverse cricothyroid artery via perforating branches. We document that several branches of the transverse cricothyroid artery pierced the membrane through small foramina. Numerous blood vessels were found within the membrane on histology. Presence of perforating vessels that connect intralaryngeal with extralaryngeal vessels has been documented. Donald and Bernstein present a case in which a patient experienced an acute, brisk, endolaryngeal hemorrhage following an attempt at translaryngeal aspiration of tracheobronchial secretions through the cricothyroid membrane [3]. They state that the bleeding was due to injury of vessels in the submucosa of larynx in the region of the cricothyroid membrane. They also state that

Fig. 2 **a** and **b** Shows Verhoeff's stain specific for elastin at 4× and 10 × magnifications. Elastin fibrils stain black to blue black; Collagen fibrils stain red. Note the equal proportions of collagen and elastin

fibers. **c** and **d** Shows Mallory's trichrome stain (4× and 40×)—collagen fibrils are blue and elastin fibrils are pink or yellow

endolaryngeal arteries in the submucosa of larynx anastomose with the cricothyroid arteries via perforating branches. Dover et al. in their landmark article report that several branches of the cricothyroid artery penetrate the cricothyroid membrane and ascend along the posterior surface of the thyroid cartilage to supply the laryngeal mucosa [6]. Reidenbach on studying the histology of cricothyroid membrane found that the membrane is pierced by small blood vessels close to its attachments to the thyroid and cricoid cartilages [14].

Carcinoma of the larynx spreads extralaryngeally by invading the cricothyroid membrane [13]. Presence of blood vessels in the cricothyroid membrane has also been implicated in the extra-laryngeal spread of laryngeal cancer [14, 15]. Reidenbach suggested the importance of perforating vessels in the spread of laryngeal tumors. Sato et al. investigated the cricoid area using computer graphics and whole organ serial sections. They found the cricoid area to have many vessels which penetrated the anteroinferior portion of the conus elasticus extending into the prelaryngeal region [19]. Ortug G et al. studied the vascular

anatomy of the cricothyroid space in the Turkish population and found numerous vascular structures in front of the cricothyroid membrane that passed through foramina in the membrane into the intralaryngeal area [15]. They stress on the importance of this region with regard to surgical procedures, spread of laryngeal cancer, and traumatic lesions of the larynx.

Children usually require a needle cricothyroidotomy, whereas surgical cricothyroidotomy is done in adults. Dysphonia and hoarseness of voice following surgical cricothyroidotomy has been reported by few authors. Boyd et al. reports that hoarseness is due to a small amount of granulation tissue formed below the vocal cords after cricothyroidotomy [8]. John V. and Charles E. state that damage to the cricothyroid membrane causes alterations of the vocal ligament, resulting in changes in the quality of voice [11]. Bennett et al. report that dysphonia can occur secondary to a tracheal fracture, usually due to insertion of an oversized tube, or if the incision is made close to the vocal cord [12]. The incision is usually made along the upper border of cricoid cartilage. McGill et al. report a case

where a longitudinal fracture of the thyroid cartilage occurred when an oversized tube was passed through the cricothyroid membrane causing severe dysphonia [5].

Conclusion

The cricothyroid membrane is observed to be fibroelastic, containing numerous fibroblast nuclei, and equal proportions of collagen and elastic fibres. The ventral dense band of fibroelastic tissue represents the median cricothyroid ligament. Numerous blood vessels traverse the membrane. This profuse communication of endolaryngeal vessels with extralaryngeal tissues may be implicated in the extralaryngeal spread of laryngeal tumors.

Conflict of Interest There is no conflict of interest.

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