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The Comparative Morphological Evaluation of Human Male and Female Vocal Folds, A Cadaveric Study.

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ABSTRACT

The age related structural changes in human vocal fold are known, but gender related differences in middle aged group are not precisely documented. This study is aimed to compare and evaluate the collagen, elastic fibre content of the lamina propria of middle aged male and female vocal folds. Cross sectional analysis of human cadaveric male and female vocal folds were performed. Haematoxylin & Eosin staining was used for the morphometric analysis and Verhoeff's & van Giesen stain for the collagen and elastic content evaluations. The collagen and elastic fibres morphology in the lamina propria, and thickness of the epithelium, the lamina propria were compared using the trinocular microscope, which was configured with image analysis software. It was observed that, there was a considerable difference in the lamina propria thickness, the female vocal cords being thinner. The content of collagen fibres were higher in the deeper and superficial lamina propria layers in both the sexes without any gender related differences. The elastic fibre content was more in the intermediate layer and better pronounced in females. The knowledge of lamina propria in middle aged group is important in understanding the pathophysiology of vocal fold and surgical procedures associated with it.

Keywords: Middle aged vocal folds, Lamina propria, Collagen content, Elastic content

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INTRODUCTION

The human vocal folds consist of the lining epithelium, lamina propria and vocalis muscle. They are lined by the stratified squamous epithelial cells. Superiorly and inferiorly, it continues with respiratory, pseudo stratified ciliated columnar epithelium. The lamina propria consists of triple layers. It consists of deep, intermediate and superficial layers, based on the histological composition. The more superficial layer is termed as 'cover' and the deeper tissue as 'body'. The superficial and medial tissues slide and move over the more rigid body tissue [1]. The quality of phonation depends on the vocal fold structure, which is defined by its cellular and extracellular components. The extracellular components of the lamina propria consist of collagen and elastic fibres and also fibrillar and interstitial proteins [2]. Human vocal folds, consists of collagen and comprises 43% of the total tissue protein [3]. There are a couple of studies which shown that the density of the lamina propria collagen fibres is maximum in the deeper and superficial layers [4, 5]. According to Gray et al. [2], the density increases from superficial to deep layers. The arrangement of the lamina propria collagen fibres in the vocal fold is believed to have a significant effect on phonation [6]. Hence in the present study, we further evaluate the density of collagen fibres in Indian middle aged male and female cadavers. Elastin content of the lamina propria is highly localized in the intermediate lamina propria layer [2]. This layer is known to contain more mature elastic fibres [3]. The vocal ligament is formed by the deeper and intermediate layers of the lamina propria, with an increase in fibrous and interstitial proteins. The elastic and collagen fibres comprise the deeper layer of lamina propria. The loose areolar tissue is found in the superficial lamina propria, which make this layer pliable and flexible and this region is called Reinke's space.

There is little in the literature assessing the gender differences in the histomorphological constituents of the lamina propria, however age related differences in collagen and elastic contents are very well addressed [7]. Gender related differences for the collagen content [4] and hyaluronic acid content [8] was reported. There are not adequate studies regarding the histo morphological structure of the vocal folds in the middle aged Indian population. The vocal fold lesions like laryngeal polyps, singers (teachers) nodules, vocal cysts, vocal cord edema and granuloma are all based on vocal fold extracellular components. Hence the lamina propria histo morphological knowledge is vital in dealing with vocal fold lesions and also in surgical intervention. Hence the present study focus on human vocal fold histomorphology in middle aged. The gender based comparison was also done in the vocal fold morphology.

MATERIALS AND METHODS

Human larynges were collected from the fresh cadavers collected at the department of Anatomy, KMC, Mangalore, India. The present study included male and female larynges. The mean age of the subjects were 44.33±3.33 (SD) with no statistical difference between male and female larynges. Subjects with neck manipulations, such as oral or nasal intubation, tracheotomy, laryngeal surgery, or head and neck irradiation, were excluded from the present study. The right sided vocal fold was removed from the cadaveric larynx and fixed in a 10% formalin solution for a day. Subsequently, 5mm thickness coronal sections were carefully obtained from the membranous middle part of the vocal fold. The sections were dehydrated in alcohol and paraffin embedding was done. Tissue specimens were cut into 4 micrometer thick histological sections and eosin-hematoxylin staining was done for the initial analysis. For further analysis of collagen & elastic fibre contents, sections were also stained with Verhoeff's & van Gieson stain.

For qualitative analyses of the elastic and collagen fibre distribution and the epithelial, lamina propria morphometric analysis, the lamina propria was divided into 3 layers according to the model proposed by Butler et al. [8]. The histomorphological analysis was performed using Nikon trinocular microscope (H600L) under 20X magnification using imaging software NIS Elements Br version 4.30. The data for histo morphometric analysis was given as mean ± SD. The statistical significance among the comparison of male and female groups was performed by the unpaired t test using a graph pad software version 4. The p values< 0.05 were considered as significant.

RESULTS

The epithelial lining at the mid vocal fold consists of stratified squamous cells arranged in 3 to 4 layers and towards upper and lower end the cells were smaller and thickness was also reduced. The mid vocal fold

region presented epithelial papillae projecting into the lamina propria. The statistically significant difference was not observed between the male and female vocal fold epithelial morphology (Figs. 1 and 2).

The lamina propria is clearly divisible into 3 layers-superficial, intermediate and deep (Figs. 3A and 3B). The average lamina propria thickness of male vocal folds was 547.85±11.39 µm and that of the female was 518.74±2.03 µm with a statistically significant difference (p>0.001). The superficial layer in both the sexes consists of loose fibrous tissue. During tissue processing some of the loose fibrous tissues were lost giving vacuole like appearance (Figs. 4A and 4B). It was observed that in both male and female vocal folds the diameter of the deeper lamina propria was highest and that of superficial lamina was smallest. The intermediate lamina propria is basically consists of elastic fibres, while the deepest part is primarily consists of collagen fibres (Figs. 5A and 5B). The elastic fibre content in the intermediate layer is more pronounced in female compared to male vocal folds.

Figure 1: Epithelium of male vocal fold (Haematoxylin & Eosin staining, 40X magnification).

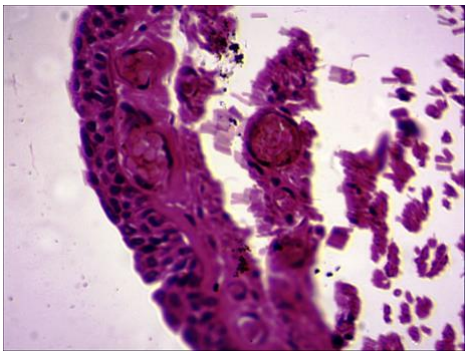

Figure 2: Epithelium of female vocal fold (Haematoxylin & Eosin staining, 40X magnification).

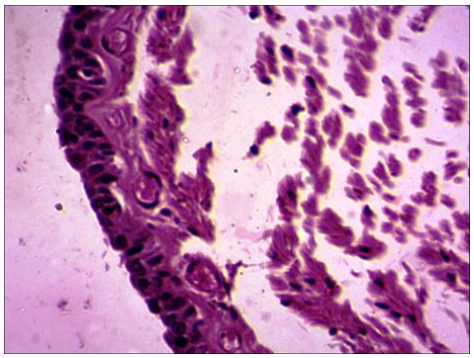

Figure 3A: Cross section through the male vocal fold showing the different layers of lamina propria (Haematoxylin & Eosin staining, 4X magnification).

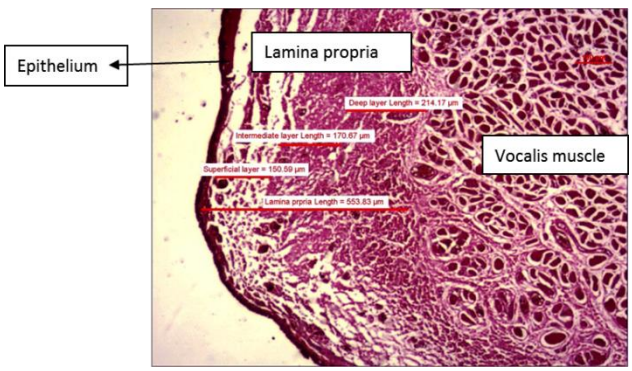

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Figure 3B: Cross section through the female vocal fold showing the different layers of lamina propria (Haematoxylin & Eosin staining, 4X magnification).

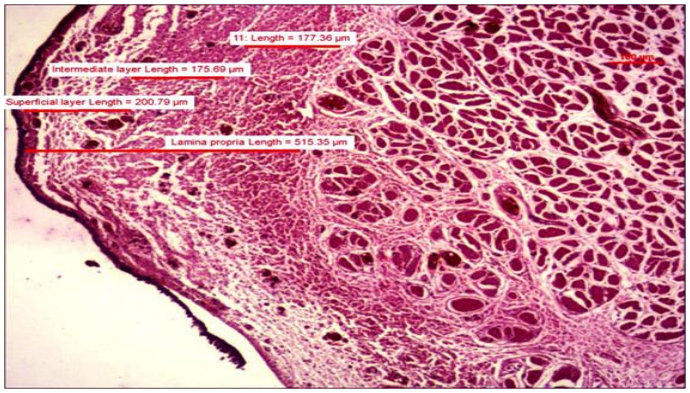

Figure 4A: Lamina propria of male vocal fold (Haematoxylin & Eosin staining, 10X magnification).

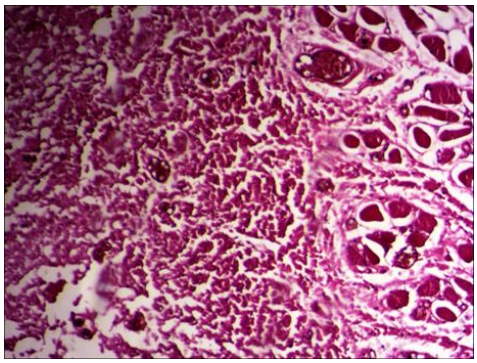

Figure 4B: Lamina propria of female vocal fold (Haematoxylin & Eosin staining, 10X magnification).

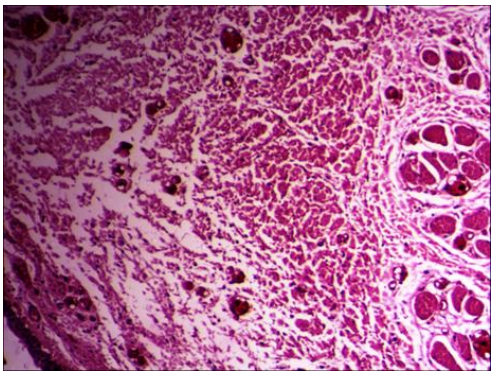

Figure 5A: Lamina propria of male vocal fold (Verhoeff's & van Gieson staining, 4X magnification).

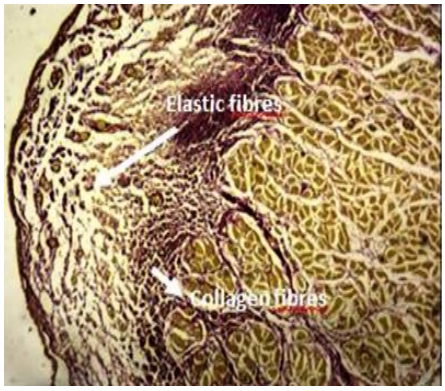

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Figure 5B: Lamina propria of female vocal fold (Verhoeff's & van Gieson staining, 4X magnification).

DISCUSSION

There is very little literature about assessing the gender differences in the histomorphological constituents of lamina propria of middle aged Indian population. In newborns, the entire lamina propria is uniform in structure resembling the adult superficial lamina propria [7]. The present study from the middle aged population observed the significant difference between the lamina propria width in male and female vocal folds. It is observed that the female vocal folds are comparatively thinner than that of the males. Gender-related differences have been earlier reported for collagen fibres [4]. Adult males have greater amounts of collagen than that of females. The present study observed that, there were clusters of collagen fibers, straight and coiled elastic fibers in the superficial layer. The thick collagen bundles and longitudinal elastic fibers and coiled elastic fibers were obvious in the intermediate layer. The deep layer exhibited coiled elastic fibers and denser collagen fibers. The density of the collagen fibres varies within the lamina propria. Earlier studies [3, 5] have shown that the density of total collagen is highest in the superficial and deep layers of the lamina propria. The arrangement of the collagen fibres within the vocal fold lamina propria is known to have significant effect on the phonation [6].

In the present study elastic fibre content was found to be more in female compared to males. Sato and Hirano [9] found less elastin with age, whereas Hammond et al. [10] found increased elastin with age. Understanding the sex-related differences in the composition of elastic fibres and collagen fibres in the lamina propria is relevant, since it can provide insights to the greater vulnerability of women lamina propria scarring and also to certain vocal fold disorders [3]. Further knowledge of the extracellular matrix with different interstitial protein distribution within the lamina propria, is essential to understand the mechanics of phonation and disease pathogenesis of vocal folds in middle aged population.

It has been reported that, an animal model of a dog gives good comparative study for the implications in the human vocal fold operations. However no animal model has laryngeal structure which is similar to the humans [11]. In the clinical setup, during the flap surgeries, a ventrally placed pedicular flap of vocal fold is best over the superficial lamina propria [12]. The postoperative voice quality is expected to be great from vocal cord surgeries. It was described that the epithelium and lamina propria of the vocal folds have to be preserved in order to minimize the scarring [13]. The minimal scarring is essential for the better postoperative phonation.

The elderly voice is different from that of a young individual. It is believed that the human lamina propria thickness of the vocal folds decreases with the age [14]. There will be a huge difference in the human voice once we cross the middle age [15]. It is believed that the change in the voice in the elderly individuals is due to the damage to the histological composition of the collagen fibres and their ratio with lamina propria and the musculature [6]. It was described that the human male vocal fold is stiffer than the female. This is due to the testosterone hormone which generates stiffness in the vocal fold epithelium [16]. However the false vocal folds of the monkeys show no gender variation [17]. The usual vocal fold vibration depends upon the vocal fold morphology and the lamina propria content along with the extracellular components [18].

Due to all these clinical implications, the present study was undertaken. We believe that the morphometric data of the present study will provide insight to the comparative anatomy of the vocal cord of the man. The results of the present investigation may be important to the oto rhino laryngologists,

anaesthesiologists and the endoscopic surgeons. The data are enlightening to the respiratory scientists and therapists. The details may be important to understand the pathophysiology of the conditions like scarred vocal folds and sulcus vocalis. The results have got implications in the management of the pathological conditions like laryngeal carcinoma, laryngeal polyps and singer nodules.

CONCLUSION

This investigation aimed to compare and evaluate the collagen, elastic fibre topography and morphology of the lamina propria of middle aged male and female vocal folds. The gender based comparison was done. It is observed that the arrangement of collagen fibers, in the different lamina of the lamina propria was similar in both males and females, however elastic fibre content was more in the intermediate layer of lamina propria of the female lamina propria. The limitation of the present study is the smaller number of sample size. The results can be better hypothesized with the larger number of specimens.

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