Original Article

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Importance of Hyaluronic Acid in Vocal Fold Lesions

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Abstract

Objective: We aimed to examine the influence of hyaluronic acid (HA) and basement membrane (BM) thickness alterations on biochemical properties of vocal fold lesions including vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema.

Methods: Archival material of 56 laryngoscopic vocal fold biopsies from 49 patients reported between July 1, 2005 and February 27, 2007 were retrospectively evaluated. Hematoxylin-eosin, alcian blue pH 2.5, and Periodic acid–Schiff (PAS) stainings were histochemically performed.

Results: We detected that the HA component in the extracellular matrix and BM thickness had increased in vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema compared with those in the control group.

Conclusion: We believe that HA plays an important role in determining the biochemical properties of vocal fold lesions including vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema. Especially the increase in the HA component in all these lesions indicates that it plays a role in reparative processes.

Keywords: Human vocal fold, larynx, extracellular matrix, hyaluronic acid

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INTRODUCTION

Normal vocal fold vibration depends critically upon the composition of the lamina propria (LP) extracellular matrix (ECM). Human vocal folds are unique structures comprising thin outer epithelia, a LP (superficial, intermediate, and deep layers) rich in ECM, and the vocalis muscle. The superficial layer of LP is also known as Reinke's space. LP is a relatively small area with high concentrations of cell-secreted matrix substances such as glycosaminoglycans (GAGs), proteoglycans, and fibrous proteins (type III collagen and elastin) (1). A GAG, hyaluronic acid (HA), is one of the main components in the vocal fold LP (2). HA modulates tissue hydration, cell proliferation and differentiation, inflammation, angiogenesis, and wound healing (3). It is also responsible for the viscoelasticity of the vocal folds (1, 2). Vocal fold trauma such as vocal overuse is an important factor in the development of vocal fold nodules. We examined the influence of HA and basement membrane (BM) thickness alterations on biochemical properties of vocal fold lesions including vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema.

METHODS

Archival material of 56 laryngoscopic vocal fold biopsies from 49 patients reported between July 1, 2005 and February 27, 2007 were retrospectively evaluated. Of the 56 cases, 27 were of vocal fold polyps, 7 of sulcus vocalis, 13 of vocal fold cysts, and 9 of Reinke's edema. Ten normal vocal fold mucosa samples taken from different autopsy cases were included in the study as control group. Signed consent forms for all patients were taken. To evaluate HA in ECM and BM alterations, hematoxylin-eosin, alcian blue pH 2.5, and PAS stainings were histochemically performed. We eval-

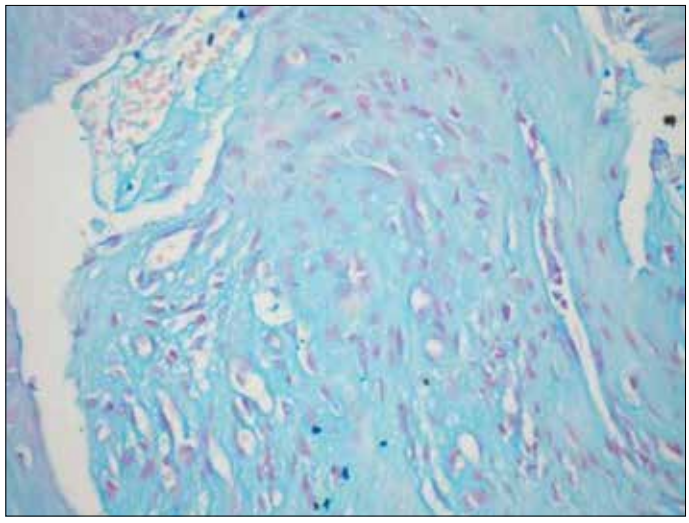

Figure 1. Severe hyaluronic acid accumulation in sulcus vocalis (Alcian blue pH 2.5, ×200)

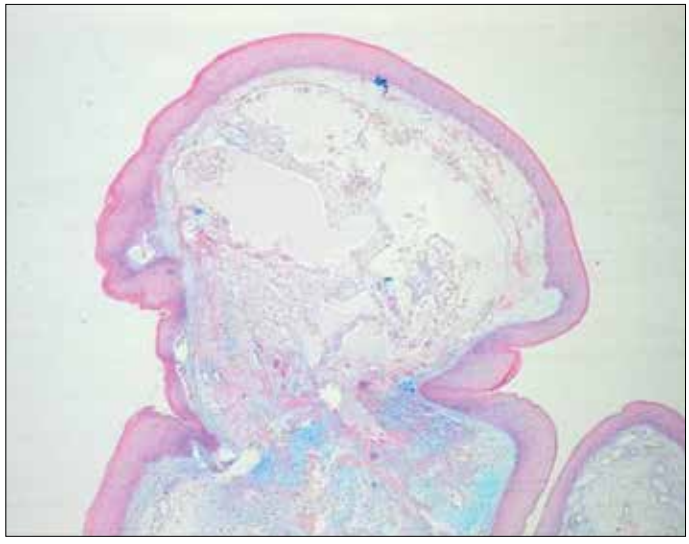

Figure 2. Moderate hyaluronic acid accumulation in vocal fold polyp (Alcian blue pH 2.5, ×40)

uated the staining pattern for each lesion and compared it with that of the control group. We quantified each case using a semi-quantitative score according to increase in the HA staining intensity with alcian blue pH 2.5 (4) (Score-1 for a mild increase, Score-2 for a moderate increase, and Score-3 for a severe increase).

Statistical Analysis

Data analysis was performed using Statistical Package for the Social Sciences version 8.0 (SPSS Inc.; Chicago, IL, USA) statistical package. Frequency data were evaluated using Kruskall-Wallis test. A p value ≤0.01 was considered to be significant.

RESULTS

We detected that HA in ECM had been increased with neomatrix formation in vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema compared with that in the control group (Figures 1-3). Results are summarized in Table 1. The difference was significant (p<0.01), and the increase was more

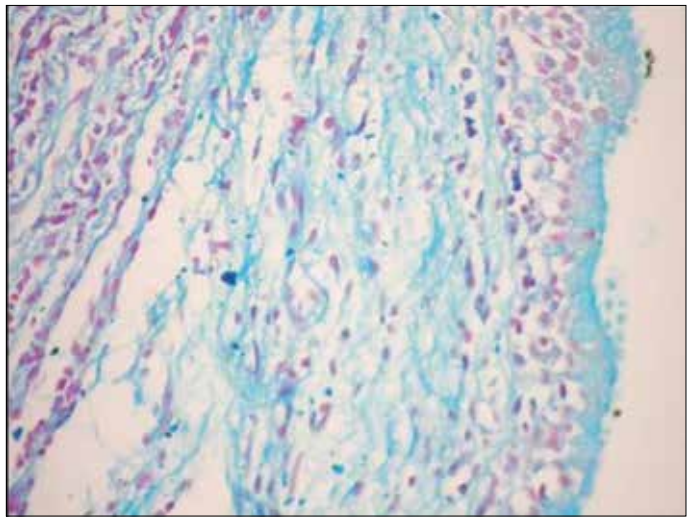

Figure 3. Moderate hyaluronic acid accumulation in vocal fold cyst (Alcian blue pH 2.5, ×200)

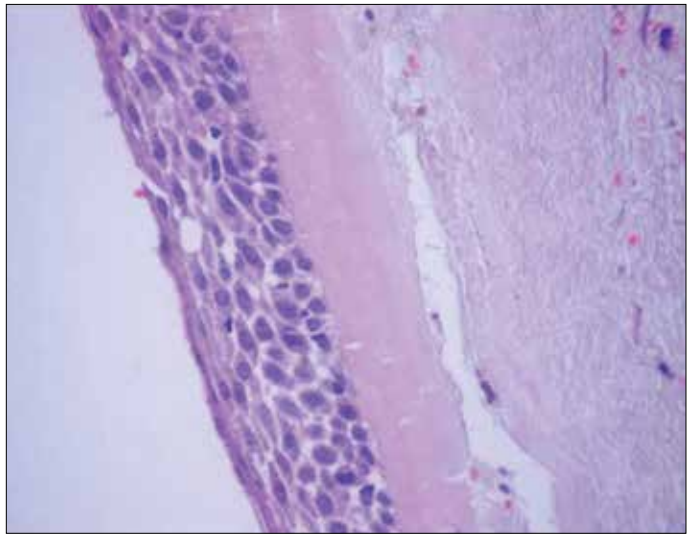

Figure 4. Increased basement membrane thickness in sulcus vocalis (H&E, ×200)

Table 1. Hyaluronic acid component distribution in vocal fold lesions

Hyaluronic acid component in ECM	Sulcus vocalis	Vocal fold polyps	Vocal fold cysts	Reinke's edema
Mild increase (Score-1)	0	8	4	3
Moderate increase (Score-2)	1	9	4	1
Severe increase (Score-3)	6	10	5	0
Case number	7	27	13	9

ECM: extracellular matrix

remarkable in sulcus vocalis lesions than in vocal fold polyps, vocal fold cysts, and Reinke's edema. BM thickness was found to be significantly increased in the four pathologic lesion groups compared with that in the control group (p<0.01)

(Figure 4). BM thickness was measured as 10-14 µm in sulcus vocalis lesions, 6-10 µm in vocal fold polyps, 6-8 µm in vocal fold cysts, and 6-7 µm in Reinke's edema. This increase in BM thickness was most remarkable in sulcus vocalis lesions.

DISCUSSION

Voice disorders represent a significant clinical problem. ECM alterations play an important role in the pathogenesis of vocal fold lesions. In the histochemical and immunohistochemical studies regarding vocal folds, ECM alterations in the normal mucosa and reactive changes against injury were evaluated in animal studies (3). However, comparative ECM alterations in human vocal folds have not been definitively detected.

Reinke's space has sparse fibroblasts, sparse elastic and collagenous fibers, rarely sparse seromucinous glands, and few capillaries, and it lacks lymphatics (5, 6). It has been suggested that because lymphatic drainage of Reinke's space is poor, collection of edema-like fluid plays a role in the development of vocal cord nodules and polyps (6). However, Sato et al. have reported that Reinke's space had a three-dimensional structure of fibrillar collagen type III, and slender fibrils of these had formed a delicate three-dimensional network. The authors claimed that within these networks, innumerable potential spaces were present (1, 5). They suggested that these extracellular interstitial spaces were made up of minute chambers or compartments occupied by ECM (1, 5). It has been reported that the stroma of vocal cord nodules shows strong alcianophilia at pH 2.5 (7). Our results also suggested that HA plays an important role in determining the biochemical properties of vocal fold lesions including vocal fold polyps, sulcus vocalis lesions, vocal fold cysts, and Reinke's edema. Especially the increase in the HA component in all these lesions indicates that HA plays a role in reparative processes.

Increase in BM thickness has been reported (8, 9); this degeneration can occur because of vocal fold trauma.

CONCLUSION

The extracellular interstitial spaces filled with ECM may be responsible for the formation of these vocal fold lesions. Further ultrastructural studies may explain ECMs' role in pathogenesis.

Ethics Committee Approval: Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013)

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: Authors have no conflicts of interest to declare.

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