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Laryngeal Mucosal Reaction during Bronchial Histamine Challenge Test Visualized by Videolaryngostroboscopy

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Summary: Objectives/Hypothesis. To examine the changes in the larynx, as well as self-reported voice and throat symptoms, among patients undergoing a histamine challenge test. Thus, to understand the possible clinical effects of histamine on the larynx.

Study design. Controlled, open prospective study.

Methods. Thirty adult patients with prolonged cough and suspicion of bronchial asthma underwent a histamine challenge test. Videolaryngostroboscopy was performed immediately before and after the challenge. Voice and throat symptoms immediately before and after the challenge test were assessed using a visual analog scale.

Results. Videolaryngostroboscopy after exposure showed significant increases in edema ($P < 0.001$) as well as redness ($P < 0.001$) of the vocal folds after the exposure. Self-reported voice complaints increased significantly for 8 of 11 symptoms. A moderate positive correlation was found between the increase in edema of the vocal folds and reported heartburn/regurgitation symptoms ($r = 0.42$, $P < 0.05$). Atopy, asthma, nasal symptoms, or bronchial hyperreactivity during the histamine challenge test were not associated with laryngeal reactions.

Conclusions. According to the results, the laryngeal mucosal reaction during a histamine challenge test can be objectively visualized. Videolaryngostroboscopy findings, together with an increase in self-reported voice and throat symptoms, show that histamine has potential effects on vocal folds. The mucosal reaction seems to be pronounced among patients with reflux symptoms, probably reflecting the permeability features of the vocal folds.

Key Words: Permeability–Edema–Vocal fold–Allergy–Reflux.

INTRODUCTION

The histamine challenge test is a method used to demonstrate nonspecific bronchial hyperreactivity in asthma diagnostics. Histamine challenge has also been used in some studies to distinguish laryngeal hyperreactivity from bronchial hyperreactivity among the patients with cough, wheezing, and dyspnea by measuring the decrease in inspiratory flows during the challenge test.[1–3] In another study, voice reactions to histamine provocation were studied among asthmatic and nonasthmatic subjects.[4] In that study population, histamine provocation induced voice changes in some asthmatic patients; voice reactions were not related to the degree of bronchial obstructions, however, leading authors to suggest that laryngeal and bronchial reactions may occur independently of each other. A more recent *in vitro* study on freshly excised porcine vocal fold epithelium demonstrated that histamine compromises the tight junction-related paracellular barrier needed in vocal fold hydration.[5]

These findings support the clinical observation that some patients develop voice and throat symptoms after exposure to histamine. These symptoms are temporary and tend to disappear within a few hours of exposure. This reaction mimics the clinical picture of the laryngeal allergic reaction, which gave us the motivation to investigate the voice symptoms and reactions of the vocal folds during a histamine challenge test, along with the possible background factors.

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Aim of the study

To examine changes in the larynx and self-reported voice and throat symptom changes among patients undergoing a histamine challenge test.

SUBJECTS AND METHODS

Subjects

The study population comprised 30 randomly selected adult patients presenting to the Skin and Allergy Hospital of Helsinki University Hospital with a prolonged cough and suspicion of bronchial asthma. Patients underwent the histamine challenge test in 2012 (May–December). Subjects were either steroid naïve or had not used inhaled glucocorticosteroids in the previous 4 weeks.

Methods

Bronchial hyperresponsiveness (BHR) was evaluated with a dosimetric histamine challenge test; the procedure is described elsewhere.[6] An inhalation-synchronized dosimeter with controlled tidal breathing (Spira Elektro 2, Respiratory Health Care Centre, Hämeenlinna, Finland) was used to nebulize increasing inhaled doses (0.025, 0.1, 0.4, and 1.6 mg) of buffered histamine diphosphate. By using the dose-response curve, the provocative dose of inhaled histamine producing a decrease of 15% in FEV_1 ($PD_{15}FEV_1$) was determined. The severity of BHR was classified as mild ($PD_{15}FEV_1$ 0.41–1.6 mg) or moderate ($PD_{15}FEV_1$ 0.11–0.40 mg), the latter being indicative of asthma. If a patient's FEV_1 was near the 15% decrease but did not reach it after a particular histamine dose, only half the amount of the next dose was given (ie, 0.2 or 0.8 mg).

Before the challenge test, subjects filled in a questionnaire regarding their medical history and airway symptoms. To describe their voice and the effects of their voice in their life, subjects answered the Voice Handicap Index (VHI). This is a 30-item questionnaire assessing the functional, emotional, and psychosocial

consequences of a possible voice disorder, with a frequency scale ranging from 0 to 4 (from never to always) for each question.[7]

Videolaryngostroboscopy was performed immediately before and after the challenge test with a portable videolaryngoscope (rpSzene-Mobile, Rehder/Partner GmbH, Germany) composed of a small 1/3″ CCD camera (model rpCam250, Rehder/Partner GmbH) mounted with a 28- to 35-mm focus zoom lens, combined with a 70° laryngeal telescope (model 4450,47, Richard Wolf, Germany) and a light source (model rp 150, Rehder/Partner GmbH).

Recordings were made digitally on a personal laptop that included rpSzene software. The subjects were seated leaning forward with their chin up during the examination. The recording was performed during an intermittent and sustained "ee" vocalization. Laryngoscopy was done without local anesthesia to avoid the effects that may result from it on the voice and throat symptom questionnaire (visual analog scale [VAS], see below).

Videolaryngoscopy videos were assessed by two experienced phoniatricians and one specializing phoniatrician during the final phase of the training. Videos were assessed in a blinded manner but the samples were presented in a paired arrangement (each pair of samples belonged to the same subject but the timing of the samples and identification of the patient were blinded). The physicians assessed edema and redness of the vocal folds, edema of the interarytenoid area, edema elsewhere in the larynx, and the amount of mucus in the larynx using a four-point scale from none to severe, with the option to label as nonassessable as well. Other possible laryngeal findings were also recorded with an open question.

The interrater reliabilities were $r = 0.77$ (when ratings of redness before the challenge test by raters 1 and 2 were compared), $r = 0.54$ (for raters 1 and 3), and $r = 0.46$ (for raters 2 and 3). Accordingly, the grading of the most experienced rater (rater 1) was chosen for analysis of the data.

Voice and throat symptoms immediately before and after the challenge test were assessed using a VAS composed of 11 parameters; each parameter was assessed using a continuous point scale from 0 to 10, with 0 being no symptom and 10 being the worst possible symptom[8] (Table 3).

Ethical considerations

The study design was approved by the Ethics Committee of the Helsinki and Uusimaa Hospital District, Department of Surgery Dnr 61/13/03/02/2012. The participants received information concerning the study at the time of recruitment and gave their written consent for study participation.

Statistical analysis

Statistical analyses were carried out using SPSS for Windows, Version 21.0.0 Statistical Software (SPSS Inc., Chicago, IL, USA). Descriptive statistics served to describe the demographic characteristics of the study and control groups. Subjects served as their own controls pre- and postchallenge.

Differences in the results before and after the challenge test were compared using the Wilcoxon signed-rank test. Spearman correlation tests were used to statistically analyze the correlation between the patient-reported symptoms and medical history in relation to laryngeal findings. Interrater reliability of the videolaryngostroboscopy was measured using Spearman correlation between each pair of reviewers. The previously mentioned tests were chosen due to the skewed distribution of the parameters.

RESULTS

The mean age of the 30 subjects (22 females) was 40.9 years, ranging from 21 to 66. The mean body mass index (BMI) was 26.1 (standard deviation [SD] 4.8). In the study population, never-smokers were 67% (n = 20), ex-smokers were 20% (n = 6), and current smokers were 13% (n = 4). Based on the clinical diagnostic examinations, 40% of the study subjects (n = 12) were diagnosed with probable or clear asthma, and 63% (n = 19) were atopic (skin prick test positive to common aeroallergens). A variety of airway symptoms was also reported by patients (Table 1).

Out of 30 subjects, 12 (40%) did not have heartburn or regurgitation, whereas 13 (43%) reported to have it occasionally and 5 (17%) reported it often. Regarding reflux medication, most subjects (n = 23, 77%) never took medication, although five took it occasionally and two used it regularly.

Out of 30 patients, three (10%) had moderate bronchial hyperreactivity according to the histamine challenge test and seven (23%) had mild bronchial hyperreactivity, whereas the others did not show any hyperreactivity (ie, $PD_{15}FEV_1 > 1.6$ mg). The maximum histamine dose (1.6 mg) was inhaled by 25 subjects. In five subjects, a significant histamine-induced bronchoconstriction was reached with a lower histamine dose (two with 0.8 mg, two with 0.4 mg, and one with 0.2 mg).

VHI scores varied from 0 to 59 (out of 120), with a mean value of 16 points ± SD 17.7. The mean scores of the subscales were "functional" 4.3 ± SD 5.6, "physical" 8.1 ± SD 7.6, and "emotional" 3.6 ± SD 6.0.

Assessment of the videolaryngostroboscopy recording of the subjects

Two subjects showed unexpected laryngeal findings: one with a small vocal fold polyp and one with vocal fold paralysis. These two subjects were not excluded from the analysis, however, because we were interested in the change in laryngeal function. Three other patients' stroboscopy videos, however, were not assessable due to throat sensitivity resulting from local anesthesia not being used. These patients were excluded from analyses of videolaryngostroboscopy results.

TABLE 1.
Airway Symptoms Reported by the Patients (n = 30)

Symptom	n (%)
Exercise-induced dyspnea	20 (67)
Dyspnea during inspiration	12 (40)
Wheezing during inspiration	8 (27)
Wheezing during expiration	5 (17)
Hoarseness during or after physical exercise	5 (17)
Choker around the neck during exercise	3 (10)
Nasal congestion or runny nose	19 (63)

TABLE 2.
Findings From Videolaryngostroboscopy Before and After the Histamine Challenge Test Presented in Median (IQR) With *P* Value

Parameter	Before (IQR)*	After (IQR)*	*P* Value
Edema of vocal folds	1.0 (1.0–2.0)	2.0 (2.0–2.0)	<0.001
Redness of vocal folds	1.0 (1.0–1.0)	2.0 (1.0–2.0)	<0.001
Edema of the interarytenoid area	1.0 (1.0–2.0)	1.0 (1.0–2.0)	0.564
Edema elsewhere in the larynx	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.000
Amount of mucus	1.0 (1.0–2.0)	2.0 (1.0–2.0)	0.248

* Parameters were assessed on a scale of 1 to 4 where 1 is none and 4 is the most severe form.
Abbreviation: IQR, interquartile range.

Edema and redness of the vocal folds were found to significantly increase after histamine exposure (Table 2). Still photos of patients with examples of these changes are in Figure 1.

The laryngeal findings that increased significantly after the challenge test (vocal fold edema and redness) did not correlate with the subject's gender, age, BMI, current smoking status, asthma status, atopy status, bronchial hyperreactivity ($PD_{15}FEV_1$) measurements, or maximum inhaled histamine dosage. A moderate correlation was seen between the recurrence of heartburn or regurgitation symptoms and change in edema of the vocal folds, referring to an increase in edema of the vocal folds in subjects who had more heartburn ($r = 0.42$, $P < 0.05$).

When airway symptoms (see Table 1) were analyzed for correlations with vocal fold edema, only hoarseness during or after

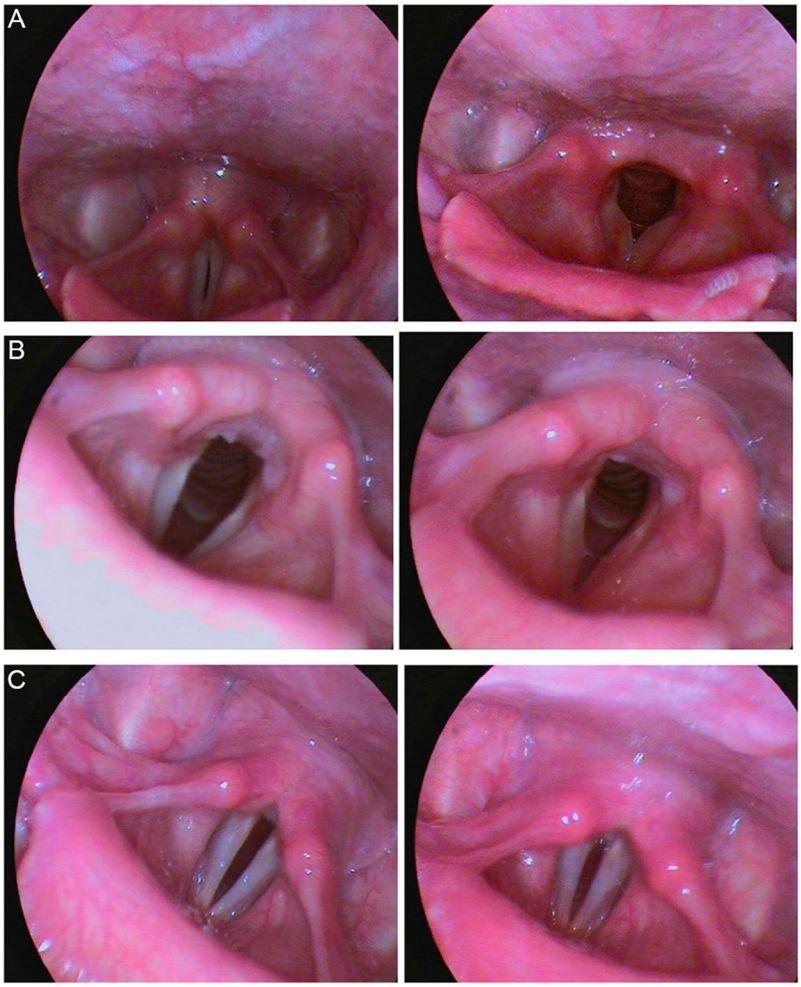

FIGURE 1. **A–C.** Three cases showing the larynx before the histamine challenge test (left picture) and after (right picture). All three subjects inhaled the maximum histamine dose of 1.6 mg. **A.** A male subject with prominent mucosal reaction (edema and erythema) of the vocal folds. **B.** A female subject with prominent mucosal reaction (edema and erythema) of the vocal folds. **C.** A female subject with mild mucosal reaction of the vocal folds.

TABLE 3.
Self-Reported Voice and Throat Symptoms (VAS) Before and After the Challenge Test Presented in Median (IQR) With *P* Value

VAS Symptom	Before* (IQR)	After* (IQR)	*P* Value
1. My voice is overstrained.	2.0 (1.0–4.3)	3.5 (2.0–7.0)	0.001
2. My voice is hoarse or husky.	3.0 (1.8–5.0)	6.0 (3.0–8.0)	<0.001
3. I feel like I have a lump in my throat.	2.0 (1.0–4.0)	2.0 (1.0–4.3)	0.139
4. I feel like I have a choker around my neck.	1.0 (0.0–2.0)	1.0 (0.0–3.3)	0.073
5. I feel like I have mucus in my throat and/or I need to clear my throat frequently.	5.0 (2.0–6.3)	6.0 (3.0–8.0)	0.070
6. My throat is dry and/or itchy.	3.0 (1.0–6.0)	5.0 (3.0–7.3)	0.006
7. My voice is weak/My voice does not resonate.	1.0 (0.0–5.3)	2.5 (1.0–6.3)	0.048
8. My voice is tense or I feel I must make an effort to speak.	1.0 (0.0–2.3)	2.5 (1.0–6.0)	0.005
9. My voice is creaky.	1.5 (0.0–4.0)	2.5 (1.0–5.3)	0.023
10. My voice often breaks when I speak.	1.0 (0.0–4.0)	3.0 (1.0–6.0)	0.004
11. I feel short of breath/I need to gasp for air.	1.0 (0.0–3.0)	2.5 (1.0–6.3)	0.005

* Ranging from 0 to 10.
Abbreviations: IQR, interquartile range; VAS, visual analog scale.

physical exercise had a moderate positive correlation ($r = 0.40$, $P = 0.045$). Additionally, laryngeal findings did not correlate with VHI score.

VAS assessment

By analyzing the VAS scores (ranging from 0 to 10) before and after the challenge test, we found 8 parameters out of 11 that changed significantly after the test (Table 3).

VAS symptom changes resulting from the challenge test did not correlate with the vocal fold edema or vocal fold redness.

One of the 11 VAS symptom changes showed a moderate positive correlation with the VHI total score, namely "my throat is dry and/or itchy" ($r = 0.40$, $P < 0.05$).

The change in the VAS symptom "my throat is dry and/or itchy" correlated positively with the maximum histamine dose ($r = 0.40$, $P < 0.05$). Age correlated positively with the change in the VAS symptom "my voice is creaky" ($r = 0.43$, $P < 0.05$). VAS symptom changes did not correlate with subjects' gender, BMI, asthma or atopy status, nasal symptoms, bronchial hyperreactivity ($PD_{15}FEV_1$) measurements, or heartburn or regurgitation symptoms.

DISCUSSION

Edema and redness of the vocal folds increased significantly after the histamine challenge test. Self-reported voice complaints increased significantly as well, especially for having an overstrained voice and voice being hoarse or husky. Of the 30 subjects, 60% reported heartburn or regurgitation symptoms; additionally, heartburn or regurgitation symptoms and change in edema of the vocal folds showed a moderate positive correlation ($r = 0.42$, $P < 0.05$).

Histamine is an inflammatory mediator that increases vascular permeability and causes fluid to escape from capillaries into the tissues. Zhang and Fisher previously demonstrated, in animal tissue, that histamine treatment compromises the tight junctions related to the paracellular barrier across vocal fold epithelium in freshly excised ovine larynges in a time- and dose-dependent fashion.[5] According to our study, a similar reaction is clinically demonstrable in the human vocal folds during a histamine challenge test. Edema and redness of the vocal folds, visualized by videolaryngostroboscopy, increased significantly after the histamine challenge test when examining the overall study population. In our study population, mucosal reaction did not correlate with the maximum inhaled histamine dose (1.6 mg), which is understandable due to the fact that all but five patients received the maximum dose. Interestingly, among patients receiving the maximum dose, the vocal fold reaction still varied from very mild to severe. Thus, it appears that reaction to histamine is not just dose dependent, but also individual.

Healthy vocal fold stratified squamous epithelium provides a barrier against exogenous and endogenous toxins getting into the underlying lamina propria. Factors that may interfere with the vocal fold epithelial barrier and permeability have been investigated in a few previous studies and the subject of a recent review.[9] In one of these studies, acidic pepsin and acid-only challenges characteristic of gastric reflux in *ex vivo* porcine vocal folds reduced transepithelial resistance, compromising the epithelial barrier function.[10] Among our study patients, the vocal fold edema during the challenge test correlated moderately with heartburn or regurgitation reported by the patients, referring to more apparent edema of the vocal folds among subjects with more heartburn or regurgitation. This suggests that reflux symptoms predispose the more pronounced effects of histamine on vocal fold mucosa. However, a recent *in vivo* study demonstrated that liquid-acidified pepsin applied to healthy porcine vocal folds did not significantly compromise epithelial structure or function, suggesting that healthy vocal folds are able to effectively defend against acidified pepsin challenges.[11] Therefore, the effects recorded in our study and attributed to reflux symptoms may be occurring among the subset of patients included in this study and cannot be generalized to all patients. In another previous study, the effects of the surface drying on the porcine vocal fold epithelial barrier were examined by hypertonic challenges, which were found to increase permeability of the vocal fold epithelium.[12] Vocal folds are at risk for surface dehydration during oral breath-

ing, which can increase the tonicity of surface fluid. Among our study patients, airway symptom "hoarseness during or after physical exercise" had a moderate positive correlation with vocal fold edema resulting from the histamine challenge. Hoarseness during the exercise might be due to dryness of the vocal folds, which may make the vocal folds more vulnerable to substances like histamine.

Atopy, asthma, nasal symptoms, or bronchial hyperreactivity during the histamine challenge test were not associated with laryngeal reactions of our study patients, so allergy and other inflammatory processes of the upper and lower airways do not seem to directly affect the vocal fold mucosa permeability. This is in line with a previous study where voice reactions to histamine provocation were studied among asthmatic and nonasthmatic subjects.[4] In that study population, histamine provocation did induce a voice change in some asthmatic patients, but the voice reaction was not related to the degree of bronchial obstruction, leading authors to suggest that laryngeal and bronchial reactions may occur independently of each other. Conversely, a study of 441 patients did report a connection between chronic diseases of the upper respiratory tract and extrathoracic hyperresponsiveness, measured by a decrease in inspiratory airflow rates during the histamine challenge test, suggesting the inflammation to be the triggering factor for the functional abnormality.[3] In another study by this group, fiberoptic laryngoscopy was performed during the histamine challenge test on nine patients and three controls.[1] After histamine challenge, seven patients with hyperresponsiveness of the extrathoracic (upper) airway showed marked mucosal edema, pharyngoconstriction, and adduction of the vocal folds during the forced inspiration. No significant change upon laryngoscopy was observed in the three controls or the two patients with bronchial hyperresponsiveness only. In line with our study, a varied mucosal reaction was also demonstrated.

The self-reported voice and throat complaints (VAS) during the challenge increased significantly for 8 of 11 symptoms, pointing out that inhaled histamine causes a spectrum of laryngeal symptoms. However, a correlation between the laryngeal findings and VAS symptoms was not found. It is possible that the vocal tract can compensate for short-term changes in the vocal folds by maintaining the perceptual end product (voice) and thus the voice symptoms did not correlate.[13] It is also possible that a larger study group or differently formulated VAS symptoms are needed to find the connection between the symptoms and edema of the vocal folds resulting from the challenge test.

VAS symptom's "my throat is dry and/or itchy" change showed a moderate positive correlation with the VHI total score. It seems that subjects experiencing more throat irritation due to the histamine suffer from more voice complaints in everyday life. Among our study patients, the VHI total score did not correlate with the laryngeal mucosal reaction during the challenge. However, it must be noted that overall VHI scores remained relatively low (mean value: 16 points ± SD 17.7), probably because study participants were examined due to a prolonged cough and suspicion of asthma and not due to a voice disorder. The reaction of vocal fold mucosa and changes in voice and throat symptoms during the histamine challenge among the patients with voice disorders would be an interesting subject for future research.

The maximum histamine dose during the challenge correlated with the VAS symptom "my throat is dry and/or itchy," pointing out, logically, that more inhaled histamine causes more pharyngeal or laryngeal irritation. Older subjects experienced a creakier voice after the challenge, which might be a sign of the better compensatory mechanisms of the younger persons' voice apparatus.

Histamine is mainly generated and stored in granules in the mast cells. In specific immunoglobulin E (IgE)-mediated allergy, the antibodies on the surface of mast cells in sensitized subjects trigger degranulation and the release of histamine. In a previous immunohistochemical study of seven excised human larynxes, a large number of mast cells were found in the epiglottis, arytenoid, and subglottis, with a distribution similar to that found in the nasal mucosa.[14] On the contrary, only a few mast cells are found in the vocal folds.[14,15] This suggests that similar mucosal allergic reactions, such as in allergic rhinitis, can be induced in the epiglottis, arytenoid, and subglottis areas, but is less likely in true vocal folds.

In line with the immunohistochemical study, additional previous studies were unable to find a direct association between exposure to an antigen during a challenge test and vocal fold reaction.[8,16,17] However, there are opposite reports of provocation tests indeed finding a direct association, including one that was a continuation of the study by Reidy where no direct association was found. This study had to be prematurely terminated after only 2 patients because of the adverse effects following treatment with the highest concentration of the antigenic suspension.[18] In both patients, mild coughing, hoarseness, and vocal fold edema and redness occurred. In another study, the immediate allergic IgE-mediated laryngeal reaction of redness and edema of the vocal cords during the specific provocation test was demonstrated among a group of patients with occupational laryngitis.[19] Differences in methodology likely led to these contradictory findings regarding direct associations of antigen exposure and vocal fold reaction. Allergic reaction of the vocal folds is still a controversial subject. Histamine challenge tests can be hypothesized as an experimental design of mucosal hypersensitivity reaction of the vocal folds, and the reaction of the vocal fold mucosa (edema and redness) is objectively visible on videolaryngostroboscopy.

A limitation of our study is a lack of a control group with placebo inhalation, which would have provided stronger statistical evidence. The patients were aware that they are inhaling potentially irritating substance, which might have an impact on VAS symptoms. On the other hand, the patients did not see their own prechallenge answers after the challenge test to avoid the comparison, which increases the reliability of the findings. Regarding the laryngeal findings, it is unlikely that the mucosal changes were explained by the patients' awareness of the inhaled substance. Still, without a control group it is impossible to exclude the partial effect of the inhalation procedure or other artifacts. Laryngostroboscopy videos were assessed in a blinded manner with paired arrangement, because we were interested in the possible change of the laryngeal status of each subject. Such paired but blinded assessment was used to enable finding minimal and subtle changes in the laryngeal status that would otherwise be missed, if all videos were grouped together.

On the basis of this study, it remains to be elucidated whether the mucosal reaction during the histamine challenge test reflects the vocal folds' reactivity to other inhaled environmental irritants. The reaction of vocal fold mucosa and changes in voice and throat symptoms during the histamine challenge among patients with voice disorders, especially among the patients experiencing sudden voice complaints due to nonspecific irritating agents (eg, odors, fumes), would be an interesting subject for future research.

CONCLUSION

Our study demonstrates that the laryngeal mucosal reaction during a histamine challenge test can be objectively visualized with videolaryngostroboscopy. The reaction is specific to each individual, probably due to permeability features of each subject's vocal fold epithelium. This histamine-produced reaction could be used as an experimental design in future studies when examining the reactions of vocal fold mucosa to specific or nonspecific irritants.

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