

Effects of sequential *Dermatophagoides pteronyssinus* antigen stimulation on anatomy and physiology of the larynx

James P. Dworkin, PhD; Patrick M. Reidy, MD; Robert J. Stachler, MD;
John H. Krouse, MD, PhD

Abstract

The goal of this investigation was to study the effects of sequential dust mite antigen stimulation on the appearance and function of the larynx. To that end, we designed a randomized, placebo-controlled, double-blind, prospective analysis of adults who had tested positive for perennial dust mite allergy. The larynx of patients who received the active antigen was challenged directly with a low (1:100) and a high (1:40) concentration of the dust mite allergen via an oral nebulizer. Voice laboratory assessment tools included voice and allergy questionnaires, videostroboscopic examination of the larynx, acoustic and speech aerodynamic analyses, and digital audio voice recordings. The study was prematurely terminated after 2 patients had been treated with the highest concentration of the antigenic suspension because of adverse effects, including chest tightness, coughing, and voice difficulties. Both of these patients had demonstrated viscous endolaryngeal secretions and vocal fold edema on videostroboscopy. No reactions were noted at the lower concentration of antigen exposure or in 1 control patient who completed the study. We believe that our findings, as preliminary as they are, may serve as an initial template for the differential diagnosis and treatment of other patients with inhalant allergies who present with chief complaints suggestive of allergic laryngitis.

Introduction

A great deal of debate has taken place over the past 40 years regarding the effects of allergy on the larynx. These discussions have centered on whether the laryngeal disturbances that have been noted in some allergic individuals represent (1) local end-organ responses or (2) manifestations of systemic and multiple end-organ sequelae.¹⁻³ Chadwick reported that the vast majority of studies of laryngeal allergy have not demonstrated a true cause-and-effect relationship between antigen exposure and pathophysiologic laryngeal reactions in patients with either seasonal or perennial allergies.⁴

In our previously published research, we challenged dust-mite-allergic patients with a fixed amount of an active antigenic suspension via a nebulizer.⁵ We then obtained measures of phonation and respiration subsystem responses to study potential disturbances in laryngeal biomechanics, acoustics, and speech aerodynamics that might accrue with such exposure. In that prospective investigation, we used a low concentration of antigen (1:200 weight/volume [w/v] ratio). Although we observed no statistically significant adverse effects, we did see a tendency toward higher-than-normal degrees of baseline and postexposure endolaryngeal secretions in many of our patients. The fact that these results were consistent with findings reported by others prompted us to undertake the current investigation, the purpose of which was to increase the levels of dust mite antigen stimulation and obtain objective and subjective measurements of both laryngeal and pulmonary functional reactions in allergic patients. We hypothesized that antigenic suspensions administered at higher concentrations than those used in our previous study would elicit a significant response in the larynx and that we would

From the Department of Otolaryngology—Head and Neck Surgery, Wayne State University, Detroit.
Corresponding author: James P. Dworkin, PhD, 37000 Fox Glen, Farmington Hills, MI 48331. Phone: (313) 745-5673; fax: (313) 577-8555; e-mail: aa1544@wayne.edu

be able to observe this response on acoustic analysis, speech aerodynamic testing, videostroboscopy, and audio voice recordings.

Patients and methods

Our investigation was designed as a randomized, placebo-controlled, double-blind, prospective study. Consistent with our earlier investigation, we used a two-group pretest/post-test methodology in which patients were challenged with either the active antigen for *Dermatophagoides pteronyssinus* or a placebo suspension. To control for bias or expectancy factors, the patients, examiners, and judges were blinded to all experimental conditions.

Patients. We obtained approval from the Human Investigation Committee at Wayne State University to recruit 20 otherwise healthy, dust-mite-allergic patients between the ages of 18 and 70 years from the patient population at the university's Department of Otolaryngology-Head and Neck Surgery in Detroit; patients were also recruited from University Otolaryngology, PC, a private-practice outpatient clinic in Southfield, Mich. Because of the potential risks involved in directly stimulating the bronchopulmonary tree and larynx with an allergen, we established extensive exclusion criteria. Evidence of any one of the following factors excluded a patient from study participation: pregnancy (determined by a urine human chorionic gonadotropin [hCG] assessment before testing), a history of smoking within the past 2 years, asthma, systemic corticosteroid treatment in the past 6 months, immunotherapy for allergy within the past 2 years, current use of topical or systemic antihistamines, current use of topical nasal or oral inhalers, or a forced expiratory volume at 1 second (FEV₁) of less than 80% of predicted value. All potential risks of antigen stimulation on the larynx were explained, and informed consent was obtained from all patients prior to the study.

Allergy testing. The presence of allergy to *D pteronyssinus* was assessed with a screening skin-prick test (Multi-Test II; Lincoln Diagnostics; Decatur, Ill.). Testing was done with 0.01 ml of a 1:20 w/v concentration of Der p1 antigen placed into the superficial dermis. To qualify for participation, a patient had to exhibit a skin wheal response of between 5 and 11 mm. These patients were then judged to be positive for the antigen without the need for further quantification of the degree of reactivity. A positive histamine control was used to ensure normal skin reactivity, and a glycerin control was used to ensure that patients were reacting to the antigen and not to the glycerin preservative.

Antigen challenge. The active antigenic suspension was made by a technician by combining 1 ml of a 1:20 w/v Der p1 antigen concentrate (Antigen Laboratories; Liberty, Mo.) in 9 ml of 0.4% phenolated normal saline, resulting in a 1:200 w/v suspension. The placebo was made by placing 1 ml of colored 50% glycerin in normal saline into 9 ml of 0.4% phenolated normal saline. Colorization was accomplished by inserting 3 drops of yellow food coloring into a 50-ml vial of 50% glycerin in saline. Then two-fold and five-fold dilutions were made, resulting in suspensions of 1:100 and 1:40 w/v, respectively. All antigen and placebo suspensions were prepared within 1 week of use.

For laryngeal challenge, 0.5 ml of either the Der p1 antigenic suspension or the control suspension was administered via a DeVilbiss nebulizer (DeVilbiss Healthcare; Somerset, Pa.). All testing was conducted in the afternoon. Patients were instructed to breathe normally through their mouths as the suspension was nebulized. They inspired the material transorally over a period of 5 minutes, and they were observed throughout this period for any adverse reactions. If after 30 minutes any patient demonstrated no response to the inspired challenge and the FEV₁ remained greater than 80% of predicted, the entire testing battery was repeated with the highest concentration.

Laboratory testing battery. The study was conducted in the Speech Physiology Laboratory at the Department of Otolaryngology-Head and Neck Surgery's outpatient clinical facility. The 7-step testing sequence for each patient was as follows:

1. Spirometry for FEV₁
2. Completion of the Voice Handicap Index (VHI) questionnaire
3. Completion of the Sinus Symptom Questionnaire[®] (SSQ) to ascertain allergy symptoms
4. Videostroboscopic examination of the larynx
5. Analyses of acoustic characteristics (fundamental frequency, harmonics/noise, jitter, and shimmer) and speech aerodynamic characteristics (maximum phonation time, mean transglottal airflow, glottal resistance, and subglottal air pressure)
6. Digital audio voice recordings
7. Antigen or placebo exposure via a nebulizer

Steps 1 through 6 were performed at baseline and 30 minutes after both antigen challenges. In order to progress to challenge with the highest concentration of antigen, a patient had to register an FEV₁ of greater

Table 1. Categories of videostroboscopic and audio findings

Videostroboscopy (larynx)	Audio (voice)
1. Supraglottic edema	1. Prosodic sufficiency
2. Supraglottic erythema	2. Speech intelligibility
3. Supraglottic secretions	3. Voice quality
4. Viscosity of supraglottic secretions	4. Vocal pitch/loudness
5. Presence and size of vocal fold polyps	
6. Glottic edema	
7. Glottic erythema	
8. Glottic secretions	
9. Viscosity of glottic secretions	
10. Vocal fold mobility	
11. Glottic competence	

than 80% of the predicted level after challenge with the lower concentration. (Detailed explanations of these procedures are described in our previous article.³)

Examiners and judges. The examiners—who were responsible for administering the skin-prick screen, test battery, and nebulizer challenge—were blinded as to whether the patient was inhaling the antigen or the placebo. Following termination of the study, two independent judges (a board-certified otolaryngologist and an American Speech-Language-Hearing Association-certified speech pathologist) analyzed the videostroboscopic and digital audio voice recordings. The judges were unaware of the identities of the patients on the recordings, and they were also blinded as to whether the recordings had been made before or after exposure to the antigen or placebo.

Videostroboscopic findings were rated for the presence, nature, and degree of supraglottic and glottic edema, erythema, and secretions; for the presence and size of vocal fold polyps; and for the degree of vocal fold mobility and glottic competence (table 1). Digital audio recordings of connected discourse, picture descriptions, and prolonged vowel productions were rated by the judges for prosodic sufficiency, speech intelligibility, voice quality characteristics, and vocal pitch/loudness features on the same 6-point scale (table 1). The judges

Table 2. Subjective rating scale for videostroboscopic and audio findings

1. Normal
2. Mild disturbance
3. Mild to moderate disturbance
4. Moderate disturbance
5. Moderate to severe disturbance
6. Severe disturbance

rated all their subjective findings on a scale of 1 (normal) to 6 (severe disturbance) (table 2). Each judge was required to evaluate each finding on two separate occasions in order to maximize intra- and inter-judge reliability. Both intra- and inter-judge ratings had to be within 1 point of each other to be considered reliable.

Results

In all, 3 patients (all men) completed all the challenges. We had planned to conduct this study on 20 patients, but we were forced to terminate the investigation prematurely after

serious adverse pulmonary reactions were experienced by the first 2 patients who received the active antigen; in both of these patients, these reactions occurred following exposure to the highest antigen concentration. No reactions were noted at the lower concentration of antigen exposure or in the 1 control patient who was exposed to the placebo.

With the exception of their allergies, all 3 patients were in excellent health. They denied problems with acid reflux, and they had never smoked tobacco products.

Despite the premature termination of the study, we are still able to make some observations about the findings obtained for the 3 patients who underwent testing.

Patient 1. This patient served as the control. Prior to exposure, he listed numerous complaints about his voice on the VHI; these complaints included a varying quality of voice during the day, vocal fatigue, and difficulty projecting voice in a loud environment. He also noted a snoring problem on the SSQ. His VHI and SSQ responses revealed no change in status following the placebo challenges.

The control patient's acoustic characteristics (figure 1) and speech aerodynamic characteristics (figure 2) were all unremarkable prior to and following the placebo challenges (figure 1).

On videostroboscopic analysis, the judges did not perceive any substantial anatomic or biomechanical abnormalities in the larynx or vocal folds prior to or following each challenge (figure 3, A; figure 4, A and B). However, mildly increased levels of supraglottic and glottic mucus accumulation were observed both at baseline and during challenge conditions.

After listening to the audio voice recordings, the judges perceived patient 1's voice abilities to be within normal limits at the start of the study and throughout its duration.

Patient 2. At baseline, this individual reported mild voice complaints on the VHI and no complaints on the SSQ. His questionnaire responses remained unchanged following the lower-concentration challenge. However, after exposure to the highest concentration of antigen, he did experience chest tightness, episodic coughing, and hoarseness. His pulmonary capacity as reflected in the FEV₁ measurement declined from more than 100% at baseline and after the first challenge to 93% after administration of the highest antigen concentration.

Acoustic (figure 1) and speech aerodynamic (figure 2) analyses did not reflect any notable differences across the entire experiment.

Analysis of the videostroboscopic recordings revealed that at baseline, patient 2 had very mild vocal fold edema, erythema, and standing endolaryngeal secretions (figure 3, B; figure 4, C). Following exposure to the highest concentration of antigen, he exhibited evidence of mild supraglottic edema and erythema, mildly increased levels of vocal fold edema and erythema, and more abundant and viscous endolaryngeal secretions (figure 3, B; figure 4, D).

Patient 2's voice characteristics, which were normal at baseline, were not perceived by the judges to have changed throughout the study (figure 5, B).

Patient 3. At baseline, patient 3 did not complain of any voice difficulties (VHI) or sinus symptoms (SSQ), and following the lower-concentration challenge, his responses to the questionnaires remained unchanged. But immediately after exposure to the highest concentration of antigen, he struggled with significant wheezing and shortness of breath in addition to chest tightness, coughing, and voice

difficulties. His pulmonary capacity dropped markedly from well above 100% at the first two measurements to 47%. Fortunately, this severe pulmonary reaction did not require treatment in the emergency room; it resolved over the course of 30 minutes while the patient was carefully monitored by the examiners.

Findings on acoustic analysis (figure 1) remained within normal limits throughout the study, but speech aerodynamic analysis (figure 2) showed that patient 3's mean maximum phonation time (figure 2, A) decreased from more than 20 seconds after the lower-concentration challenge to 8 seconds following the highest-concentration challenge. The remaining measurements were

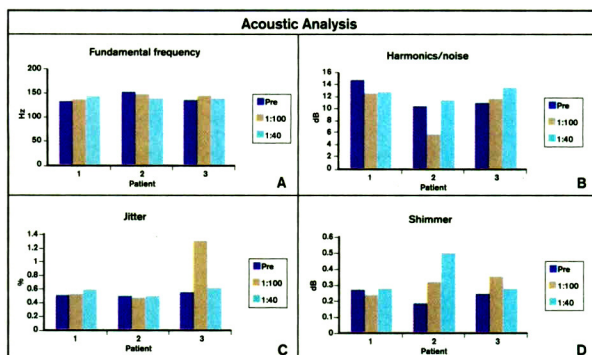


Figure 1. Charts show the acoustic findings in the control patient who received placebo (patient 1) and the 2 patients who received active antigen (patients 2 and 3).

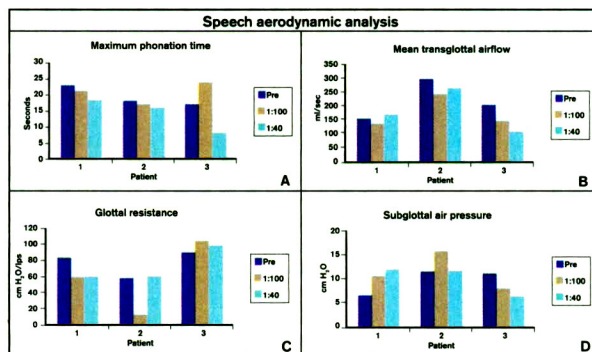


Figure 2. Charts show the speech aerodynamic findings in the control patient who received placebo (patient 1) and the 2 patients who received active antigen (patients 2 and 3).

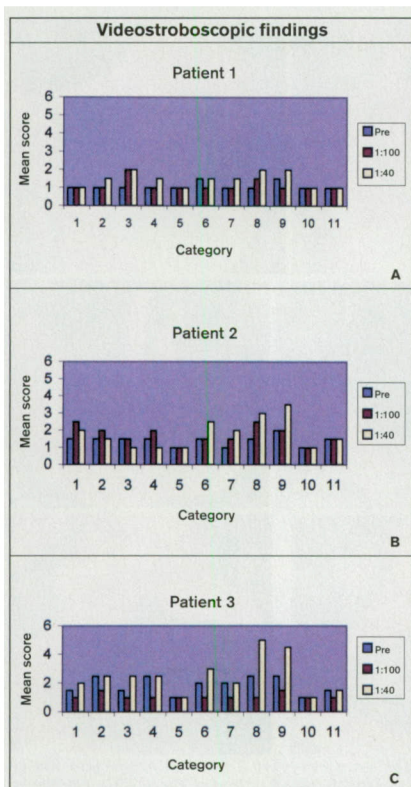


Figure 3. Charts show the judges' subjective ratings of laryngeal findings on the videostroboscopic recordings of the control patient who received placebo (patient 1) and the 2 patients who received active antigen (patients 2 and 3) prior to and following both challenges. The judges rated each of 11 categories (table 1) on a scale of 1 (normal) to 6 (severe disturbance).

within normal limits throughout the study.

Videostroboscopy showed that patient 3 had very mild supraglottic and glottic edema, erythema, and endolaryngeal secretions at baseline (figure 3, C; figure 4, E). After administration of the lower concentration of antigen, he experienced a very slight increase in the degree of endolaryngeal secretions. But after exposure to the highest concentration, he experienced marked increases in vocal fold edema and the degree and viscosity of endolaryngeal secretions (figure 4, F).

Voice analysis revealed that after exposure to the highest concentration of antigen, patient 3 experienced a perceptible decrease in voice quality; at baseline, his speech and voice characteristics had been judged to be within normal limits (figure 5, C).

Judges. Both intra- and inter-judge reliability exceeded 90% for all of the videostroboscopic and digital audio ratings.

Discussion

No study to our knowledge has conclusively shown that a cause-and-effect relationship exists between antigen exposure and direct changes in the anatomy and function of the larynx. However, previous investigators have suggested that there is a high correlation between allergy and laryngeal signs and symptoms, including throat irritation, soreness, burning, and laryngitis.⁷⁻¹⁰ These authors have also reported successful alleviation of these signs and symptoms with the administration of various empiric treatments, including allergen-specific immunotherapy.

The chief aim of the present study was to obtain objective and subjective evidence of laryngeal pathophysiologic reactions to dust mite exposure in individuals with known allergies to *D pteronyssinus*, a perennial antigen. We used a sequentially higher concentration of this stimulant for this study because our previous study failed to detect any such sequelae with low-concentration (1:200 w/v) exposure.⁵ However, because of the onset of marked respiratory distress and acute coughing following administration of the highest concentration of antigen (1:40 w/v), the study was terminated after only 3 patients had participated—2 who underwent active treatment and 1 placebo control.

Even so, the responses of the 2 patients who did receive the highest antigen concentration were particularly noteworthy. Patient 2 evidenced about a 10% decrease in his FEV₁ level following such exposure, and patient 3 experienced a reduction of about 50% along with severe wheezing and chest tightness symptoms. In both patients, mild coughing, hoarseness, and vocal fold edema and erythema occurred. Throat clearing was observed in association with abundant and viscous endolaryngeal standing secretions, which seemed to trigger these reactions and which were not present at baseline. What remains uncertain is whether such mucus production originated in the larynx or was actually coughed upstream from the lower respiratory tract secondary to concomitant bronchospasms.

It is important to interpret the findings of this pre-

liminary investigation with caution, particularly in view of the limited number of patients studied. The data obtained do not definitively demonstrate a cause-and-effect relationship between allergen exposure and laryngeal pathology. However, the results do lend support to our experimental hypothesis that adverse physiologic phonation and respiration subsystem reactions would be provoked in dust-mite-allergic patients if their larynges were deliberately exposed to high concentrations of *D pteronyssinus*. We suggest that the laryngeal abnormalities noted in our patients occurred secondary to substantial lower respiratory stimulation and irritative tracheobronchial responses. These combined reactions helped to illustrate both the unified functional relationship between the anatomically interconnected respiration and phonation subsystems and their co-occurring vulnerability to inhaled allergens.

The 2 patients in the present study who were exposed to the dust mite antigen experienced a series of upper and lower respiratory tract symptoms in a specific sequence immediately following the administration of the highest antigen concentration (table 3). We believe that the proposed allergy model represented by this sequence helps to explain the specific findings in these 2 patients, and it may serve as an initial template for the differential diagnosis and treatment of other patients with inhalant allergies who present with chief complaints suggestive of allergic laryngitis.

In conclusion, the role of allergy in chronic laryngeal inflammation and dysfunction is becoming increasingly clear. Results from the present study demonstrate exposure to increasing concentrations of antigen provokes both upper and lower airway signs and symptoms. A combination of direct inflammatory changes in the endolarynx and secondary transport of mucus to the larynx results in coughing, throat clearing, and other

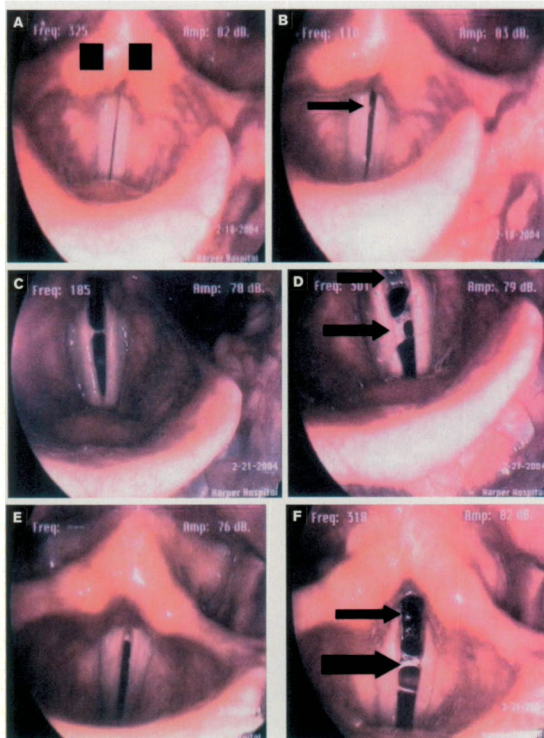


Figure 4. Videostroboscopic images show patient 1 (the control) at baseline (A) and following the final placebo challenge (B); patient 2 at baseline (C) and following the highest-dose antigen challenge (D); and patient 3 at baseline (E) and following the highest-dose antigen challenge (F). Note: The arrow in image B (post-placebo challenge) illustrates no significant mucus accumulation at the glottis; the arrow in image D (post-high-dose antigen challenge) illustrates substantial mucus accumulation at the glottis; and the arrows in image F (post-high-dose antigen challenge) illustrate substantial mucus accumulation at the glottis.

effects. While these acute findings can be seen within minutes of significant antigen exposure, it is likely that a prolonged exposure to antigen among susceptible individuals results in chronic laryngeal changes secondary to dysfunctional voice behaviors. It is probable that these chronic effects are responsible for a significant portion of the dysphonia observed in allergic patients with chronic laryngitis.

Additional research is necessary to segregate the laryngeal findings that are attributable to allergic stimulation

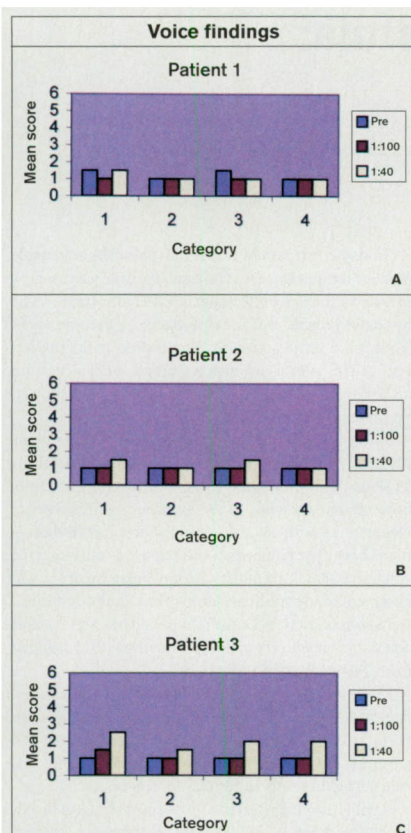


Figure 5. Charts show the judges' subjective ratings of voice findings on digital audio recordings of the control patient who received placebo (patient 1) and the 2 patients who received active antigen (patients 2 and 3) prior to and following both challenges. The judges rated each of 4 categories (table 1) on a scale of 1 (normal) to 6 (severe disturbance).

from the irritative and inflammatory effects that are related to other known laryngeal pathologies. Of note, laryngeal dysfunction related to laryngopharyngeal reflux and chronic vocal abuse must be evaluated in patients with allergic disease to determine if it plays an additive or synergistic role in the development of chronic laryngitis. We are currently in the process of implementing prospective protocols in our labora-

Table 3. Sequence of symptoms observed following administration of the highest concentration of antigen

1. Nasal congestion
2. Reactive runny nose
3. Resultant postnasal drainage
4. Itchy palate
5. Globus or foreign-body sensation
6. Bronchospasms that cause vigorous coughing
7. Pulmonary congestion
8. Decrease in FEV₁
9. Shortness of breath
10. Upward migration of mucus and pulmonary secretions
11. Laryngeal mucus accumulation
12. Reactive throat clearing
13. Resultant vocal fold edema
14. Ensuing vocal fatigue
15. Dysphonia

tory that are designed to address the relative impact of these known pathologic processes so that we may more clearly define the role of allergy in the pathogenesis of laryngeal dysfunction. Finally, studies aimed at measuring the influences of antigen stimulation on cytologic and histopathologic indicators would help refine our proposed model of laryngeal allergy.

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