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Correlation of pepsin-measured laryngopharyngeal reflux disease with symptoms and signs

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ABSTRACT

OBJECTIVE: Pepsin detection in throat sputum has been posited as a reliable biological marker of laryngopharyngeal reflux disease (LPRD). This study was designed to further correlate pepsin concentration with symptoms and signs of LPRD.

STUDY DESIGN: Cross-sectional study.

SETTING: Nanfang Hospital of Southern Medical University.

SUBJECTS AND METHODS: Fifty-six laryngitis patients were divided into a reflux laryngitis group and a chronic laryngitis group based on the reflux symptom index (RSI), reflux finding score (RFS), and proton pump inhibitor treatment for two weeks. Oral and hypopharyngeal secretions from the study patients and from 15 healthy subjects were collected. Thirty-six obstructive sleep apnea (OSA) patients were divided into a mild-moderate group and a severe group by the apnea-hypopnea index (AHI). Bedtime and first-morning oral secretions were collected. Enzyme-linked immunosorbent assay was used to measure the pepsin concentration.

RESULTS: In laryngitis patients, the total score of RSI and RFS ($P < 0.05$), and the symptoms, including clearing throat often, coughing, and sensing a lump in the throat ($P < 0.006$), were more severe in the pepsin-positive group. No significant differences were found between the oral and hypopharyngeal secretions. In OSA, pepsin levels in the first-morning oral secretions were correlated with AHI, mean SaO_2, and mini SaO_2 ($P < 0.01$). However, RSIs were not significantly correlated with these indicators.

CONCLUSION: Higher levels of pepsin in sputum were associated with higher RSI and RFS in cases of laryngitis. There was no relationship between pepsin levels and RSI in cases of OSA. There were no differences of pepsin concentration in sputum collection methods or in collection timing.

Laryngopharyngeal reflux (LPR), the backflow of gastric contents into the larynx, pharynx, trachea, and bronchus, contributes to several upper airway inflammatory disorders, which are referred to as laryngopharyngeal reflux disease (LPRD). This term was adopted by the American Academy of Otolaryngology–Head and Neck Surgery in its 2002 position statement on LPR.[1] LPR is associated with reflux laryngitis, obstructive sleep apnea (OSA), laryngeal cancer, contact laryngeal ulcers, laryngomalacia, posterior glottic edema and erythema, laryngeal granuloma, laryngospasm, stridor, subglottic stenosis, and otitis media with effusion.[2-4]

To our knowledge, no method has been validated to perform a quantitative analysis of LPRD,[5] which might be a main cause of many misdiagnosed LPR patients. The method suggested by Ford[6] to combine a set of measurements to assess and manage LPRD has become increasingly accepted. Ford suggested that patients be first clinically evaluated with the reflux symptom index (RSI; history, symptoms > 13), the reflux finding score (RFS; videolaryngoscopy > 7), and then be engaged in an empirical trial of lifestyle and dietary changes. After an initial assessment, clinicians should treat LPR for three months using proton pump inhibitors, the most potent form of acid suppression therapy available. Multi-channel impedance and pH monitoring are to be used in only those patients whose symptoms are unchanged or have worsened after three months of proton pump inhibitor therapy. This method not only confirms reflux using clinical characteristics, but also implements treatment for suspected cases.

Pepsin plays an important role in the development of many reflux-related disorders. Pepsin has been posited as a reliable diagnostic marker of LPRD[7] because it can be easily detected in airway secretions when gastric reflux has occurred for an extended period of time. The recently published data on the assessment of throat sputum samples has showed that pepsin immunoassay is as sensitive and specific for LPRD as 24-hour double-probe (esophageal and pharyngeal) pH monitoring.[8] Although pepsin is inactive at pH 6.5 and above, it remains stable up to pH 8.0 and can be reactivated when the pH is reduced by a subsequent acidic reflux event. In addition, pepsin is stable for at least 24 hours at pH 7.0 at 37°C and retains 79 percent (SD 11%) of its original activity after re-acidification at pH 3.0.[9] Bulmer et al[10] reported that laryngeal tissues are essentially resistant

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to damage up to pH 4.0 but are damaged when pepsin is present in the porcine larynx model. The subglottic mucosa and the vocal folds are damaged more easily than the posterior commissure and ventricular mucosa. However, we still have limited information about how pepsin contributes to the laryngeal inflammation expressed by the symptoms and by laryngeal findings in patients.

There were two objectives in this study: 1) to evaluate the correlation between the severity of clinical characteristics and sputum pepsin concentration in two diseases often associated with LPR: reflux laryngitis and OSA; and 2) to compare the methods and timing of sputum collection.

Materials and Methods

The study protocol was approved by the Committee of Nanfang Hospital. Before initiation of any procedure, signed informed consent was obtained from all participants.

Subjects

We recruited 56 subjects (27 men, 29 women; mean age 37.77 ± 10.59 years) enrolled in the Department of Otolaryngology of Nanfang Hospital from April to October 2007 with unspecific laryngopharyngeal symptoms such as chronic cough, throat clearing, globus sensation, and hoarseness. Thirty-six subjects (34 men, 2 women; mean age 40.67 ± 7.42 years) registered in the Center for Sleep Disorders of Nanfang Hospital during April to June 2008, were likewise recruited as the study's OSA patients. All patients with acute infections of the upper respiratory tract, chronic tonsillitis, chronic sinusitis, malignant tumor, or a history of antireflux medical therapy or continuous positive airway pressure treatment for one month or more were excluded. The normal group consisted of 15 healthy subjects (9 men, 6 women; mean age 25.07 ± 3.24 years).

Study Design

The laryngitis patients and healthy subjects were asked to complete the self-administered nine-item RSI questionnaire[11] and were examined by videolaryngoscopy to reveal any laryngeal mucosal abnormalities. The video recordings were evaluated using RFS[12] by two otolaryngologists, each with more than five years of experience in the field, who worked independently and were blind to the patient data. The mean score for the subjects was used for analysis of the results. The laryngitis patients were treated with 20 mg of omeprazole twice daily for two weeks. A diagnosis of reflux laryngitis (16 men, 16 women; mean age 38.75 ± 9.96 years) was confirmed by RSI $>$ 13, RFS $>$ 7, and by the improvement of characteristic laryngoscopic findings or symptoms after two weeks of proton pump inhibitor therapy.[6,13] The patients who did not meet the criteria were categorized into the chronic laryngitis group (11 men, 13 women; mean age 36.46 ± 11.45 years).

The OSA patients were first asked to complete the Epworth Sleepiness Scale (ESS) and the RSI questionnaire. Polysomnography was then performed using a Sandman Elite diagnosis recording system (Tyco, Inc., Ottawa, Ontario, Canada). Polysomnography reports established OSA severity using the apnea-hypopnea index (AHI) and measurement of mini SaO_2 and mean SaO_2 levels. Thirty-six OSA patients were divided into two groups according to the severity of their sleep apnea:[14] 1) the "mild-moderate group," AHI 5 to 30 (10 men; mean age 39.30 ± 7.60 years), and 2) the "severe group," AHI ≥ 30 (24 men, 2 women; mean age 41.19 ± 7.43 years) (Table 1).

Throat Sputum Collection

Two different samples consisting of oral secretions and hypopharyngeal secretions were collected from the laryngitis patients and the healthy subjects. The subjects were asked to spit into a container at least 0.5 mL of sputum to be used for enzyme-linked immunosorbent assay (ELISA). These samples were defined as the oral secretions. The videolaryngoscopy and a disposable sputum aspiration catheter were inserted into patients through different nostrils after superficial anesthesia. Once these catheters had been advanced to the sinus piriformis or postcricoid area, the system was connected to a vacuum pump to extract the sputum defined as the hypopharyngeal secretions. One hundred six oral and hypopharyngeal secretions from 56 laryngitis patients and 30 samples from 15 healthy subjects were successfully collected. Unfortunately, two hypopharyngeal secretions from the reflux laryngitis group and four hypo-

Table 1
Characteristics of the groups

		Laryngitis group (n = 56)		OSA group (n = 36)	
	Normal (n = 15)	Reflux laryngitis (n = 32)	Chronic laryngitis (n = 24)	Mild-moderate (n = 10)	Severe (n = 26)
Age, yrs					
Mean ± SD	25.07 ± 3.24	38.75 ± 9.96	36.46 ± 11.45	39.30 ± 7.60	41.19 ± 7.43
Range	17-30	18-60	21-59	27-50	26-63
Sex (M:F)	9:6	16:16	11:13	10:0	24:2

OSA, obstructive sleep apnea.