Salivary Pepsin Lacks Sensitivity as a Diagnostic Tool to Evaluate Extraesophageal Reflux Disease

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Objectives To determine the sensitivity of salivary pepsin compared with multichannel intraluminal impedance with pH testing (pH-MII), endoscopy, and gastroesophageal reflux disease (GERD) questionnaires.

Study design We prospectively recruited 50 children from Boston Children's Hospital who were undergoing pH-MII to evaluate for GERD. The patients completed 24-hour pH-MII testing, completed symptom and quality of life questionnaires, and provided a saliva specimen that was analyzed using the PepTest lateral flow test. A subset of patients also underwent bronchoscopy and esophagogastroduodenoscopy. Receiver operating characteristic curve analyses were performed to determine the sensitivity of salivary pepsin compared with each reference standard.

Results Twenty-one of the 50 patients (42%) were salivary pepsin-positive, with a median salivary pepsin concentration of 10 ng/mL (IQR, 10-55 ng/mL). There was no significant difference in the distributions of acid, nonacid, total reflux episodes, full column reflux, or any other reflux variable in patients who were pepsin-positive compared with those who were pepsin-negative ($P > .50$). There was no significant correlation between the number of reflux episodes and pepsin concentration ($P > .10$). There was no positive relationship between salivary pepsin positivity, any extraesophageal symptoms or quality of life scores, or inflammation on bronchoscopy or esophagogastroduodenoscopy ($P > .30$).

Conclusion Salivary pepsin measurement has a low sensitivity for predicting pathological gastroesophageal reflux in children. *(J Pediatr 2016;177:53-8).*

G astroesophageal reflux disease (GERD) is commonly attributed to such symptoms as chronic cough and wheezing.[1-3] GERD has been reported in up to 80% of patients with a chronic respiratory condition, such as asthma and cystic fibrosis, and has been linked to poorer outcomes and exacerbations in children.[4-6] Proving causality between respiratory symptoms and reflux events is challenging, however. Much debate exists on whether esophageal reflux burden actually correlates with the amount of reflux that reaches the oropharynx and the airways. The current reference standard for measuring reflux burden and respiratory symptom correlation is combined pH and multichannel intraluminal impedance (pH-MII) testing, but these studies are costly, time-consuming, and invasive. New diagnostic tests are needed to assess for full column reflux that may impact the airways.

Salivary pepsin has been proposed as a promising biomarker for this purpose.[7,8] Pepsin is a proteolytic enzyme produced in the stomach, so its presence in the oropharynx and tracheobronchial tree suggests reflux and resultant aspiration. Higher pepsin levels have been reported in tracheal aspirates and bronchoalveolar lavage (BAL) fluid from children with chronic cough and proximal reflux (as measured by pH-metry), and may represent more severe pediatric lung disease.[9-12] Bronchoscopy is an invasive diagnostic procedure, and thus alternative methods to measure pepsin have been sought. Although salivary pepsin appears to be an attractive option because of ease of sampling, no pediatric studies comparing salivary pepsin with pH-MII have been performed to date.[13-16]

The objective of the present study was to test the sensitivity of salivary pepsin concentration for diagnosing reflux-related lung disease compared with combined pH-MII testing, endoscopy, and GERD symptom scores. We hypothesized that salivary pepsin may be detected more frequently in children with full-column reflux, which predisposes to reflux-related lung disease.

Methods

This was a prospective cross-sectional study of children aged 1-19 years undergoing pH-MII testing and esophagogastroduodenoscopy for the evaluation of GERD. Patients who had undergone fundoplication or previous esophageal or gastric surgery

BAL	Bronchoalveolar lavage
GER	Gastroesophageal reflux
GERD	Gastroesophageal reflux disease
pH-MII	pH-multichannel intraluminal impedance
ROC	Receiver operating characteristic

were excluded. Approval was granted by our hospital's Institutional Review Board, and informed consent was obtained from each patient or adult guardian.

Recruited patients were asked to provide a random saliva sample for pepsin testing. Alternatively, for young patients who were unable to spontaneously produce a salivary sample, a saliva aspirate was obtained from the oropharynx. All samples were obtained after a minimum 2 hours of fasting before pH-MII testing. Patients or their guardians completed a baseline symptom questionnaire as well as 2 validated questionnaires, the Pediatric Quality of Life Questionnaire and the Pediatric Gastroesophageal Reflux Disease Symptom and Quality of Life Questionnaire.

Salivary Pepsin Measurement

Each saliva sample was refrigerated at 4 °C in 0.5 mL of 0.01 M citric acid and processed within 1 week of collection. Saliva specimens were analyzed using the PepTest lateral flow device (RD BioMed, Hull, United Kingdom),[16] a colorimetric assay containing 2 unique human monoclonal antibodies that capture and detect pepsin protein. A valid positive PepTest result consists of control and test lines appearing on the assay strip. A negative result produces only 1 line (control), and an invalid result produces no lines. Pepsin concentration was measured using a lateral flow device reader, which uses optical detection to provide a precise quantification of the positive test line intensity. Pepsin concentration was then extrapolated for each positive test strip using standard curves provided by RD BioMed that allow conversion of intensity readings to concentrations (ng/mL).

Reflux Definitions

pH-MII tracings were manually reviewed by either of 2 investigators (F.D. and R.R.) using BioView Analysis 5.3.4 dedicated software (Sandhill Scientific, Denver, Colorado). A reflux episode was defined as a >50% drop from baseline impedance measured at least in the distal 2 sensors. A pH sensor at the distal end of the catheter measured pH drop (defined as <4) separately. An acid reflux episode involved a decrement in both pH and impedance readings, whereas nonacid events involved impedance declines only. An impedance study was considered abnormal overall if there were >73 episodes of impedance decline during a minimum study period of 18 hours.[17] The pH portion was defined as abnormal if pH was <4 for > 6% of the study period.[18]

Statistical Analyses

Continuous data are displayed as mean ± SD if normally distributed and as median (IQR) otherwise, and were compared using the Student t test and Wilcoxon rank-sum test, respectively. Proportions were compared using the Pearson χ^2 test or Fisher exact test when any expected cell count was <5. The association between pepsin concentrations and the number of acid reflux episodes was assessed by Spearman rank correlation. Receiver operating characteristic (ROC) curve analysis was used to determine an optimal pepsin concentration cutpoint for predicting pH-MII, the reference standard. The

optimal cutpoint was chosen using the Youden index criterion.[19] Logistic regression was used to investigate the independent association of esophagitis (determined endoscopically), symptom index for cough, and total number of reflux episodes with positive PepTest results. All tests of significance were 2-sided, with $P < .05$ considered statistically significant.

Results

Fifty patients, including 34 boys (68%), with a mean age of 8.7 ± 5.3 years, were recruited. Eleven patients (22%) had abnormal impedance studies, and 19 patients (38%) had abnormal pH monitoring. Twenty-four patients (48%) remained on acid-suppression therapy while undergoing pH-MII testing; there was no significant difference across reflux variables between patients receiving and those not receiving these medications ($P > .05$). Twenty-one patients (42%) had pepsin detected in the saliva specimen. There were no differences in the number of patients with abnormal pH testing (pepsin-positive, 38% vs pepsin-negative, 38%; $P = .99$) or abnormal MII testing (pepsin-positive, 29% vs pepsin-negative, 17%; $P = .49$). There also were no differences in reflux profiles between patients who were pepsin-positive and pepsin-negative (Table I). Patients who were pepsin-positive were less likely than patients who were pepsin-negative to have a history of recent cough (57% vs 89%; $P = .01$), but no other between-group differences in extraesophageal symptoms and quality of life scores were found (Table I).

The use of a positive PepTest to predict abnormal pH-MII test results (defined as either abnormal pH or abnormal MII) was associated with 42% sensitivity, 58% specificity, and 50% accuracy. When using ROC curve analysis to determine an optimal cutpoint for pepsin concentrations, the sensitivity of salivary pepsin was still lower than that of reflux testing using pH-MII (Table II). Logistic regression showed no independent associations between pepsin positivity and esophagitis, symptom index for cough, and total number of reflux episodes (data not shown).

The relationship of abnormal pH-MII test results and symptoms with salivary pepsin concentrations is shown in Table III. Pepsin concentrations were lower in patients with a recent history of daily chronic cough compared with those without cough (median, 0 [IQR, 0-10] vs 18 [IQR, 5-49]; $P = .007$). No other differences between these 2 groups were found. In addition, there were no significant relationships between pepsin concentration and the numbers of acid ($r = 0.06$; $P = .67$), nonacid ($r = 0.11$; $P = .46$), pH only ($r = -0.10$; $P = .47$), and total ($r = 0.14$; $P = .32$) reflux events. There was also no significant correlation between pepsin concentration and the percentages of total proximal reflux ($r = 0.02$; $P = .88$), proximal acid reflux ($r = 0.09$; $P = .55$), or proximal nonacid reflux ($r = 0.02$; $P = .88$).

None of the patients exhibited endoscopic evidence of erosions; however, 28% of the patients (14 of 50) had histological evidence of esophagitis with eosinophils on biopsy. In the patients with esophagitis, there was no significant difference in the proportion of patients who were pepsin-positive (21%;