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

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Abstract

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. Patients completed 24 hour pH-MII testing, symptom and quality of life questionnaires, and provided a saliva specimen which was analyzed using the PepTest® lateral flow test. A subset of patients also underwent bronchoscopy and esophagogastroduodenoscopy. ROC analyses were performed to determine the sensitivity of salivary pepsin compared with each reference standard.

Results—21 patients (42%) were salivary pepsin positive, with median concentration of pepsin in the saliva 10 ng/mL (IQR 10 – 55). There was no significant difference in the distributions of acid, nonacid, total reflux episodes, full column reflux or any other reflux variable in patients that were pepsin positive compared with pepsin negative ($p>0.5$). There was no significant correlation between the number of reflux episodes and pepsin concentrations ($p>0.1$). There was no positive relationship between salivary pepsin positivity, any extraesophageal symptoms or quality of life scores, or inflammation on bronchoscopy or esophagogastroduodenoscopy ($p>0.3$).

Conclusions—Salivary pepsin measurement has a low sensitivity for predicting pathologic gastroesophageal reflux in children.

Keywords

Gastroesophageal Reflux; Impedance; Pepsin

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The authors declare no conflicts of interest.

Gastroesophageal reflux disease (GERD) is commonly attributed to symptoms such as chronic cough and wheezing[1–3]. GERD has been reported in up to 80% of patients with chronic respiratory conditions, such as asthma and cystic fibrosis, and has been linked to poorer outcomes and exacerbations in children[4–6]. However, proving causality between respiratory symptoms and reflux events is challenging. 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. Taken together, 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[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, therefore alternative methods to measure pepsin are sought. Salivary pepsin seems an attractive option because of ease of sampling, but no pediatric studies have been performed so far with comparison with pH-MII[13–16]. The objective of this study is to test the sensitivity of salivary pepsin for diagnosing reflux-related lung disease, compared against combined pH-MII testing, endoscopy, and GERD symptom scores. We hypothesize that salivary pepsin may be more frequently detected in children with full-column reflux, which predisposes to reflux-related lung disease.

Methods

This is a prospective cross-sectional study of children aged 1–19 years undergoing pH-MII testing and esophagogastroduodenoscopy (EGD) for the evaluation of gastroesophageal reflux disease. Patients were excluded if they had undergone fundoplication or had prior esophageal or gastric surgery. Approval was granted by the Institutional Review Board, and informed consent was obtained from each patient or adult guardian.

Recruited patients were asked to provide a random saliva samples 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 hour period of fasting, prior to pH-MII testing. Patients or their guardians completed a baseline symptom questionnaire as well as two validated questionnaires, the Pediatric Quality of Life Questionnaire (PedsQL) and the Pediatric Gastroesophageal Reflux Disease Symptom and Quality of Life Questionnaire (PGSQ).

Salivary pepsin measurement

Each salivary sample was refrigerated at 4°C in 0.5mL of 0.01M citric acid and processed within one week of collection. Saliva specimens were analyzed using the PepTest® lateral flow device (LFD) (RD BioMed, Hull UK) [16]. The PepTest® is a colorimetric assay containing two unique human monoclonal antibodies that capture and detect pepsin protein

respectively. A valid positive PepTest® result consists of control and test lines appearing on the assay strip. A negative result produces only one line (control) and an invalid result produces none. Measurement of pepsin concentration was performed using a lateral flow device reader, which utilizes optical detection to provide an exact quantification of the positive test line intensity. Pepsin concentrations were then extrapolated for each positive test strip using standard curves provided by RDBioMed that allow conversion of intensity readings to concentrations (ng/mL).

Reflux Definitions

pH-MII tracings were manually reviewed by either of two investigators (FD and RR) using BioView Analysis 5.3.4 dedicated software (Sandhill Scientific, Denver CO). A reflux episode is defined as more than 50% drop from baseline impedance measured at least in the distal two sensors. A pH sensor at the distal end of the catheter measures pH drops (defined as <4) separately. An acid reflux episode involves a decrement in both pH and impedance readings, whereas non-acid events involve impedance declines only. An impedance study is considered abnormal overall if there are greater than 73 episodes of impedance decline during a minimum study period of 18 hours[17]. The pH portion is defined as abnormal if pH <4 for > 6% of the study period[18].

Statistical analyses

Continuous data are displayed as mean ± standard deviation if normally distributed, or median (interquartile range; IQR) otherwise, and compared using Student t-test or Wilcoxon rank-sum test, respectively. Proportions were compared by Pearson Chi-square 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) analysis was used to determine an optimal pepsin concentration cutpoint for predicting pH-MII, the reference standard. The optimal cutpoint was chosen using Youden's 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®. All tests of significance were two-sided with $P<0.05$ 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 variable between patients who were on and off these medications (p>0.05). Twenty-one patients (42%) had pepsin detected in their saliva. There were no differences in the number of patients with abnormal pH testing (pepsin positive 38% versus pepsin negative 38%, $P=0.99$) or abnormal MII testing (pepsin positive 29% versus pepsin negative 17%, $P=0.49$). No differences in reflux profiles between pepsin positive and negative patients were found (Table I). Patients who were pepsin positive were less likely to have a history of recent

cough compared with pepsin negative patients (57% versus 89%, *P*=0.01), but no other differences in extraesophageal symptoms and quality of life scores were found (Table I).

The use of a positive PepTest® for predicting abnormal pH-MII testing (defined as either abnormal pH or MII measurements) resulted in 42% sensitivity, 58% specificity, and 50% accuracy. Using ROC analysis to determine an optimal cutpoint for pepsin concentrations, the sensitivity of salivary pepsin was still low compared against reflux testing using pH-MII (Table II). Logistic regression showed no independent association between esophagitis, symptom index for cough, and total number of reflux episodes with pepsin positivity (data not shown).

The relationship of abnormal pH-MII testing and symptoms with salivary pepsin concentrations is shown in Table III. Pepsin concentrations were lower among patients with a recent history of daily chronic cough than those without cough [median (IQR): 0 (0, 10) versus 18 (5, 49), *P*=0.007]. No other differences were found. We found no significant relationship between pepsin concentration and the number of acid (r = 0.06, *P*=0.67), nonacid (r = 0.11, *P*=0.46), pH only (r = −0.10, *P*=0.47) and total (r = 0.14, *P*=0.32) reflux events. There was also no significant correlation between pepsin concentration and the % of total proximal reflux (r = 0.02, *P*=0.88), % proximal acid reflux (r = 0.09, *P*=0.55) or proximal nonacid reflux (r = 0.02, *P*=0.88).

None of the patients had endoscopic evidence of erosions. However, 28% of patients (14/50) had histologic evidence of esophagitis with eosinophils on biopsy. There were no significant differences between the proportion of patients who were pepsin positive (6/14, 21%) or pepsin negative (8/14, 28%, p=0.18) in the patients with esophagitis.

Thirty-four patients underwent bronchoscopy. Although not statistically significant, patients who were salivary pepsin positive tended to have a higher median percentage of neutrophils [10.0 (0.0, 40.0)] than patients who were pepsin negative [0.0 (0.0, 3.0), *P*=0.07]. There were no differences in the proportion of patients who had positive bacterial (*P*=0.43) or fungal cultures (*P*=0.46) in patients who were pepsin positive or negative. In this group of 34 patients, there were no differences between patients that were pepsin positive versus negative with respect to reflux variables (number of reflux events, % time pH<4, percentage of proximal reflux, p>0.56) or to quality of life scores (p>0.33). We did not find any significant differences in the proportion of patients with an abnormal pH probe or abnormal impedance study in the pepsin positive versus negative groups as well (p=1.0).

The median pepsin concentration of suctioned saliva was 0 (0,0) compared with spontaneous spit whose median concentration was 10 (0,34) (*P*=0.002). Eighty-four percent (21/25) of suctioned samples were pepsin negative compared with 32% (8/25) of spontaneous spit samples (*P*=0.0002).

Discussion

The paradigm that gastroesophageal reflux (GER) is one of the most common causes of chronic cough has greatly influenced clinical practice for over two decades[20–22]. It has spurred an enormous increase in acid suppression therapy among children, even when

clinical trials have failed to find a consistent relationship between measured reflux and clinical response[23–26].

Cough and GER are separately commonplace occurrences in children and therefore establishing causality is extremely difficult. This link has been supported by findings that GER often precedes respiratory symptoms, although a handful of studies have also shown that cough can incite reflux in adults[1,27,28]. To prove this association, reviews and guidelines routinely recommend children with suspected extra-esophageal symptoms to undergo multiple, often invasive, diagnostic tests[18,29].

Currently, combined pH-MII is considered the best diagnostic method available for extra-esophageal reflux burden, as pH-MII monitoring assesses for proximal reflux burden and for both acid and nonacid reflux, which increases the yield of GER-symptom associations in children[30,31]. However, despite these benefits, pH-MII testing involves placing a catheter in the nose and requires a long recording period which can be uncomfortable for the patient and inconvenient for families. The measurement of salivary pepsin as a proxy for full-column reflux therefore seems promising.

Prior to the development of salivary pepsin testing, pepsin was measured by western blot analyses or ELISA in bronchoalveolar lavage fluid (BAL). Previous studies evaluated salivary pepsin for predicting reflux burden. However, although initial studies suggested a relationship between BAL pepsin and proximal acid reflux measured by pH-metry, studies using pH-MII failed to corroborate these earlier findings[10,12,32]. One of the limitations of using BAL pepsin as a biomarker is that sampling requires bronchoscopy under anesthesia, which is again both expensive and invasive.

In our cohort, the overall prevalence of salivary pepsin was 42% (21 of 50 patients). Pepsin was detected in the saliva of 6 of 11 (54.5%) of children with abnormal impedance testing, and in 8 of 11 (42.1%) of children with abnormal pH-metry. Our positive rate of detection was similar to that reported by Yuksel et al who had 43% of samples testing positive in adults with GERD symptoms and abnormal pH findings[16]. In this study, saliva was collected at a single time point and both time and concentrations were not specified. In a similar study, Kim et al reported 55% positive pepsin samples in adults with GERD symptoms and abnormal pH-metry, but saliva was collected at the time of symptoms[15]. A strategy of serial saliva collections was employed by Hayat et al for every patient in a large adult cohort, and they reported pepsin detection in 67.5% of patients with GERD symptoms. Moreover, among those with prolonged esophageal acid exposure time, 77.6% had at least one positive saliva sample[8]. The higher prevalence of salivary pepsin in the latter adult studies compared with ours may have been because their saliva sampling was timed with symptoms. In a study by Na et al, salivary pepsin concentrations were highest after an overnight fast, before any meals were ingested and without any associated symptoms [33]. In our study, all patients had salivary samples obtained after a 2 hour minimum NPO period. However, because of this potential sampling variability, the ideal window for salivary pepsin collection in children merits additional studies. An alternative explanation is that adult patients are able to produce saliva spontaneously whereas in our pediatric cohort, half of our patients required oral

suctioning and therefore we may have observed a dilution effect – which limits the utility of this test in children.

Sensitivity and specificity analyses of salivary pepsin performed in adult patients have yielded inconsistent results. Ocak et al studied 20 adult patients with positive reflux symptom indices and found that the sensitivity and specificity of salivary pepsin was 33% and 100%, respectively, when compared against 24-hour esophageal pH monitoring[34]. Hayat et al found that, if pepsin was detected in at least one saliva sample, the test had a sensitivity and specificity of 78.6% and 64.9% respectively[8] compared with pH-MII. In our study, we assessed the ability of both PepTest® and pepsin concentration using an optimal cutpoint from our data to predict abnormal impedance by pH-MII. The sensitivity and specificity of a positive pepsin sample by PepTest® was just 42% and 58%, respectively, which is lower than the adult values. An optimal cutpoint of >74 ng/mL provided 17% sensitivity and 100% specificity.

Recognizing that pH-MII is not the only tool to assess reflux burden, we also looked at the relationship between pepsin positivity and reflux symptoms, quality of life scores, and the presence of esophagitis – and still did not find a significant association. These results suggest that either salivary pepsin is not a sensitive enough tool to diagnose extraesophageal reflux disease or our gold standard tools (pH-MII, endoscopy, GER symptoms) are inadequate reference tools for comparison. We have previously shown that bronchoscopy pepsin has similarly low sensitivity relative to esophageal reflux monitoring, which again raises the possibility that either our current reference standards are inadequate or that pepsin measured beyond the gastrointestinal tract is not an appropriate test for the diagnosis of reflux[12]. The other possibility is that GER, measured either by salivary pepsin or pH-MII testing, is not a significant contributor to pulmonary symptoms.

There are several limitations to this study. First, our sample size is drawn from a tertiary care center where patients harbor significant pulmonary symptoms. Therefore these children may not be representative of patients typically cared for in primary care settings. However, our patients do reflect those seeking subspecialty care in pediatric pulmonary and otorhinolaryngology clinics. Furthermore, all of the patients included in this study had symptoms significant enough to warrant pH-MII testing. Therefore, even though we could compare pepsin positivity between patients with and without pathologic reflux, we did not determine the rate of pepsin positivity in healthy control patients.

Second, one-half of the patients in our pediatric series had difficulty providing a saliva sample. In our cohort, patients who were not able to provide samples were younger than those who were able (6.2±4.9 versus 11.2±4.6; P=0.0005). Obtaining technically-adequate samples of saliva from younger children is challenging as many patients cannot provide adequate saliva; therefore oral suctioning (with subsequent clearance of the saliva from the catheter with 0.5 cc of sterile water) is required. Because of this dilution effect, rates of pepsin positivity may be lower in the pediatric population compared with adults. However, when we did isolate our analyses to just children who were able to provide a saliva sample spontaneously, there was still no significant relationship between reflux by pH-MII and

salivary pepsin (data not shown). Therefore, use of this technique in patients who cannot produce saliva spontaneously may have a limited role.

Third, we relied on single, random saliva collection rather than at symptomatic times which may have reduced our rate of pepsin positivity. However, because this test will likely be used in the outpatient clinic setting when patients may or may not be acutely symptomatic, we feel that these results are generalizable to patients presenting to specialists.

Finally, the lack of association between pH-MII testing measurements and salivary pepsin may not be a limitation of the salivary pepsin test but rather a limitation of the reference standard (pH-MII testing) itself. pH-MII and pH probe testing traditionally relies on the measurement of esophageal reflux burden which may or may not reflect the amount of reflux that travels into the oropharynx. Another possibility is that salivary pepsin positivity is common and even one-third of healthy asymptomatic adults have pepsin detected in their saliva. Thus the lack of relationship between esophageal reflux and salivary pepsin could reflect the fact that some patients may not have pathologic reflux but still be pepsin positive. In our study, we did note that 38% (15/39) of children with normal impedance results had detectable salivary pepsin. Finally, the other possibility is that the amount of reflux needed to turn salivary pepsin positive is not known and that the traditional reflux cutoffs do not apply.

In conclusion, salivary pepsin detection using an immunoassay has been proposed as a rapid, convenient, noninvasive, and easily-interpretable means of diagnosing GER – particularly as it relates to extraesophageal symptoms[8,16,34,35]. However, based on this study, single-point-in-time salivary pepsin does not appear to correlate well with pathologic reflux by pH-MII testing in children. Additional studies are still needed to determine if repeated salivary sampling increases the sensitivity of the test or if a different reference standard for extraesophageal reflux needs to be considered.

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Abbreviations

BAL	bronchoalveolar lavage
EGD	esophagogastroduodenoscopy
GER	gastroesophageal reflux
GERD	gastroesophageal reflux disease
IQR	interquartile range
LFD	lateral flow device
PedsQL	Pediatric Quality of Life Questionnaire

PGSQ	Pediatric Gastroesophageal Reflux Disease Symptom and Quality of Life Questionnaire
pH-MII	pH-multichannel intraluminal impedance
ROC	receiver operating characteristic

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Table 1

Reflux characteristics and quality of life scores in patients with and without salivary pepsin.

Measure	Pepsin − (n=29)	Pepsin + (n=21)	*P*
Abnormal pH-metry	11 (38%)	8 (38%)	0.99
Abnormal impedance	5 (17%)	6 (29%)	0.49
Reflux			
Total no. of reflux episodes (acid + non-acid)	43.0 (32.0, 53.0)	45.0 (19.0, 91.0)	0.55
No. of acid reflux episodes	26.0 (6.0, 38.0)	19.0 (11.0, 46.0)	0.69
No. of non-acid reflux episodes	11.0 (5.0, 26.0)	14.0 (6.0, 33.0)	0.77
No. of pH only episodes	12.0 (7.0, 17.0)	11.0 (3.0, 17.0)	0.55
No. of full column episodes	17.0 (8.0, 25.0)	16.0 (4.0, 29.0)	0.96
% proximal (total)	0.4 (0.3, 0.7)	0.3 (0.2, 0.9)	0.88
% proximal (acid)	0.2 (0.0, 0.5)	0.3 (0.1, 0.7)	0.63
% proximal (non-acid)	0.1 (0.1, 0.3)	0.1 (0.1, 0.2)	0.85
% of time pH<4	4.0 (1.3, 7.4)	2.0 (0.3, 13.6)	0.70
Cough symptom index	37.0 (8.0, 55.0)	37.0 (20.0, 58.0)	0.66
Symptom burden			
History of asthma	20 (71%)	15 (71%)	1.00
History of croup	16 (59%)	8 (38%)	0.15
History of chronic cough (≥1 × per week)	27 (93%)	15 (71%)	0.06
Ear infection in the past 6 months	9 (32%)	3 (15%)	0.18
Sinus infection in the past 6 months	6 (22%)	2 (10%)	0.44
Pneumonia in the past 6 months	6 (21%)	4 (20%)	1.00
Asthma or wheezing in the past 6 months	17 (63%)	10 (48%)	0.29
Daily chronic cough in the past 6 months	25 (89%)	12 (57%)	0.01
Quality of life			
PGSQ (total score)	0.76 (0.42, 1.19)	0.61 (0.42, 1.19)	0.48
PGSQ (symptom score)	0.65 (0.41, 1.29)	0.56 (0.35, 0.82)	0.43
PGSQ (everyday life impact score)	0.79 (0.43, 1.43)	0.64 (0.00, 1.71)	0.45
PDQL (total score)	84.5 (68.0, 91.3)	78.3 (61.0, 91.3)	0.66
PDQL (physical score)	78.0 (59.0, 94.0)	81.3 (64.3, 96.9)	0.61
PDQL (psychosocial score)	83.9 (73.0, 93.0)	76.7 (63.5, 86.7)	0.21

Table 2

Receiver-operator characteristics for optimal cutpoint of salivary pepsin concentration or pepsin positivity to predict (A) abnormal MII, (B) abnormal pH, and (C) abnormal pH-MII (by either criteria) for all salivary pepsin samples combined, for suctioned samples, and for spontaneous spit samples. (Se: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: Area Under Curve)

Group	Reference	Optimal cut-point	Se	Sp	PPV	NPV	Accuracy	AUC (95%CI)
All								
	pH	PepTest® positive	0.42	0.58	0.38	0.62	0.52	0.50 (0.36, 0.65)
		Pepsin >132 ng/mL	0.11	1.00	1.00	0.65	0.66	0.50 (0.35, 0.66)
	MII	PepTest® positive	0.55	0.62	0.29	0.83	0.60	0.58 (0.41, 0.75)
		Pepsin >10 ng/mL	0.46	0.87	0.50	0.85	0.78	0.63 (0.44, 0.83)
	pH-MII	PepTest® positive	0.42	0.58	0.48	0.52	0.50	0.50 (0.36, 0.64)
		Pepsin >74 ng/mL	0.17	1.00	1.00	0.57	0.60	0.52 (0.38, 0.67)
Suction								
	pH	PepTest® positive	0.13	0.82	0.25	0.67	0.60	0.53 (0.37, 0.68)
		Pepsin >132 ng/mL	0.12	1.00	1.00	0.71	0.72	0.49 (0.32, 0.65)
	MII	PepTest® positive	0.20	0.85	0.25	0.81	0.72	0.53 (0.31, 0.74)
		Pepsin >10 ng/mL	0.20	0.95	0.50	0.83	0.80	0.53 (0.31, 0.75)
	pH-MII	PepTest® positive	0.18	0.86	0.50	0.57	0.56	0.52 (0.37, 0.67)
		Pepsin >10 ng/mL	0.18	1.00	1.00	0.61	0.64	0.53 (0.38, 0.69)
Spit								
	pH	PepTest® positive	0.64	0.29	0.41	0.50	0.44	0.54 (0.35, 0.73)
		Pepsin >115 ng/mL	0.09	1.00	1.00	0.58	0.60	0.43 (0.20, 0.66)
	MII	PepTest® positive	0.83	0.37	0.29	0.88	0.48	0.60 (0.40, 0.80)
		Pepsin >10 ng/mL	0.67	0.79	0.50	0.88	0.76	0.72 (0.46, 0.99)
	pH-MII	PepTest® positive	0.62	0.25	0.47	0.38	0.44	0.57 (0.38, 0.76)
		Pepsin >74 ng/mL	0.15	1.00	1.00	0.52	0.56	0.45 (0.22, 0.67)

Table 3

Association of impedance, pH study and symptom history with pepsin concentration.

	N	Range	Median (IQR)	*P*
Abnormal impedance study				0.14
No	39	0 – 187	0 (0, 10)	
Yes	11	0 – 300	10 (0, 115)	
Abnormal pH study				0.96
No	31	0 – 132	0 (0, 10)	
Yes	19	0 – 300	0 (0, 10)	
Asthma/wheezing in the past 6 months				0.40
No	21	0 – 300	10 (0, 10)	
Yes	27	0 – 187	0 (0, 10)	
Daily chronic cough in the past 6 months				0.007
No	12	0 – 300	18 (5, 49)	
Yes	37	0 – 187	0 (0, 10)	
Ear infection				0.28
No	36	0 – 300	0 (0, 10)	
Yes	12	0 – 187	0 (0, 5)	
Sinus infection				0.37
No	39	0 – 300	0 (0, 10)	
Yes	8	0 – 187	0 (0, 5)	
Pneumonia				0.88
No	39	0 – 300	0 (0, 10)	
Yes	10	0 – 187	0 (0, 34)	

P value from Wilcoxon rank-sum test.