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Threshold level of Peptest in diagnosing gastroesophageal reflux disease with extraesophageal symptoms: Evidence from Vietnam

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Abstract

Background and Aim: We aimed to evaluate the application of Peptest, a novel technique to detect pepsin in the saliva, and identify its threshold level for the diagnosis of gastroesophageal reflux disease (GERD) with extraesophageal symptoms.
Methods: A cross-sectional study was conducted in two groups: patients with extraesophageal GERD symptoms (symptomatic group divided into GERD and non-GERD groups according to 24-h esophageal pH-impedance monitoring [pH-I] results) and healthy controls. For the symptomatic group, endoscopy, pH 24 h, high-resolution manometry (HRM), and salivary Peptest were performed. For the healthy control group, only Peptest was done. The accuracy of Peptest was compared with that of pH-I by the Lyon consensus criteria.
Results: Chronic laryngitis was the most frequent extraesophageal symptom. On saliva testing, the GERD group had a higher prevalence of positive samples and pepsin concentration than the control group. Between GERD and non-GERD groups, the optimal threshold level was 31.2 ng/mL, with a sensitivity of 86.7% and specificity of 27.5%. The optimal threshold level was 31.4 ng/mL to differentiate GERD from healthy controls, with a sensitivity of 86.7% and specificity of 66.0%. Age, number of total refluxes, DeMeester score, post-reflux swallow-induced peristaltic wave (PSPW) index, and mean nocturnal baseline impedence (MNBI) were associated with pepsin concentration. Regarding HRM metrics, there was no significant difference of pepsin concentration between low/normal upper esophageal sphincter (UES) resting pressure, low/normal lower esophageal sphincter (LES) resting pressure, low/normal 4-s integrated relaxation pressure (IRP4s), and hypomotility/normal motility.
Conclusion: Patients with extraesophageal symptoms had a higher prevalence of positive Peptest. The optimum threshold level of 31.4 ng/mL had high sensitivity and moderate specificity to differentiate between patients with GERD and healthy controls.

Introduction

Gastroesophageal reflux disease (GERD) is a common functional gastrointestinal disease with a prevalence of 13.98% worldwide and shows a rising trend in the number of cases and years of life lived with disability (YLDs).[1,2] A study on outpatients with upper gastrointestinal symptoms in southern Vietnam reported that 26.2% patients were diagnosed with GERD.[3] The hallmark presentations of GERD are troublesome regurgitation and heartburn. However, there is a subset of patients who have the evidence of underlying GERD but

mostly complain about extraesophageal symptoms rather than typical ones, making the diagnosis difficult.[4] According to the updated American College of Gastroenterology (ACG) guidelines, patients who have extraesophageal manifestations without typical GERD symptoms are recommended to undergo pH monitoring before acid suppression therapy.[5] For those who have combined typical symptoms and extraesophageal symptoms, conventional testing such as proton pump inhibitor (PPI) trial and endoscopy can be considered as preliminary methods to establish a diagnosis of GERD.

In clinical practice, these current methods impose some limitations on diagnosing GERD, such as the long time taken (at least 2 weeks for PPI trials, 24 h for esophageal pH monitoring), invasive nature and poor tolerance (endoscopy and pH monitoring), high cost, and requiring advanced training (pH monitoring). The development of novel techniques has been of great concern, especially noninvasive methods that can be used widely. Among these, Peptest is a promising method using pepsin, a proteolytic enzyme produced only in the stomach,[6] so the presence of pepsin in saliva could imply the reflux of the gastric contents into the laryngopharynx. Therefore, salivary pepsin testing is expected to be a noninvasive and reasonable method of detecting GERD in those having extraesophageal symptoms. Many studies have been conducted to evaluate the diagnostic value of Peptest and its optimal cut-off in diagnosing GERD and laryngopharyngeal reflux (LPR) but mainly in European and Chinese population.[7–10] In addition, the standard sample collection and the threshold level are still different within studies. In this study, we aimed to evaluate the application of Peptest and the possible threshold level of pepsin concentration for the diagnosis GERD with extraesophageal symptoms.

Methods

Subject. A cross-sectional study was conducted in Hoang Long Clinic and the Institute of Gastroenterology and Hepatology, Hanoi, Vietnam, from June 2019 to January 2022.

For the symptomatic group, the inclusion criteria were as follows: Participants aged ≥18 years who visited the clinic and had extraesophageal symptoms suspected as due to GERD, including chronic cough, globus sensation, chronic laryngitis (diagnosed by an ENT doctor), dyspnea, and chest pain, were eligible for enrolment. Patients should not have been on PPIs or antacids for 72 h. Exclusion criteria were as follows: Patients who did not give consent and those with respiratory and cardiac diseases mimicking GERD symptoms as confirmed by a specialists to exclude cofounding organic diseases and expected achalasia cases.

For the control group, the inclusion criteria were as follows: Asymptomatic healthy volunteers, recruited from the same study location, ≥18 years old, no suspected GERD symptoms within 1 month, and GerdQ score <8.[11] The exclusion criteria were a history of previous gastrointestinal surgery or known esophageal motor, or psychiatric disorders.

Procedure. On enrolment, participants were interviewed according to a previously designed case report form to collect data including demographic information (sex, age), body mass index (BMI), clinical symptoms, gastroesophageal reflux disease questionnaire (GerdQ score), and frequency scale for the symptoms of GERD (FSSG). Endoscopy results within 3 months were collected from patients' medical records: hiatal hernia was classified by Hill Grade, Barrett's esophagus, and reflux esophagitis was classified according to Los Angeles (LA) classification.[12] All participants in the symptomatic group afterward underwent high-resolution manometry (HRM), 24-h pH-impedance (pH-I) monitoring, and Peptest. For the control group, only demographic information, BMI, and GerdQ score were collected.

High-resolution manometry. This technique was performed after overnight fasting by using a water-perfused HRM catheter with 24 pressure channels (Solar GI medical measurement system [MMS], Poland). The procedure includes 10 supine liquid swallows (5 mL/swallow) and 2 multiple rapid swallows (5 continuous swallows of 2 mL of water spaced at intervals of 2–3 s). The records were analyzed by the MMS software according to the Chicago Classification v3.0.[13] The normal range of upper esophageal sphincter (UES) resting pressure is 34–104 mmHg, therefore pressure lower than 34 mmHg was classified as low pressure. The normal value of LES resting pressure is 10–45 mmHg; according to this, the lower esophageal sphincter (LES) resting pressure lower than 10 mmHg was defined as low pressure. 4-s integrated relaxation pressure (IRP4s) ≥19 mmHg (for water-perfused system) was classified as high pressure, the rest was definitively normal. Ineffective esophageal motility and absent contractility were defined as hypomotity in this study. Patients with other motility disorders (distal esophageal spasm, esophagogastric junction outflow obstruction, fragmented peristalsis) are not counted as hypomotility or normal motility.

24-h esophageal pH-I monitoring. Before monitoring pH-I, all participants were instructed to discontinue PPIs for at least 7 days. Then they underwent high-resolution esophageal manometry to locate the LES position. The study used the Ohmega device from Laborie (The Netherlands), and the record was transferred to MMS computer software for analysis. The catheter had one pH sensor (located at 5 cm above the tip) and six impedance channels positioned 3, 5, 7, 9, 15, and 17 cm above the sphincter. The catheter was nasally placed to the esophagus in an upright position. While using the device for 24 h, patients were instructed to press the button to record the mealtimes, bedtimes, drug use, and the time of having clinical symptoms.

The study group used Lyon consensus criteria for 24-h pH-I to diagnose GERD.[14] GERD was confirmed when the acid exposure time (AET) was ≥6%. Patients with AET<6% could have functional heartburn or esophageal hypersensitivity, and both groups in this study were classified as not having GERD. Other parameters on pH-I were collected for analysis (total reflux episodes, mean nocturnal baseline impedance [MNBI], post-reflux swallow-induced peristalsis wave [PSPW], DeMeester score). MNBI was calculated by means of esophageal impedances at 1 a.m., 2 a.m., and 3 a.m. PSPW was counted if the peristalsis appeared after the reflux event within 30 s. MNBI <2292 Ω and the percentage of PSPW (PSPW index) <61% were considered as supportive metrics for GERD.[14]

Salivary pepsin. Salivary sample collection was done as follows: In the symptomatic group, patients were asked to stop PPIs and antacids for at least 3 days. All participants were given two tubes containing citric acid to collect at least 2 mL saliva each time after waking up in the morning (before brushing teeth, drinking, or eating) and within 1 h after dinner. All participants were instructed to write the time of sample collection outside each tube, and then store them in a refrigerator at 4°C until taking them to the medical site the next day.

The Peptest (RD Biomed Ltd., UK) was used to determine whether pepsin was present in the saliva samples, and the Peptest Cube was used to measure the pepsin concentration (ng/mL) in case of a positive result from the Peptest. Peptest is a rapid test that uses two types of antibodies. Pepsin in the sample is bound at the test line by two antibodies, and a colored line is seen through the viewing window in the plastic case. According to the manufacturer's instruction, the test is considered positive when the concentration of salivary pepsin is above 16 ng/mL. The Peptest Cube is a measuring device based on reflectance measurements to identify the level of pepsin in a positive sample.[15]

Statistical analysis. Data were analyzed by SPSS statistical software version 20.0. Quantitative variables are presented as mean $\pm$ SD, and qualitative variables are presented as count (frequency). The differences were tested by the Chi-square test for qualitative variables; independent *t*-test was used for comparing two groups and the Kruskal–Wallis test for three groups. The

Table 1 Demographic and clinical characteristics of participants

	Symptomatic group			
Characteristics	GERD ($n = 30$)	Non-GERD ($n = 69$)	Control group ($n = 50$)	*P*-value
Age (years), mean $\pm$ SD	44.33 ± 11.19	43.46 ± 12.62	43.92 ± 13.77	0.949
Sex (male), *n* (%)	16 (53.3%)	24 (34.8%)	25 (50.0%)	0.118
BMI, mean $\pm$ SD	22.78 ± 3.02	21.56 ± 2.81	21.61 ± 2.24	0.095
BMI classification, *n* (%)				
Underweight (<18.5 kg/m^2)	1 (3.3%)	12 (17.4%)	4 (8%)	0.079
Normal ($\geq$18.5 and <23 kg/m^2)	18 (60.0%)	36 (52.2%)	36 (72%)	
Overweight/obese ($\geq$23 kg/m^2)	11 (36.7%)	21 (30.4%)	10 (20%)	
Clinical symptoms				
Regurgitation	73.3%	69.6%	N/A	0.705
Heartburn	43.3%	50.7%	N/A	0.499
Chronic laryngitis	60.0%	43.5%	N/A	0.131
Chest pain	46.7%	27.5%	N/A	0.064
Globus sensation	36.6%	55.1%	N/A	0.092
Dysphagia	23.3%	24.6%	N/A	0.889
Dyspnea	26.7%	30.4%	N/A	0.705
Chronic cough	20.0%	14.5%	N/A	0.494
FSSG score, mean $\pm$ SD	12.93 ± 8.19	12.26 ± 7.56	N/A	0.692
GerdQ score, mean $\pm$ SD	7.97 ± 2.68	7.93 ± 2.73		0.948

BMI, body mass index; FSSG, frequency scale for the symptoms of GERD; GERD, gastroesophageal reflux disease; GerdQ, gastroesophageal reflux disease questionnaire.

Table 2 Endoscopic and pH-I results

	Symptomatic group		
Characteristics	GERD ($n = 30$)	Non-GERD ($n = 69$)	*P*-value
Endoscopy findings, *n* (%)	$n = 28$	$n = 65$	
No esophagitis	9 (32.1%)	21 (32.3%)	0.824
LA grade A	18 (64.2%)	43 (66.2%)	
LA grade B/C/D	1 (3.6%)/0/0	1 (1.5%)/0/0	
Barrett's esophagus (short segment)	2 (7.1%)	4 (6.2%)	0.859
Hiatal hernia	3 (10.7%)	0 (0%)	0.007
pH-I monitoring			
AET (%), median (IQR)	17.3 (9.1–38.3)	0.9 (0.3–2.2)	<0.001
Total reflux episodes, median (IQR)	68.5 (38.9–96.5)	62.0 (43.0–82.5)	0.888
Acid reflux, median (IQR)	50.0 (30.8–66.5)	38.0 (19.5–50.5)	0.016
Non-acid reflux, median (IQR)	12.0 (1.8–35.3)	28.0 (15.0–39.0)	0.039
DeMeester score, median (IQR)	63.9 (31.1–122.9)	4.1 (1.9–9.0)	<0.001
PSPW index (%), mean $\pm$ SD	24.43 ± 12.11	34.76 ± 11.02	<0.001
PSPW index <61%, *n* (%)	30 (100%)	69 (100%)	—
MNBI (Ω), mean $\pm$ SD	1001.72 ± 637.83	2331.0 ± 687.88	<0.001
MNBI >2292 Ω, *n* (%)	2 (5.6%)	48 (57.1%)	<0.001

AET, acid exposure time; GERD, gastroesophageal reflux disease; IQR, interquartile range; LA, Los Angeles; MNBI, mean nocturnal baseline impedance; pH-I, pH-impedance; PSPW, post swallow-induced peristalsis wave.

receiver operating characteristic (ROC) curve was used to determine the optimum threshold level of pepsin concentration in diagnosing GERD, which was chosen using the Youden index method. For those who had two values of pepsin concentration at two different times of collection, the higher value was used for the ROC curve analysis. The regression model was used to find the relationship between the variables and pepsin concentration.

Ethical consideration. The study was approved by the Institutional Review Board of Dinh Tien Hoang Institute of Medicine (decision No: IRB-1909 dated 1 March 2020) and was a part of the national project of the Institute of Gastroenterology and Hepatology (Hanoi) (Grant number: ĐTĐLCN.04/20).

Results

One-hundred and forty-nine participants were included in the study (symptomatic group $n = 99$, and control group $n = 50$). The symptomatic group was then subdivided into—based on the diagnosis on pH-I—the GERD group ($n = 30$) with confirmed diagnosis of GERD, and the non-GERD group ($n = 69$). Their demographic and clinical features are given in Table 1. The mean age, sex, and the mean BMI were not significantly different

Table 3 Characteristics of Pepsin quantitative and qualitative testing.

Characteristics	Comparison between GERD and non-GERD group			Comparison between GERD group and control group		
	GERD ($n = 30$)	Non-GERD ($n = 67$)	*P*-value	GERD ($n = 30$)	Control group ($n = 50$)	*P*-value
Sample 1 (morning)						
Positive, *n* (%)	27 (90.0%)	60 (87.0%)	0.947	27 (90.0%)	30 (60%)	0.004
Concentration, median (IQR)	56.80 (32.00–88.30)	90.75 (27.85–126.41)	0.162	56.80 (32.00–88.30)	29.40 (16.00–64.18)	0.115
Sample 2 (evening)						
Positive, *n* (%)	25 (83.3%)	54 (80.6%)	0.749	25 (83.3%)	21 (42%)	<0.001
Concentration, median (IQR)	41.10 (16.00–116.95)	59.45 (29.23–101.10)	0.915	41.10 (16.00–116.95)	16.00 (16.00–41.35)	0.021
Proportion of those having at least one positive samples, *n* (%)	29 (96.7%)	66 (95.7%)	0.814	29 (96.7%)	35 (70%)	0.004
Proportion of those with both positive samples, *n* (%)	23 (76.7%)	48 (69.6%)	0.471	23 (76.7%)	16 (32%)	<0.001

GERD, gastroesophageal reflux disease; IQR, interquartile range.
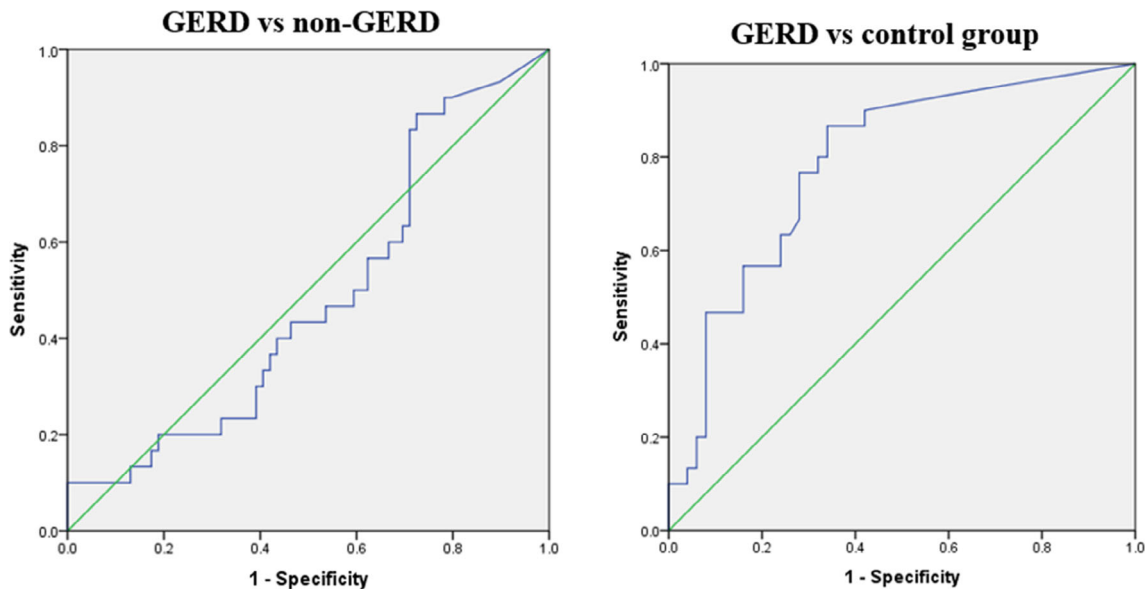

Figure 1 Receiver operating characteristic curve of pepsin concentration in diagnosing gastroesophageal reflux disease with extraesophageal symptoms.

Table 4 Comparison of pepsin concentration between five different HRM metric groups.

	Low UES resting pressure	Normal UES resting pressure	*P*-value
Sample 1 (morning)	83.97 ± 53.35	79.53 ± 56.78	0.952
Sample 2 (evening)	88.19 ± 71.69	69.29 ± 54.33	0.070
	Low LES resting pressure baseline	**Normal LES resting pressure baseline**	
Sample 1 (morning)	86.07 ± 73.25	80.40 ± 52.22	0.523
Sample 2 (evening)	90.71 ± 56.75	70.91 ± 59.52	0.469
	Low LES resting pressure when swallowing	**Normal LES resting pressure when swallowing**	
Sample 1 (morning)	92.25 ± 78.26	79.33 ± 50.49	0.543
Sample 2 (evening)	95.95 ± 71.82	70.74 ± 55.84	0.088
	Low IRP4s	**Normal IRP4s**	
Sample 1 (morning)	85.84 ± 60.74	76.82 ± 50.15	0.304
Sample 2 (evening)	85.02 ± 64.44	62.24 ± 51.21	0.224
	Hypomotitity	**Normal motility**	
Sample 1 (morning)	72.73 ± 51.69	93.81 ± 60.20	0.523
Sample 2 (evening)	77.32 ± 57.37	73.90 ± 63.23	0.881

HRM, high-resolution manometry; IRP4s, 4-s integrated relaxation pressure; LES, lower esophageal sphincter; UES, upper esophageal sphincter.

between the groups. Regarding the clinical symptoms, regurgitation had the highest prevalence in both GERD and non-GERD groups. Among the extraesophageal symptoms, chronic laryngitis was the most frequent (60% in GERD, and 43.5% in non-GERD patients). Both clinical scores (FSSG and GerdQ) did not differ between the GERD and non-GERD groups.

The results of upper gastrointestinal endoscopy and pH-I in the GERD and non-GERD groups, shown in Table 2, indicate that both groups had a high prevalence of esophagitis (69.7%) with the predominance of Los Angeles grade A. The GERD group had a significantly higher prevalence of hiatal hernia. On 24-h pH-I, the non-GERD group had higher values of acid reflux number, PSPW index, MNBI, and DeMeester score, but a smaller number of non-acid reflux incidents than the GERD group.

On saliva testing, in both samples in the morning and in the evening, the positive rate of pepsin was significantly different between the GERD and control groups, as shown in Table 3. The GERD group had a higher prevalence of positive samples (in both the morning and evening samples) and pepsin concentration (in evening sample) than in the control group. However, there was no significant difference in the positive rate of pepsin and pepsin concentration between the GERD and non-GERD groups.

The ROC curve of Peptest is shown in Figure 1 when using pH-I as the gold standard to differentiate GERD from non-GERD patients and the GERD group from controls. Between the GERD and the non-GERD groups, the optimal threshold level was determined as 31.2 ng/mL with a sensitivity of 86.7% and specificity of 27.5%. The threshold level of 34.3 ng/mL has a sensitivity of 83.3% and specificity of 29.0%. The area under the ROC curve was higher when using Peptest to differentiate GERD patients with extraesophageal symptoms from healthy controls (0.787); the optimum threshold level was 31.4 ng/mL with a sensitivity of 86.7% and specificity of 66.0%.

Comparing pepsin concentration between low and normal UES resting pressure, or low and normal LES resting pressure baseline/swallow, or low and normal IRP4s, or hypomotility and normal motility revealed no significant difference in pepsin concentration (Table 4).

Table 5 Relationship between some factors and pepsin concentration.

Variables	Coefficients	*P*-value	95% CI
Sex	−0.102	0.368	−41.90 to 15.72
Age	−0.244	0.037	−2.51 to −0.08
FSSG score	0.016	0.893	−1.87 to 2.14
GerdQ score	−0.186	0.129	−10.04 to 1.30
AET	−2.034	0.029	−14.16 to −0.78
Number of total refluxes	−0.325	0.024	−1.06 to −0.08
Number of acid refluxes	−0.188	0.159	−1.23 to 0.21
Number of non-acid refluxes	−0.046	0.661	−11.86 to 7.56
DeMeester score	2.167	0.023	0.351 to 4.499
PSPW index	−0.310	0.019	−3.01 to −0.28
MNBI	0.374	0.030	0.003 to 0.05
UES resting pressure	−0.1235	0.245	−0.90 to 0.234
LES resting pressure baseline	−0.062	0.629	−2.23 to 1.56
IRP4s	−0.166	0.219	−5.73 to 1.33

AET, acid exposure time; IRP4s, 4-s integrated relaxation pressure; LES, lower esophageal sphincter; MNBI, mean nocturnal baseline impedance; PSPW, post-swallow-induced peristalsis wave; UES, upper esophageal sphincter.

By using linear regression (Table 5), age, DeMeester score, and MNBI were the factors positively associated with pepsin concentration, while AET, the number of total refluxes, and PSPW index were negatively associated with pepsin concentration. There was no significant correlation between pepsin concentration and the number of acid refluxes, number of non-acid refluxes, UES resting pressure, LES resting pressure baseline or when swallow, or IRP4s.

Discussion

Many methods have been proposed to diagnose GERD with extraesophageal symptoms, especially laryngopharyngeal reflux

(LPR), which vary from simple to complex.[4] Currently, methods used to detect pepsin concentration are diverse, including Peptest, western blot, and enzyme-linked immunosorbent assay, among which Peptest is the fastest and expected to be a promising tool in diagnosing GERD and LPR.[10,16,17] The ACG guideline 2021 has proposed salivary pepsin testing as a method of detecting LPR.[5] However, due to the lack of a standard protocol regarding the time of collecting saliva samples and the variety of techniques used to measure the concentration, a recommended cut-off value of salivary pepsin has not been globally proposed. This study was conducted among patients with extraesophageal reflux symptoms in comparison to a healthy control group to evaluate the application of Peptest in diagnosing GERD and the possible threshold level of salivary pepsin concentration in differentiating GERD from non-GERD or healthy individuals.

In this study, the sensitivity of Peptest was 86.7% and 27.5% and the specificity was 86.7% and 66.0%, respectively, when using threshold level of 31.2 ng/mL (GERD *vs* non-GERD) and 31.4 ng/mL (GERD *vs* controls). This finding is consistent with previous findings indicating that Peptest had a moderate diagnostic value for LPR and GERD. A preliminary study from China conducted in 250 patients with at least 8 weeks of symptoms suggestive of GERD used Peptest to determine salivary pepsin level at three time points: morning on waking, after lunch, and after dinner. Similar findings as found here were seen that patients with GERD had a higher prevalence of pepsin and higher pepsin concentration than patients with non-GERD and healthy controls. However, the optimal cut-off value was much higher (76 ng/mL) with a sensitivity of 73% and a specificity of 88.3%.[8] In another study on 111 patients with heartburn showed that 37.9% of healthy asymptomatic subjects had at least one sample positive for pepsin and 21% of all samples were positive for pepsin; the corresponding figures for GERD patients were 67.6% and 40.1%, respectively.[9] In a systematic analysis of 16 articles that included 2401 patients and 897 controls, the pooled sensitivity and specificity for the diagnosis of GERD/LPR with Peptest were 62% and 74%, respectively, and the diagnostic odds ratio and area under the ROC curve were 5.0 and 7.0, respectively.[7]

In our study, age had a slightly negative correlation with pepsin concentration, although the result of Zihao Gou's study showed that age had no relation with pepsin concentration.[18] When comparing the metrics, pH-I, AET, the number of total refluxes, PSPW index, MNBI, and DeMeester score were factors associated with the pepsin level. However, in contrast to previous studies, in our study, AET and the number of total refluxes had a negative correlation with the concentration of pepsin. In the study of Hayat, both AET and the total number of reflux episodes had a weak but significant correlation with the concentration of pepsin in saliva.[9] Although the detection of pepsin in saliva could imply a reflux from stomach moving into the oral or laryngopharyngeal area, this phenomenon was also seen in healthy individuals. Then, it is difficult to distinguish whether pepsin detected in the Peptest was pathologic or physiologic. In our study, the parameters that were detected in HRM, including UES resting pressure, LES resting pressure (both baseline and when swallowing), IRP4s, and motility patterns, can affect pepsin concentrations reflux into esophagus. A study by Xing Du also found a low correlation between pepsin concentration and

LES pressure, but no correlation was found between pepsin concentrations and UES pressure.[8] The similar results in the study of Zihao Guo showed that salivary pepsin concentrations had no significant correlation with LES pressure and hypomotility conditions.[18]

This was the first study conducted on Vietnamese people using Peptest to determine an optimal threshold level of pepsin concentration. However, there were several limitations of this study. First, only two samples were recruited in both groups, and these were not related to the symptom appearance shown, which could have led to positive samples being missed. Second, the "healthy control" is defined only on the basis of the symptoms at the time of evaluation, and no further exploration tests were done. There was no collection of symptoms' frequency information, as the guide of the Peptest recommends that if patients only have episodic symptoms, the time when the saliva samples were collected should be recorded for the episodic symptoms; healthy controls did not undergo 24-h esophageal pH-I monitoring, so we could not confirm whether the subjects were completely free of GERD.

Conclusion

In summary, our findings showed a higher prevalence of positive Peptest in patients with extraesophageal reflux symptoms. The optimal threshold level of 31.4 ng/mL had high sensitivity and moderate specificity to differentiate GERD and healthy controls, while the threshold level of 31.2 ng/mL had high sensitivity but low specificity in differentiating GERD and non-GERD individuals.

Acknowledgments

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