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Saliva pepsin measurements in the detection of gastroesophageal reflux disease in laryngopharyngeal reflux patients: a cohort study

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Abstract

Objective To study the diagnostic value of salivary pepsin measurement (Peptest) for detecting gastroesophageal reflux disease (GERD) in laryngopharyngeal reflux (LPR) patients.

Methods Patients with reflux symptoms were consecutively recruited from January 2020 to November 2022. Patients benefited from hypopharyngeal–esophageal impedance-pH monitoring (HEMII-pH), fasting and bedtime saliva collections to measure pepsin. Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values were evaluated for GERD and LPR patients considering the highest values of pepsin tests at ≥ 16, ≥ 75, and ≥ 216 ng/mL cutoffs. The relationship between HEMII-pH, endoscopic and clinical findings, and pepsin measurements was studied.

Results Saliva was collected in 109 LPR patients and 30 individuals with both LPR and GERD. The total number of pharyngeal reflux events was significantly higher in GERD-LPR patients compared with LPR patients ($p = 0.008$). The mean fasting and bedtime pepsin saliva concentrations were similar between groups. The sensitivity of Peptest in LPR patients was 30.5%, 70.2%, and 84.0% at cutoffs ≥ 16, ≥ 75 and ≥ 216 ng/mL. In GERD-LPR group, Peptest was 80.0%, 70.0%, and 30.0% sensitive. At cutoff 16 ng/mL, Peptest reported PPV of 20.7% and 94.8% in LPR-GERD and LPR groups, respectively. NPV were 73.9% and 8.7% in GERD-LPR and LPR groups, respectively. The consistency analysis between Peptest and HEMII-pH was not significant. Peptest was significantly associated with the number of acid pharyngeal reflux events ($r_s = 0.182$; $p = 0.032$).

Conclusion Pepsin saliva measurements appear to be not a reliable diagnostic tool for the detection of GERD in LPR patients. Future studies are needed to determine the place of Peptest in laryngopharyngeal reflux and gastroesophageal reflux diseases.

Keywords Laryngopharyngeal · Gastroesophageal · Reflux · Voice · pH monitoring · Laryngeal · Pharyngeal · Saliva · Pepsin

Introduction

Laryngopharyngeal reflux (LPR) is a challenging condition in gastroenterology and otolaryngology regarding the lack of consensus for the diagnostic and the poor response of empirical proton pump inhibitor (PPI) treatments [1, 2]. The place of gastrointestinal (GI) endoscopy in the LPR checkup is an additional controversial issue. Indeed, erosive esophagitis is found in 10–40% of LPR patients at the hypopharyngeal–esophageal multichannel intraluminal impedance-pH monitoring (HEMII-pH) [3–5]. However, GI endoscopy and GERD detection remain important to detect GERD-complications, such as esophageal stricture, erosive esophagitis or Barrett metaplasia. The use of HEMII-pH in all patients with suspected LPR remains controversial regarding the unavailability of the catheter in some hospitals, the inconvenience, and the poor tolerability of the 24-h testing approach [6].

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These issues led to the development of non-invasive reflux diagnostic approaches such as saliva pepsin test (Peptest® kit, RD Biomed Ltd., Hull, United Kingdom) [7]. Recently, pepsin has been found in GERD patients without extra-esophageal symptoms, supporting a potential interest of this approach in GERD detection [8]. Moreover, patients with both LPR and GERD appear to have higher number of acid pharyngeal reflux event than LPR patients without GERD [5], and, theoretically, more refluxate content from the stomach, which may be evaluated by Peptest.

In this study, we hypothesized that patients with both LPR and GERD may have higher concentration of saliva pepsin than those without GERD, making the Peptest an interesting tool to detect GERD in LPR patients, and, therefore, indicate a gastroesophageal checkup.

Methods

Patients with suspected laryngopharyngeal reflux were consecutively recruited from the departments of otolaryngology and gastroenterology of two European hospitals (XX). Patients underwent 24-h HEMII-pH (off PPIs) to confirm the LPR diagnostic. Gastrointestinal (GI) endoscopy was proposed to patients. The exclusion criteria included histories of Nissen surgery, head and neck radiation, or traumatisms, active smoker, alcohol dependence, upper respiratory tract infection within the last month, active seasonal allergies, asthma, neurological and psychiatric disorders. The local ethics committee approved the study protocol (CHU Saint-Pierre, n°BE076201837630). Patients consented to participate.

Hypopharyngeal–esophageal multichannel intraluminal impedance-pH monitoring

The HEMII-pH catheter placement and analyses were described in previous publication [5]. The catheter was placed in the morning before breakfast by an experienced practitioner. The catheter was composed of 8 impedance segments and 2 pH electrodes (Versaflex Z®, Digitrapper pH-Z testing System, Medtronic, Europe). Six esophageal impedance segments were placed along the esophagus zones (Z1 to Z6) at 19, 17, 11, 9, 7 and 5 cm above the lower esophageal sphincter (LES). Two pharyngeal impedance segments were placed 1 and 2 cm above the cricopharyngeal sphincter. The pH electrodes were placed 2 cm above lower esophageal sphincter and 1/2 cm below upper esophageal sphincter, respectively. The diagnosis of LPR was based on the occurrence of > 1 acid (pH < 4.0), weakly acid (pH = 4.1–7.0) or non-acid (pH > 7.0) pharyngeal reflux event, which was defined as an episode reaching the two impedance sensors in the hypopharynx [9].

The GERD diagnostic was based on the Lyon criteria [10]. GERD diagnosis was confirmed in case of advanced esophagitis (Los Angeles grade C or D), Barrett's mucosa or peptic strictures at the GI endoscopy. At the HEMII-pH, the Lyon criteria proposed a GERD diagnosis in patients with acid exposure time in the low esophagus > 6%. Patients with esophagitis (grade A or B) or acid exposure time between 4 and 6% were considered as suspected GERD (gray area). [10]

Saliva pepsin measurement

Patients collected saliva samples in the morning (fasting) and 2 h after the dinner (bedtime) during the 24-h HEMII-pH period. The saliva was collected into a 30 mL universal sample collection tube containing a pre-established concentration of citric acid. The saliva sample collections were stored in the refrigerator. The pepsin saliva concentration was measured with Peptest® device (RD Biomed Ltd., Hull, United Kingdom) according to a standardized procedure [11]. The saliva pepsin concentration was measured with the Cube Reader® and ranged from 1 to 500 ng/mL.

Demographics and clinical data

Demographic (i.e., age, gender, body mass index (BMI)) and clinical data were collected for all patients. Symptoms were assessed with the French version of the Reflux Symptom Score-12 (RSS-12) [12], which is a validated 12-item reported-outcome questionnaire documenting frequency and severity of otolaryngological, digestive, and respiratory complaints. Oral, pharyngeal and laryngeal findings were rated with the Reflux Sign Assessment (RSA) [13], which is a validated 61-point finding score assessing laryngeal and extra-laryngeal findings.

Statistical methods

Statistical analyses were performed with the Statistical Package for the Social Sciences for Windows (SPSS version 27.0; IBM Corp, Armonk, NY, USA). The sensitivity, specificity, positive (PPV) and negative predictive value (NPV) of highest Peptest of the testing day were calculated at several cutoffs (≥ 16, ≥ 75 and ≥ 216 ng/mL) considering LPR diagnosis (HEMII-pH) and GERD diagnosis (GI endoscopy or HEMII-pH). According to data, Mann–Whitney and Chi-square tests were used to evaluate the differences between LPR patients and those with both LPR and suspected/confirmed GERD at the Lyon criteria. A p-value < 0.05 was considered as significant. Multivariate analysis was used to study the relationships between demographic features, clinical data, GI endoscopy, HEMII-pH and Peptest findings. The association was considered as low, moderate and strong for

$r_s < 0.30$, 0.30–0.60, and $r_s > 0.60$, respectively. The consistency between GI endoscopy, HEMII-pH and PepTest was evaluated with kappa-Cohen analysis.

Results

Setting and population

One-hundred and thirty-nine patients completed the evaluations. Thirty patients had both GERD and LPR and 109 had LPR only. There were 87 females and 52 males, respectively. The mean age of patients was 54.1 ± 14.4 years. The mean BMI was 26.4 ± 5.9. There were no significant differences between groups regarding age, BMI and gender ratio (Table 1). The GERD diagnosis was exclusively based on GI endoscopic findings in 8 cases (26.7%), while others reported abnormal HEMII-pH findings with or without abnormal GI endoscopy. The proportion of hiatal hernia and LES insufficiency did not differ between groups. The total number of pharyngeal reflux events was significantly higher in GERD patients compared with LPR patients ($p = 0.008$). There were no significant differences between groups regarding clinical data, morning, bedtime and the highest pepsin saliva concentrations (Table 1).

Accuracy of pepsin saliva measurements

The accuracy of Peptest diagnosis in LPR, GI-based GERD and HEMII-pH-based GERD diagnostics is

Table 1 Characteristics of patients

Characteristics	GERD ($N = 30$)	LPR ($N = 109$)	Total ($N = 139$)
Age (mean, SD)	53.1 ± 16.5	54.3 ± 13.9	54.1 ± 14.4
BMI (mean, SD)	27.0 ± 5.2	26.2 ± 6.1	26.4 ± 5.9
Male (N, %)	12 (40)	40 (36)	52 (37)
Female (N, %)	18 (60)	69 (64)	87 (63)
Gastrointestinal endoscopy			
Normal	3 (14)**	35 (41)**	38 (35)
Esophagitis	12 (57)**	0 (0)**	12 (11)
Hiatal hernia	8 (38)	35 (41)	43 (40)
LES insufficiency	9 (43)	36 (42)	45 (42)
Gastritis	3 (14)	8 (9)	11 (10)
Helicobacter Pylori	1 (5)	1 (1)	2 (2)
HEMII-pH (mean, SD)			
Distal esophageal acid events (pH < 4.0)	69.0 ± 65.9**	24.6 ± 24.1**	34.7 ± 41.8
Distal esophageal non-acid events (pH ≥ 4.0)	19.6 ± 29.6	19.2 ± 42.8	19.3 ± 40.1
Distal esophageal total events	88.7 ± 70.4*	43.0 ± 48.1*	53.3 ± 57.0
Pharyngeal acid events	13.4 ± 17.4	8.2 ± 12.7	9.3 ± 13.9
Pharyngeal non-acid events	22.1 ± 24.4	17.0 ± 36.1	18.1 ± 33.9
Pharyngeal events (total number)	35.4 ± 26.6*	25.1 ± 36.9*	27.4 ± 35.1
Pepsin saliva measurements			
Morning saliva sample	73.7 ± 89.2	89.6 ± 91.6	85.4 ± 90.3
Bedtime saliva sample	104.4 ± 140.4	116.5 ± 133.2	113.9 ± 134.3
Highest saliva sample	163.7 ± 133.0	163.6 ± 136.7	163.6 ± 135.5
Clinical data			
Reflux symptom score-12 (mean, SD)	72.7 ± 59.0	64.1 ± 48.0	65.9 ± 50.5
Reflux sign assessment (mean, SD)			
Oral score	4.7 ± 2.9	5.7 ± 2.2	5.3 ± 2.5
Pharyngeal score	8.8 ± 4.4	10.1 ± 4.3	9.7 ± 4.3
Laryngeal score	8.3 ± 4.9	10.7 ± 5.3	9.9 ± 5.2
Reflux sign assessment	18.0 ± 7.5	25.7 ± 8.0	23.1 ± 8.6

BMI body mass index, *GERD* astroesophageal reflux disease,*HEMII-pH* hypopharyngeal–esophageal multichannel intraluminal impedance-pH monitoring,*LES* ower esophageal sphincter,*N* number,*SD* standard deviation

*Data reported significant group differences ($p < 0.05$)

**clustering criteria (GERD-LPR *versus* LPR)

described in Table 2. According to thresholds, Peptest was positive in 23–76% of patients with abnormal GI endoscopy (esophagitis), while it was positive in 31.6–84.2% of patients with GERD at the HEMII-pH.

The sensitivity of Peptest in LPR patients without GERD was 30.5%, 70.2%, and 84.0% at cutoffs ≥ 16, ≥ 75 and ≥ 216 ng/mL (Table 3). Both sensitivity and specificity of Peptest were higher in LPR patients compared to those with both GERD and LPR. However, Peptest reported higher NPV in GERD patients compared to LPR patients (Table 3). The consistency analysis between Peptest, HEMII-pH, and RSS-12 > 11 was not significant (See Appendix Table 4).

Associations

There were no significant associations between Peptest, age, gender, or BMI. Peptest was significantly associated with the number of acid pharyngeal reflux events ($r_s = 0.182$; $p = 0.032$). In patients with only LPR, acid distal esophageal exposure was significantly associated with the number of pharyngeal reflux events ($r_s = 0.425$, $p = 0.001$). In GERD group, the BMI was positively associated with the number of pharyngeal reflux events ($r_s = 0.395$, $p = 0.034$) and the RSS-12 ($r_s = 0.377$; $p = 0.044$).

Discussion

Pepsin saliva measurement (Peptest) is a non-invasive approach that was developed to help practitioners in the detection of laryngopharyngeal reflux [7]. The saliva pepsin concentration is associated with adequate sensitivity and predictive values in patients with LPR disease [14–16]. However, most studies focused on LPR patients and authors did not consider the presence of GERD, which is known to be a condition that may exacerbate the LPR severity [5]. To date, the data about the accuracy, the sensitivity, the specificity and the predictive values of Peptest are lacking in patients with GERD and a related risk of esophageal complications.

The primary findings of the present study revealed that the pepsin saliva concentration was not influenced by the presence of GERD and the related highest number of pharyngeal reflux events at the HEMII-pH. This observation supported the findings of Bobin et al. who reported that the saliva pepsin level was not associated with the number of pharyngeal reflux events at the HEMII-pH and the related severity of LPR disease [17]. Fortunato et al. similarly supported that the concentration of salivary pepsin was not an accurate measure of severity of reflux at the pH-impedance testing because of the wide range of pepsin concentration measured in individuals over 24 h. [18]These statements were, however, contradicted by the findings of the pediatric study of Haddad et al. who observed a positive association between the saliva level of pepsin A and the

Table 2 Accuracy of saliva pepsin test according to thresholds and diseases

	HEMII-pH		GI endoscopy		HEMII-pH	
	GERD (Lyon criteria)		GERD (esophagitis)		LPR (> 1 HRE)	
	Pathological	Normal	Pathological	Normal	Pathological	Normal
At least 1 sample ≥ 16 ng/mL	16/19 (84.2)	7/9 (77.8)	10/13 (76.9)	8/9 (88.9)	110/131 (84.0)	6/8 (75.0)
At least 1 sample ≥ 75 ng/mL	15/19 (78.9)	6/9 (66.7)	7/13 (53.8)	8/9 (88.9)	92/131 (70.2)	5/8 (62.5)
At least 1 sample ≥ 216 ng/mL	6/19 (31.6)	2/9 (22.2)	3/13 (23.1)	3/9 (33.3)	40/131 (30.5)	1/8 (12.5)

GERD gastroesophageal reflux disease,*GI* astrointestinal,*HEMII-pH* hypopharyngeal–esophageal multichannel intraluminal impedance-pH monitoring,*HRE* hypopharyngeal reflux event,*LPR* laryngopharyngeal reflux

Table 3 Characteristics of patients according to the reflux profiles

	HEMII-pH/GI—GERD +				HEMII-pH—LPR			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	SP (%)	PPV (%)	NPV (%)
At least 1 sample ≥ 16 ng/mL	80.0	15.6	20.7	73.9	84.0	25.0	94.8	8.7
At least 1 sample ≥ 75 ng/mL	70.0	30.3	21.7	78.6	70.2	37.5	94.9	7.1
At least 1 sample ≥ 216 ng/mL	30.0	71.6	22.5	78.8	30.5	87.5	97.6	7.1

GERD gastroesophageal reflux disease,*GI* gastrointestinal,*HEMII-pH* hypopharyngeal–esophageal multichannel intraluminal impedance-pH monitoring,*HRE* hypopharyngeal reflux event,*LPR* laryngopharyngeal reflux,*SE* sensitivity,*SP* specificity,*PPV* positive predictive value,*NPV* negative predictive value

gastroesophageal reflux episodes with its peak value correlated with acidic reflux [19].However, it remains difficult to compare pediatric and adult populations according to the age-related differences in the LPR physiology, which consists of a higher prevalence of GERD and liquid LPR in pediatric patients compared with adults who have less GERD and more gaseous pharyngeal reflux event [20].

The present study reported comparable sensitivity and specificity in LPR and GERD-LPR groups. At cutoff 16 ng/mL, Peptest was 80.0% sensitive and 15.6% specific in GERD-LPR group, while the sensitivity and specificity of LPR group were 84.0% and 25.0%, respectively.

A difference was found between groups about predictive value data. Peptest reported a higher positive predictive value in LPR patients compared to individuals with both LPR and GERD, while its negative predictive value was highest in presence of GERD. Zelenik et al. investigated accuracy, sensitivity, specificity PPV and NPV in LPR and GERD-LPR patients [8]. Interestingly, they similarly observed comparable sensitivity and specificity between groups, and they corroborated our observation about group differences in predictive value data [8]. The accuracy, sensitivity, specificity, PPV, and NPV of Peptest were, respectively, as follows: 35%, 33%, 100%, 100%, and 3% in LPR patients [8]. In the GERD group, they reported accuracy, sensitivity, specificity, PPV, and NPV of 46%, 27%, 63%, 40.0%, and 48%, respectively [8]. The group differences in predictive value data may be attributed to the homogeneity of the LPR study populations, which mainly included patients with a positive diagnostic at the HEMII-pH. From an epidemiological standpoint, the lack of healthy individuals benefiting from GI endoscopy, HEMII-pH and pepsin collection, and the low number of patients with LPR symptoms but negative HEMII-pH led to inaccuracy of specificity and NPV assessments. Concerning other cutoffs, Peptest was 30.5% sensitive and 87.5% specific at cutoff 216 ng/mL, which corroborate the findings of Yu et al. who reported Peptest sensitivity and specificity of 30.0% and 93.3% at cutoff point of 219.47 ng/mL, respectively. [21]

The findings of the present study did not suggest a potential influence of GERD and related highest number of pharyngeal reflux events on the saliva pepsin concentration. This observation needs to be treated with caution because pepsin saliva concentration may be influenced by several factors, which were not considered in the present study. First, there is no agreed-upon cutoff for salivary pepsin as a diagnostic marker in LPR and GERD because variability in the levels of salivary throughout the testing day. According to Peptest studies [7, 8, 14–16], the 16, 75 and 216 ng/mL thresholds were evaluated but many other thresholds may be used to adjust the sensitivity and the specificity of the test regarding the practitioner wish. Second, saliva pepsin concentration is known to vary regarding the patient diet. Indeed, it has been suggested that foods and beverages may significantly influenced the saliva pepsin concentration of LPR patients [22]. In that way, the Peptest studies should be interpreted considering diet differences across the world region populations. Third, the inconsistencies in the associations between Peptest and HEMII-pH features may support the potential contribution of other gastroduodenal enzymes in the development of mucosa inflammation and related reflux symptoms and findings [23, 24]. A few clinical studies reported a significant higher bile salt concentration in saliva of LPR patients compared to controls [24, 25]. From a basic science point of view, bile salts appear to be involved in laryngopharyngeal inflammation and related reflux [23]. In the same vein, the inconsistencies between objective findings and RSS may be related to the lack of consideration of the mucosa sensitivity. Aviv et al. observed that some patients with LPR have laryngopharyngeal mucosa hypersensitivity, which leads to higher symptom scores compared to individuals without mucosa hypersensitivity [26]. According to the individual differences in laryngopharyngeal sensitivity, there may have no significant consistency between pharyngeal reflux events and the symptom severity at the RSS.

To the best of our knowledge, the present study is the largest cohort study investigating the sensitivity, specificity, PPV, and NPV of Peptest according to GERD, which is the main strength of our study. As mentioned above, the main limitation is the homogeneity of the study population, which may bias the analysis of predictive values.

Conclusion

Pepsin saliva measurements appears to be not a reliable diagnostic tool for the detection of GERD in LPR patients. Future studies are needed to determine the place of Peptest in laryngopharyngeal reflux and gastroesophageal reflux diseases.

Appendix

See Table 4 .

Table 4 Consistency analysis

Outcomes	GERD (GI/HEMII-pH)		LPR (HEMII-pH)	
	Kappa	p-value	Kappa	p-value
Highest pepsin test	0.074	NS	0.022	NS
RSS-12 > 11	0.043	NS	0.057	NS

GERD gastroesophageal reflux disease,*GI* gastrointestinal,*HEMII-pH* hypopharyngeal–esophageal multichannel intraluminal impedance-pH monitoring,*HRE* hypopharyngeal reflux event,*LPR* laryngopharyngeal reflux,*NS* on-significant

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Data availabiity Data are available on request.

Declarations

Conflict of interest The authors have no conflicts of interest.

Informed consent Patients consented to the study.

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