Published online: 2020-06-30

Laryngopharyngeal Reflux: Symptoms, Signs, and Presence of Pepsin in Saliva - A Reliable Diagnostic Triad

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Int Arch Otorhinolaryngol

Abstract

Introduction Twenty-four-hour multichannel intraluminal impedance with double probe pH monitoring (MII-pH), though considered the most sensitive tool for the diagnosis of gastroesophageal reflux disease (GERD), is invasive, time consuming, not widely available, and unable to detect non-acid reflux. In contrast, the presence of pepsin in the saliva would act as a marker for reflux, considering that pepsin is only produced in the stomach.

Objective To evaluate the predictive value of salivary pepsin in diagnosing laryngopharyngeal reflux (LPR) as suggested by the results of reflux symptom index (RSI $>$ 13), reflux finding score (RFS $>$ 7), and positive response to treatment with a 4-week course of proton-pump inhibitors.

Methods This prospective study was done at a tertiary care hospital on 120 adult patients attending ENT OPD with clinical diagnosis of LPR. The presence of pepsin in their pharyngeal secretions and saliva using a lateral flow device, the Peptest, was compared with RSI, RFS, and with the response to medical treatment using the Chi-squared test.

Results Salivary pepsin was found to be positive in 68% of the patients, with 87.5% of them showing positive response to treatment. Chi-squared analysis showed a significant association between positive salivary pepsin and RFS $>$ 7, RSI $>$13, a combination of RFS $>$ 7 and RSI $>$ 13 as well as with response to treatment ($p < 0.0001$).

Conclusion When considered along with the clinical indicators of RFS and RSI of more than 7 and 13, respectively, and/or with a response to treatment, a positive salivary pepsin test indicates statistically significant chance of presence of LPR.

Keywords
- reflux findings score
- reflux symptoms index
- saliva
- pepsin
- laryngopharyngeal reflux

Introduction

Gastroesophageal reflux disease (GERD) is defined as the retrograde flow of gastric contents into the esophagus or above. Laryngopharyngeal reflux (LPR) is the condition arising from the retrograde flow of gastric contents into the larynx/pharynx, thus causing tissue damage that results in a wide array of clinical symptoms and signs. Though LPR and GERD are an extension of similar diseases, they differ considerably in the pathophysiology, clinical presentation, diagnosis, and treatment. Gastroesophageal reflux disease is said to occur due to dysfunction of the lower esophageal sphincter whereas in LPR, the upper esophageal sphincter is at fault. The main symptom of GERD is heartburn due to esophagitis. In LPR, only 25% of the patients have esophagitis, and around 40% complain of heartburn.[1]

received
October 27, 2019
accepted
March 4, 2020

DOI https://doi.org/10.1055/s-0040-1709987.
ISSN 1809-9777.

The major symptoms of LPR are globus sensation, throat irritation, dysphagia, frequent throat clearing, dryness of throat, chronic cough, hoarseness of voice, and voice fatigue. These symptoms are long-term and cause intermittent concern to the patient. If severe, there can be vocal fold/subglottic edema andendolaryngeal mucus/granuloma formation.[2] It has been shown that mixed as well as non-acid reflux contribute to most of the symptoms of LPR as compared with purely acid reflux, in the case of GERD.[3] Pepsin is an enzyme in gastric juice that has been implicated in the pathogenesis of LPR as it can damage the laryngeal mucosa, even at mild acidic or alkaline pH.[4–6]

The diagnosis of LPR based on symptoms and laryngeal findings alone has poor sensitivity and specificity as most of the abnormal findings are also seen in around 86% of healthy individuals.[7] The mainstay of the diagnosis of LPR at present is a combination of symptoms, fiberoptic endoscopic findings, and 24-hour multichannel intraluminal impedance (MII) combined with double-probe pH monitoring (MII-pH). Multichannel intraluminal impedance-pH monitoring is now considered the most sensitive tool for the diagnosis and characterization of GERD and its laryngopharyngeal symptoms. It detects both acid and non-acid gastric reflux and assesses the proximal extent and nature of refluxate. However, MII-pH monitoring is invasive, time consuming and not available in many centers, with additional patient intolerability.[8–11] Moreover, non-acid reflux cannot be detected by pH monitoring alone. Recent insight into the pathophysiology of LPR with the help of MII-pH studies has demonstrated non-acid and mixed reflux to be more common than acid reflux.[3] Pepsin plays a major role in the pathogenesis of LPR. Pepsin can damage the laryngeal and pharyngeal mucosa at both acidic and alkaline pH, as it shows some activity even at pH 8.[12] Johnston et al did a prospective translational study in established porcine in vitro model to examine the effect of active/inactive pepsin on laryngeal CAIII and Sep70 protein levels. They reported detectable levels of pepsin in laryngeal epithelia after a reflux event.[12] Normally, pepsin in this site would be enzymatically inactive, as the mean pH of the laryngopharynx is 6.8. Significantly, pepsin would be reactivated by a subsequent decrease in pH, such as would occur during an acidic reflux event or possibly after uptake into intracellular compartments of lower pH. Since pepsin is produced only in the stomach, its presence in the saliva would act as a marker for reflux.[13] Pepsin, thus, has the potential to overcome the invasive,time-consuming,and expensive MII-pH studies, as it can be easily detected in the pharyngeal secretions and saliva using a lateral flow device such a Peptest.

There is a paucity of reports from the Indian subcontinent on this easily available, relatively less expensive, less time-consuming and non-invasive test utilizing salivary pepsin in the diagnosis of LPR. Hence, this study was undertaken.

Materials and Methods

This prospective study was performed at a tertiary care hospital following institutional research and ethical committee approval (No. RC/16/142). The objective of the present study was to evaluate the diagnostic value of pepsin in saliva in the diagnosis of LPR. Written informed consent was obtained from all patients. All adult patients attending the ear, nose and throat outpatient department with a history of change in voice/burning sensation in the substernal or epigastric region/regurgitation/dysphagia/throat pain/cough/foreign body sensation in throat/frequent throat clearing for more than 4 weeks with clinical diagnosis of LPR were selected for the study. They were then asked to complete a questionnaire with various possible symptoms suggestive of LPR for calculating reflux symptom index (RSI). The RSI is a 9-point questionnaire, each rated on a Likert scale from 0 to 5,considering a score > 13 suggestive of LPR.[8] All patients then underwent flexible nasopharyngolaryngoscopy for assessing reflux findings score (RFS), which is an 8-component assessment tool for quantifying thevseverity of laryngeal inflammation. An RFS score > 7 is suggestive of LPR.[9] Those with previous laryngeal surgery, neoplasm of the pharynx and larynx, chronic granulomatous lesion of the larynx/pharynx, and use of proton pump inhibitors in the previous month were excluded.

Saliva samples were collected 1 hour after meals. Patients were instructed to cough up saliva from the back of their throat and spit into 30-ml standard tubes containing 0.5mL of 0.01 mol/L citric acid at pH 2.5 to preserve the action of any pepsin present. The samples were refrigerated at 4°C. Immunoserologic pepsin analysis was performed within a week of collecting the sample, using a Peptest lateral flow device (LFD) (RD Biomed Ltd, Hull, UK) by one of the coinvestigators who was blinded to the clinical data. The collection tubes were centrifuged at 4,000 rpm for 5 minutes. EightyμL of the supernatant were drawn up using standard micropipettes and transferred to a clear screwtop microtube containing 240 μL of migration buffer solution. This tube was mixed with a vortex mixer for 10 seconds. Using a dual bulb pipette, 80 μL of this sample was placed in the well of the lateral flow device (LFD). The control line appeared within a few minutes and the test line appeared within 5 to 15 minutes if the result was positive.

All patients were given proton pump inhibitor (PPI – esomeprazole 40 mg twice a day) and prokinetic (domperidone 10 mg twice a day) for 4 weeks and the response to treatment was assessed with RSI reduction by 50%. The patients were divided into 3 groups for analysis and response to treatment group A (with RFS > 7); Group B (RFS > 7 and RSI > 13); Group C (RFS > 7, RSI > 13, and positive response to treatment) (►**Fig. 1**)

Results

A total of 120 patients were recruited for the study, of which there were 53 men and 67 women. The age range was from 21 to 68, with the average age being 40 years. The average age was 39 in those with positive salivary pepsin and 43 in those with a negative test. The most common symptoms were throat irritation (58.3%), globus sensation (46%), dry cough (24%), and regurgitation (15%). The mean RSI was 14.86 overall, 16.61 in the pepsin-positive, and 12.14 in the pepsin-negative patients. The mean RFS was 8.53 overall, 9.01 in the pepsin-positive, and 7.89 in the pepsin-negative patients. The general characteristic of both groups (pepsin-positive and negative) are shown in ►**Table 1**.

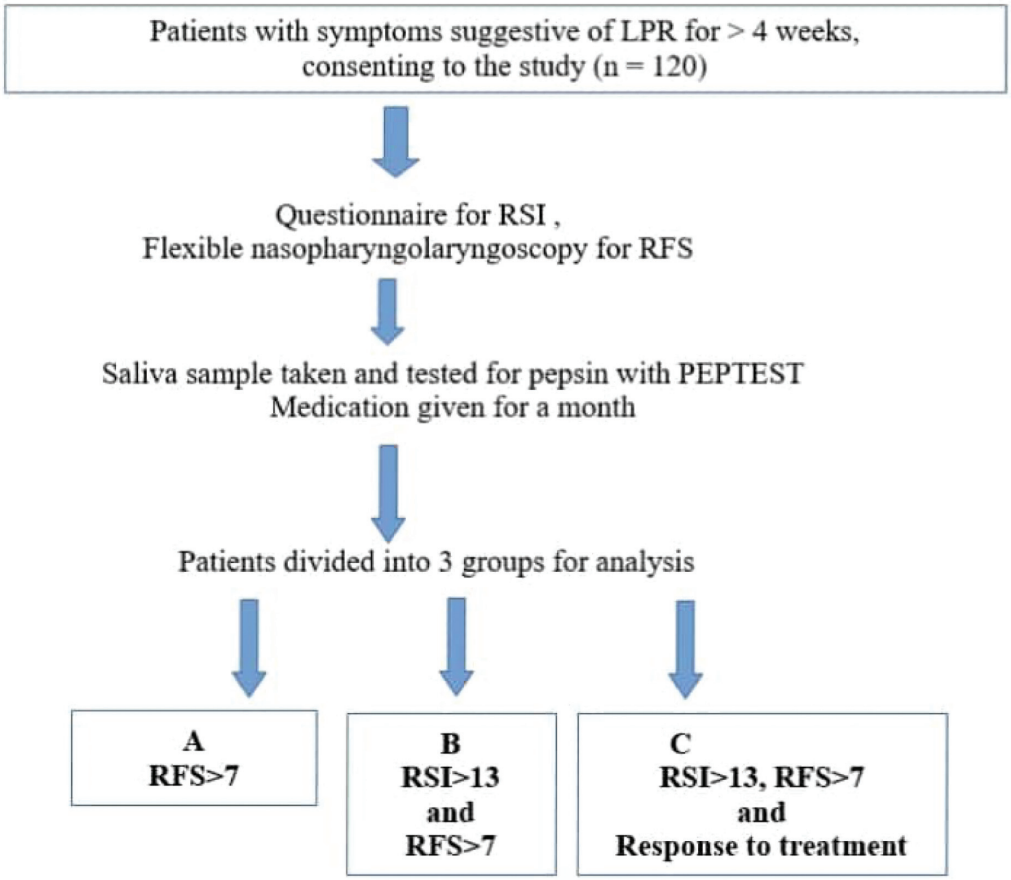

Fig. 1 Flowchart showing study procedures.

Table 1 Comparison of general characteristics of pepsin-positive and pepsin-negative groups

Characteristic	Pepsin (+)	Pepsin (-)
Age	39	43
Men	29 (55%)	24 (45%)
Women	44 (66%)	23 (34%)
Mean RSI	16.61	12.14
Mean RFS	9.01	7.89
Mean RSI after treatment	10.34	9.53
Response to treatment	54 (45%)	19 (16%)
Most common item in RSI	Globus sensation (54%)	Globus sensation (38%)
	Excess throat mucus (23%)	Dry cough (18%)
Most common sign in RFS	Arytenoid congestion (67%)	Arytenoid congestion (47%)
	Thick endolaryngeal mucus (34%)	Vocal cord erythema (26%)

Abbreviations: RFS, reflux finding score; RSI, reflux symptom index.

Of the 120 participants who enrolled in the study, 97 (80.8%) had RFS > 7, and 72 (60%) had RSI > 13. On combining these two parameters, there were a total of 68 (56.7%) participants who had both RFS >7 and RSI > 13, whereas only 19 (15.8%) had both RFS and RSI below this cut off. Salivary pepsin was found to be positive in 82 of the 120 subjects (68.1%). Seventy-two of the patients showed a positive response to the treatment, of which 63 had a positive Peptest, whereas 48 participants showed no response to the treatment,29 of whom tested negative for pepsin (►**Tables 2** & **3**).

The chi-squared analysis showed a significant association between positive salivary pepsin and RFS > 7 as well as with RSI >13. Similarly, positive associations were observed for positive pepsin and a combination of RFS > 7 and RSI > 13 alone as well as with response to treatment (►**Table 4**).

Discussion

Laryngopharyngeal reflux has become a highly prevalent condition, causing considerable concern both for the patient and the otolaryngologist.[10] It presents a diagnostic challenge owing to atypical presentation and lack of readily available sensitive diagnostic tests. More often than not, the treatment is started empirically with PPIs. In a common clinical setting, LPR is diagnosed based on symptoms and laryngoscopic findings. Reflux symptom score (RSI) is a commonly used

Table 2 Distribution of different groups based on salivary pepsin

Group	Pepsin +	Pepsin -
RFS > 7	74	23
RFS < 7	8	15
RSI >13	68	4
RSI <13	14	34
RFS > 7 & RSI > 13	65	3
RFS <7 & RSI < 13	5	14
Positive Response to treatment	63	9
No Response to treatment	19	29
RFS > 7 & RSI > 13 & positive response to treatment	56	1

Abbreviations: RFS, reflux finding score; RSI, reflux symptom index.

tool, which helps in keeping a record of symptoms for reassessment after treatment. An RSI score > 13 is highly suggestive of LPR.[9] In our study, the mean RSI score was 14.86 (overall), which showed improvement with treatment in 60% of patients (►**Table 1**).

Several studies have been conducted evaluating pepsin as a marker for LPR, all of them showing considerable variation in the sensitivity and specificity of Peptest. A cross-sectional study conducted by Ocak e al,[14] in which a two-channeled 24-hour esophageal pH monitoring catheter was placed in 20 patients with a suspicion of LPR, and each patient gave one sample of sputum for the immunoserologic pepsin detection test. This test was noted to have a sensitivity of 30% and a specificity of 100%, with a positive predictive value of 100%. The low sensitivity was attributed to single sample collection. In addition, this study also noted the proximal probe of the pepsin positive patients having an apparent acidic pH as compared with the pepsin-negative group (pH: 3.26 versus

Table 3 Latent class distribution

Group	RFS >7	RSI >13	RESPONSE +	PEPSIN +	FREQ	CUMULATIVE FREQ
1	+	−	−	−	15	15
2	−	+	−	−	1	16
3	−	−	+	−	2	18
4	−	−	−	+	5	23
5	+	+	−	−	2	25
6	+	−	+	−	5	30
7	+	−	−	+	5	35
8	−	+	+	−	0	35
9	−	+	−	+	0	35
10	−	−	+	+	0	35
11	+	+	+	−	1	36
12	+	+	−	−	9	45
13	+	−	+	+	4	49
14	−	+	+	+	3	52
15	+	+	+	+	56	108
16	−	−	−	−	12	120

Abbreviations: FREQ, frequency; RFS, reflux finding score; RSI, reflux symptom index.

Table 4 Chi-squared analysis between positive pepsin and the various clinical parameters

	RFS > 7 N (%)		RSI > 13 N (%)		RFS > 7 & RSI > 13 N (%)		RFS > 7 & RSI > 13 & positive response to treatment N (%)	
	YES	NO	YES	NO	YES	NO	YES	NO
PEPSIN +	74 (76.3)	8(34.7)	68(94.4)	14(29.2)	65 (95.6)	5(26.3)	56 (98.2)	5(29.4)
PEPSIN -	23 (23.7)	15 (65.3)	4(5.6)	34(70.8)	3 (4.4)	14(73.7)	1 (1.8)	12(70.6)
TOTAL	97	23	72	48	68	19	57	17
Chi-squared statistic (*p* Value)	14.8 (<0.00019)		56.7 (<0.0001)		45.3 (<0.0001)		42.8 (<0.0001)	

Abbreviations: RFS, reflux finding score; RSI, reflux symptom index.

pH: 6). They thus suggest that a positive pepsin test in a patient clinically suspected to have LPR can be a cost-effective, accurate, and alternative diagnostic method.[12] Another study by Alexander et al[15] suggests that salivary pepsin has a sensitivity of 78% and specificity of 53% for predicting an RFS >7. Wang et al[16] conducted a meta-analysis to assess the diagnostic value of pepsin in saliva for LPR. The pooled sensitivity and specificity were 64% and 68%, respectively. They concluded that salivary pepsin has moderate value in diagnosing LPR and requires further studies to optimize the method of detection of pepsin. The reason for such varied results across various studies could be differences in sample size, number of salivary samples collected, timing of sample collection, method of pepsin detection and criteria for diagnosis of LPR. As there is a wide range of concentration of salivary pepsin observed in an individual over a period of 24 hours, samples collected soon after reflux event are more likely to be positive.[16] In our study, 82 patients (68.1%) were positive for salivary pepsin. Similar results were observed in a study by Ianella et al to assess the correlation between obstructive sleep apnea (OSA) and LPR, where salivary pepsin was used to confirm the diagnosis of clinically suspected LPR based on positive RSI & RFS. 66.6% of the patients with clinical LPR tested positive for salivary pepsin.[17]

There are a few studies comparing treatment response to salivary pepsin, but none have studied the correlation between pepsin and combined RSI, RFS, and a positive response to treatment. A study by Wang et al noted significant association between strongly positive salivary pepsin and a good treatment response.[18] Another study by Alexander et al showed significant correlation between RFS scores and positive Peptest, but no correlation between RSI and a positive Pepstest.[15] Sereg-Bahar et al studied pepsin and bile acids in the saliva of patients with LPR and reported a significant correlation between the RSI and RFS scores and the level of total pepsin and bile acids in the saliva.[19] There was a significant association between a positive Peptest and RSI $>$ 13 and RFS $>$ 7, individually, as well as on combining RSI and RFS together. The association was significant even when combining RFS, RSI and response to treatment ($p<$ 0.05), in our study (►**Table 4**), making our study the first to compare associations between these parameters together.

This prospective study indicates that salivary pepsin test is indeed a very useful test in the diagnosis of LPR, especially in a set up in which MII-pH is not available. A positive pepsin test in combination with RSI, RFS and trial of response to treatment revealed significant association.

Conclusion

In the clinical presentation of a constellation of symptoms and signs in LPR, RSI)and RFS)are to be considered. The present prospective study of immunoserologic pepsin analysis in the saliva of patients revealed that a positive salivary pepsin test along with RSI $>$ 7, RFS $>$ 13, and positive response to treatment with PPIs indicates statistically significant chance of presence of LPR. Hence, salivary pepsin test, a relatively inexpensive, less time-consuming and patient friendly non-invasive test is being suggested as part of armamentarium in the diagnosis of LPR.

Limitations

The diagnosis of LPR could not be confirmed with MII-pH due to non-availability of the equipment.

Only a single sample of saliva was tested. The number of positives may have been more if there were multiple samples. However, multiple samples could not be obtained due to limited funds. There was a lack of control group.

Funding

The present study was possible with internal funding provided by the research committee of the Pondicherry Institute of Medical Sciences, Puducherry, India.

Conflict of Interests

The authors have no conflict of interests to declare.

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