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Is Pepsin a Reliable Marker of Laryngopharyngeal Reflux? A Systematic Review

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Abstract

Objective. Laryngopharyngeal reflux (LPR) is a common illness of otolaryngology visits. Over the past few years, pepsin has become a promising marker of LPR. The objective of the present research is to analyze the existing literature using pepsin as a diagnostic tool of LPR through a systematic review.

Data Sources. PubMed (Medline), Trip Database, Cochrane Library, EMBASE, SUMsearch, and Web of Science.

Review Methods. The outcome assessed was the presence of pepsin in LPR patients. We included articles in which pepsin was studied in LPR patients (clinically suspected or with confirmed diagnosis). Studies with no control group, comparison group, and/or a sample size lower than 20 patients were excluded.

Results. Twelve studies were included. All included studies, with the exception of 2, found statistically significant differences for pepsin in cases compared with healthy controls.

Conclusion. Pepsin might be a reliable marker in LPR patients, although questions remain about optimal timing, location, nature, and threshold values for pepsin testing. Future investigations are necessary to clarify the best method to use pepsin in the diagnostic process of LPR.

Keywords

extraesophageal reflux, laryngopharyngeal reflux, LPR, pepsin, systematic review, reflux

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Patients with gastroesophageal reflux (GERD) often display classic symptoms such as pyrosis or regurgitation, but other atypical symptoms, such as chronic cough, asthma, or laryngitis, may appear. These atypical symptoms can be identified as laryngopharyngeal reflux (LPR).[1]

LPR accounts for 4% to 10% of patients seen in otolaryngology consultations. It usually has nonspecific symptoms, making its diagnosis difficult. An important issue contributing to this difficult diagnosis is the lack of reliable diagnostic tests. Multichannel intraluminal impedance (MII) and 24-hour dual-probe pH-metry are considered the gold standard.[2] The reproducibility of pH-metry is poor[3] as its outcome is dependent on the position of the proximal sensor, and the pH cutoff value is not clear yet.[4] In addition, these tests cannot be performed in all patients suspected of presenting LPR given their invasive nature and high cost.

Instead of these diagnostic tools, otolaryngologists usually rely on clinical questionnaires such as the Reflux Symptom Index (RSI),[5] tests such as Reflux Finding Score (RFS),[6] and a proton-pump inhibitor (PPI) therapeutic trial[7] to make a diagnosis. There are some limitations for these diagnostic tests.[8-10]

The result of these limitations is that LPR is usually misdiagnosed. For this reason, the economic burden of misdiagnosing LPR has been highlighted by many authors.[11-14]

The gastric enzyme, pepsin, has been proposed as a solution to overcome the aforementioned limitations. Pepsin is a proteolytic enzyme that is secreted by the stomach's chief cells as the zymogen pepsinogen. As there is no production of pepsin in the airway, its presence is a sign of LPR.

Some investigations have demonstrated the biologic plausibility of pepsin taking part in LPR,[1,15-21] and in recent

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Table 1. Quality Score.

Item Assessed	Characteristic	Weight
Sample size	>70	2
	40-69	1
	20-39	0
Gold-standard comparison	Multichannel intraluminal impedance or dual probe	2
	pH monitoring	1
	None or others	0
Blinded study	Yes	2
	No	0
Time between gold-standard test and pepsin detection	<1 week	2
	>1 week	0
Significance of the sample	Yes	2
	Partially	1
	No	0
Total		

years, an increasing number of investigations have attempted to demonstrate that pepsin is a reliable marker for LPR. Nevertheless, different methods have been used to compare and analyze the available evidence, and results have been contradictory.

The objective of the present research article is to analyze the existing literature using pepsin as a diagnostic tool of LPR through a systematic review.

Methods

Literature Search: Inclusion and Exclusion Criteria

Recommendations of the PRISMA statement were followed. We performed a systematic review using PubMed (Medline), Trip Database, Cochrane Library, EMBASE, SUMsearch, and Web of Science. We used a predefined search strategy employing a combination of keywords (*pepsin, laryngopharyngeal reflux, saliva, sputum, nasal, airway, GERD, extraesophageal reflux*) complemented with free text terms. We also manually reviewed the reference lists of all selected articles to identify studies potentially fulfilling inclusion criteria but not found by the initial search method.

The outcome assessed was the presence of pepsin in LPR patients. The title and abstracts of the studies retrieved were thoroughly reviewed, and those fulfilling inclusion criteria were independently assessed for eligibility by 2 review team members (C.C.-H. and P.V.). Any disagreement between them was resolved through discussion with a third reviewer (A.R.-R.).

We have included studies written in both English and Spanish. There were no restrictions by date or publication type, and the last update of the search was performed in December 2016.

We included articles in which pepsin was studied in LPR patients (clinically suspected or with confirmed diagnosis). Articles were included even if pepsin measurement was not the main objective of the work. Studies with no comparison group and/or a sample size lower than 20 patients were excluded.

Assessment of the Study Quality

Due to the heterogeneity of the selected works, we developed a score to assess the quality and the risk of bias of the included studies.

The scale had 5 items, with a score range from 0 to 10 points. The items considered were the following: sample size, gold-standard comparison, blinded study, time lapse between gold-standard test and pepsin detection, and significance of the sample. Articles were included regardless of its score. The scale with the scoring of each item appears in **Table 1**.

Results

Search Results

A description of the research process appears in **Figure 1**. We obtained 146 studies, and after reading all the abstracts, 28 were selected for full-text reading. Of them, 12 fulfilled the established inclusion criteria.

Of the selected articles for full-text reading, 5 were excluded because no control group was present, 4 did not study LPR patients, 2 did not study the association between LPR and pepsin, 2 were written in Chinese, and 3 had a sample size lower than 20 patients.

Results of the Included Studies

The description of included results is shown in **Table 2**.

All included studies, with the exception of 2,[22,23] found statistically significant differences for pepsin in diagnosed cases compared with healthy controls.

Yadlapati et al[22] studied a sample of 33 participants distributed in 3 cohorts: asymptomatic controls, laryngeal symptoms, and laryngeal and esophageal symptoms. They tested for pepsin in saliva using the Peptest (RD Biomed, East Yorkshire, England) did not find statistically significant differences. However, when an estimation of pepsin concentration in each cohort was performed, statistically significant differences were then found. Differences in favor of laryngeal and esophageal cohorts symptoms were $P = .01$ and .04.

Komatsu et al[23] studied a sample of 55 patients with LPR symptoms. For the control group, 10 patients with typical GERD symptoms (no LPR symptoms) were randomly selected. This study did not find differences in pepsin concentration, but unlike the other studies included in this review, they used GERD patients, asymptomatic for LPR, and not healthy patients as the control group.

The most prominent study published to date regarding quality scores was performed by Na et al,[24] with a score of 9 points. This work is also the study with the second largest sample size of LPR patients, including 62 participants, 50 confirmed LPR patients, and 12 asymptomatic controls. They looked for pepsin in saliva via enzyme-linked immunosorbent assay (ELISA). Samples were obtained at

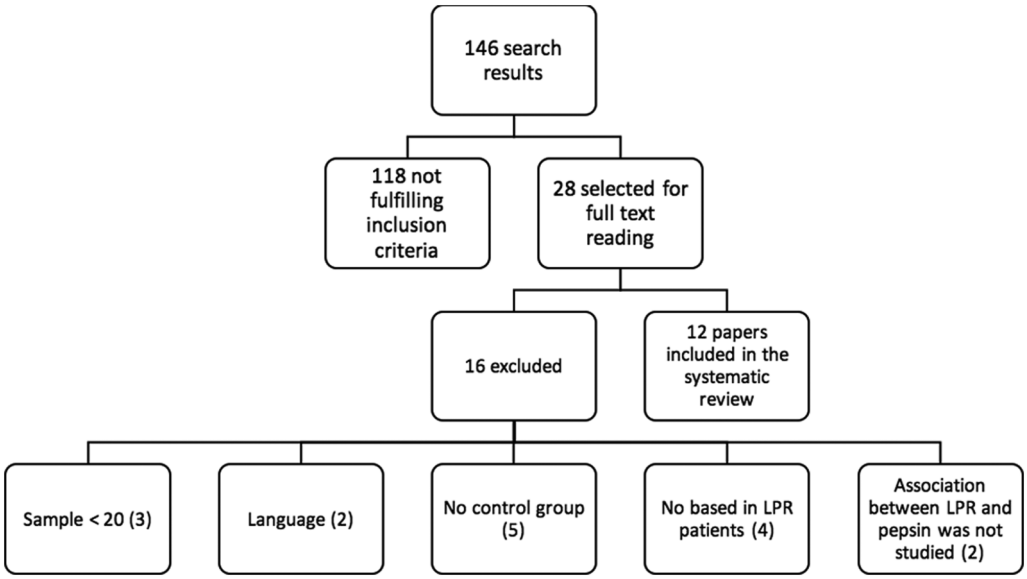

Figure 1. Flow diagram depicting the identification and selection of eligible studies for inclusion in the systematic review. LPR, laryngopharyngeal reflux.

different times of the day. They found higher levels of pepsin in patients than in controls. They also found higher levels of pepsin upon waking, whereupon they highlighted the importance of taking samples upon waking.

The study with the largest sample size was performed by Fortunato et al,[25] with 133 pediatric patients. This work and the one performed by Iannella et al[26] were the only studies performed in pediatric patients. Both used patients with a confirmed diagnosis of LPR.

There is controversy among the selected studies about the presence of pepsin in healthy controls. Eight of the included studies found pepsin in healthy controls,[2,22,24,25,27-30] while another 3 did not.[20,26,31] In the group of studies that did not find pepsin in healthy controls, 2 of 3 used controls confirmed via gold-standard probes.[20,31] On the other hand, the whole group of studies that found pepsin in controls used clinically healthy controls, not confirmed controls.

With regard to the samples to be examined, studies can be divided into 2 main groups: 9 studied fluids,[2,22,24-28,30,31] whereas the other 3 studied biopsies.[20,23,29]

Within the studies that sampled biopsies, the work of Jiang et al[29] stands out as the only one to study pepsin in biopsies taken from awakened patients. The study of pepsin using immunohistochemistry (IHQ) was first described by Johnston et al,[20] but only in anesthetized patients.

Jian et al[29] studied a sample of 15 confirmed LPR patients and 21 controls. Biopsies were taken from interarytenoid mucosa. The difference in the percentages of positive pepsin was statistically significant among 3 groups (acid, nonacid, and healthy controls): $\chi^2 = 18.6$, $P < .01$. When the cutoff point was set as moderately positive, a sensitivity of 80% and a specificity of 85.7% were found.

Quality of the Included Studies

The included studies ranged between 2 and 9 points regarding our quality score criteria (**Table 1**). Six of the 12 included studies were over 5 points, and the average scoring was 4.66 points. The main weakness was that authors did not clarify how much time had elapsed between performing a pepsin detection test and the gold-standard test. The second most common weakness was small sample size.

Discussion

Over the past few years, pepsin has become a promising marker of LPR, as we can see in the growing number of studies assessing it, rendering this review highly necessary. This is the first systematic review focused exclusively on the role of pepsin as a diagnostic tool for laryngopharyngeal reflux. The available studies suggest that pepsin is a good marker for LPR. Although 2 studies are discrepant, there is a general agreement on the valuable role of pepsin as a diagnostic marker for LPR.

Pepsin as a Diagnostic Test

In view of the evidence assessed in this study, pepsin seems to be a reliable marker for LPR. All the included studies, with the exception of 2,[22,23] found a relationship between LPR and pepsin.

Yadlapati et al[22] did not find any statistically significant differences. However, they found statistically significant differences when they performed a quantitative estimation of pepsin. Their findings could be explained because they used only RSI for the selection of patients, so there may be a possibility they had a high number of false positives. In addition, they did not define the time of day the saliva samples were taken. Other authors have highlighted the

Table 2. Description of the Included Studies.

Author	City or State and Country	Score	Sample Size	Pepsin Detection	Sample	Main Outcome	Pepsin in Patients	Pepsin in Controls	Journal	Cutoff Point
Fortunato et al[25]	Chicago, United States	7	90 confirmed LPR pediatric patients; 43 asymptomatic pediatric controls	Saliva, ELISA	Confirmed pediatric LPR patients via 24-hour combined pH-MII monitoring	Pepsin score 3 (sensitivity = 80%, specificity = 45.7%); pepsin score 4 (sensitivity = 70%, specificity = 52.8%)	86% with 1 positive sample; average concentration within 30 minutes after event 38.6 ± 156 ng/mL	9.3% with 1 positive sample	*Neurogastroenterology & Motility*	No cutoff point
Yadlapati et al[22]	Illinois, United States	3	15 clinically diagnosed LPR (6 laryngeal symptoms, 9 laryngeal and esophageal symptoms); 18 asymptomatic controls	Saliva, monoclonal antibody Peptest (RD Biomed, East Yorkshire, England)	Clinically diagnosed LPR patients (RSI and GERDq tests)	Significant differences in quantitative estimated pepsin (P = .01 and .04); no differences in percentage of positives (P = .5)	Laryngeal symptoms patients: 40% positive; estimated pepsin (7.5 ± 11.2) Laryngeal and esophageal symptoms patients: 75% positive; estimated pepsin (117.9 ± 147.4)	53% positive for pepsin; estimated pepsin (32.4 ± 41.9)	*Clinical Gastroenterology and Hepatology*	1.6 ng/mL
Na et al[24]	Seoul, South Korea	9	50 confirmed LPR patients; 12 asymptomatic controls	Saliva, ELISA (Cloud- Clone, Houston, Texas)	Confirmed LPR patients via 24-hour combined pH-MII monitoring	Statistically significant differences (P = .031 and .025, respectively)	Pepsin upon waking: 17.2 ng/mL Pepsin after lunch: 3.0 ng/mL	Pepsin upon waking: 3.7 ng/mL Pepsin after lunch: 0.0 ng/mL	*The Laryngoscope*	No cutoff point
Ianella et al[26]	Rome, Italy	3	20 confirmed LPR patients; 20 asymptomatic controls	Tears, ELISA (DRG, Marburg, Germany)	Confirmed LPR pediatric patients via 24-hour combined pH-MII monitoring	No P value	20% positive for pepsin	0% positive for pepsin	*International Journal of Pediatric Otolaryngology*	2.5 ng/mL in at least one of the eye samples
Sereg-Bahar et al[27]	Ljubljana, Slovenia	6	28 confirmed LPR patients; 48 asymptomatic controls	Saliva, enzyme immune test (USCN Life Science, Wuhan, China)	Confirmed LPR patients via 24-hour combined pH-MII monitoring	Statistically significant differences (P = .023)	Total pepsin μg/L: 29.8 (SD = 16)	Total pepsin μg/L: 9.3 (SD = 7.4)	*Clinical Otolaryngology: Official Journal of ENT-UK*	No cutoff value
Komatsu et al[23]	Pittsburgh, United States	6	20 clinically diagnosed LPR; 10 controls (GERD patients, no LPR symptoms)	Hypopharyngeal, gastric cardia, and distal esophagus biopsies; Western blot analysis (Abcam, Cambridge, UK)	Confirmed LPR patients via EGD and MII	No statistically significant differences (P = .703, .226, and .964, for each location)	Total pepsin: 1,547.58	Total pepsin: 1,815.7	*Surgical Endoscopy*	No cutoff value
Hayat et al[2]	London, United Kingdom	3	21 clinically diagnosed LPR; 10 asymptomatic controls	Saliva (5 samples in 24 hours), monoclonal antibody Peptest	Patients and controls had simultaneous MII, pH monitoring, and saliva pepsin sampling	Statistically significant differences (P = .025)	26.7% positive for pepsin	12% positive for pepsin	*Journal of Clinical Gastroenterology*	5 samples; cutoff value 25 ng/mL
Birtić et al[28]	Osijek, Croatia	5	45 clinically diagnosed LPR; 30 asymptomatic controls	Saliva, ELISA (USCN Life Science, Wuhan, China)	Clinically diagnosed LPR patients (RSI and RFS)	No P value was calculated	Total pepsin: 91.97 ng/mL	Total pepsin: 10.76 ng/mL	*International Journal Collegium Antropologicum*	No cutoff value
Jiang et al[29]	Canton, China	5	15 confirmed LPR patients (7 acid, 8 nonacid); 21 asymptomatic controls	Interarytenoid mucosal biopsies, IHQ detection of pepsin, rabbit antihuman polyclonal pepsin (USCN Life Science, Wuhan, China)	Confirmed LPR patients via 24-hour combined pH-MII monitoring	Statistically significant differences (P = .01); no difference between acid and nonacid LPR; sensitivity of 80% and a specificity of 85.7%	80% positive for pepsin	14.29% positive for pepsin	*The Laryngoscope*	Moderately positive
Wang et al[30]	Canton, China	4	32 clinically diagnosed LPR; 15 asymptomatic controls	Saliva and hypopharyngeal secretions, ELISA (USCN Life Science, Wuhan, China)	Clinically diagnosed LPR patients (RSI, RFS, and PPI treatment test)	Statistically significant differences (P = .000); no differences in pepsin concentration between oral and hypopharyngeal samples (P = .136)	93.8% positive for pepsin	20% positive for pepsin	*Otolaryngology–Head and Neck Surgery*	No cutoff value
Kim et al[31]	Suwon, Korea	3	40 confirmed LPR patients; 12 confirmed controls	Saliva, Western blot (Santa Cruz Biotechnology, Santa Cruz, California)	Confirmed LPR patients via 24-hour pH monitoring; confirmed controls via 24-hour pH monitoring	No P value; no statistically significant difference in the positive rate of a pepsin test between patients with and without typical symptoms	50% positive for pepsin	0% positive for pepsin	*Digestion*	1540 pixels, using image-pro Plus 6.0 image analysis system
Johnston et al[20]	North Carolina, United States	2	9 confirmed LPR patients; 12 confirmed controls	Laryngeal biopsy (vocal fold, posterior commissure, and ventricle region), IHQ detection of pepsin	Confirmed LPR patients via 24-hour pH monitoring; confirmed controls via 24-hour pH monitoring	Statistically significant differences ($P < .001$)	88.89% positive for pepsin	0% positive for pepsin	*The Laryngoscope*	No cutoff value

Abbreviations: EGD, esophagogastroduodenoscopy; ELISA, enzyme-linked immunosorbent assay; GERD, gastroesophageal reflux disease; GERDq, gastroesophageal reflux disease questionnaire; IHQ, immunohistochemistry; LPR, laryngopharyngeal reflux; MII, multichannel intraluminal impedance; PPI, proton pumb inhibitor; RFS, Reflux Finding Score; RSI, Reflux Symptom Index.

importance of a specific time of day for saliva sample taking to detect pepsin.[2,24,32]

Komatsu et al[23] did not find a link between pepsin and LPR, but, unlike the rest of the selected studies, their control group consisted of patients with GERD, not healthy controls. Thus, it does not follow that pepsin is not related to LPR, but it calls into question if pepsin can be used to differentiate patients with GERD from patients with LPR.

In a more recent study, Yadlapati et al[22] addressed this very question and also found that pepsin is not useful in distinguishing between patients with LPR and GERD. In the study carried out by Kim et al,[31] included in this review, there were no statistically significant differences in the positive rates of pepsin tests between patients with and without typical reflux symptoms.

Therefore, according to the evidence found, pepsin does not seem to be useful in distinguishing between patients with LPR and GERD.[22,23,31] Future comprehensive research should be carried out.

Fortunato et al[25] found a low specificity rate using pepsin detection in saliva via ELISA. Other authors highlighted the low sensitivity and specificity rate for pepsin to diagnose LPR in children.[33,34]

Pepsin in Healthy Patients

Most of the studies assessed in this review found pepsin in healthy controls, but it was at a noticeably lesser concentration than in patients (**Table 2**).

Of the 3 studies that did not find pepsin in healthy controls, 2 used confirmed controls.[20,31] On the other hand, the studies that found pepsin in controls used asymptomatic patients, not confirmed with the gold-standard test. On one hand, it could mean that pepsin yielded several false positives. On the other hand, it may suggest that asymptomatic LPR patients have LPR episodes.

Cutoff Point

There is too much variability among selected studies in the cutoff point to consider pepsin as pathologic (**Table 2**). As some authors have highlighted before, there is no consensus[30,35] on this point, and it also depends on the technique used to measure pepsin. In addition, we must question the use of nonquantitative tests, such as Peptest, and whether a patient should be diagnosed with LPR with only 1 positive sample. Most of the included studies do not explain how many samples they studied, and others did not specify how many samples were studied before they considered a patient positive for pepsin.

Saliva, Sputum, or Biopsies as Samples

Studies assessed in this review are divided in 2 main groups. One used saliva and sputum as samples, and the other used pharynx and larynx biopsies.

Biopsy tests seem to be more sensitive than saliva tests[29,32,36] but are more aggressive because they usually require sedating the patient. Most authors took biopsies from unconscious patients. However, in one of the assessed studies in this review, biopsies were taken from conscious patients, with the area numbed by local anesthesia.[29] This technique could be widely used in otolaryngology examination rooms. On the other hand, saliva/sputum tests are less aggressive. Therefore, they are more easily accepted by patients and also accessible to all practitioners.

Another problem among investigations that studied biopsies is that there is no consensus about which area to take the biopsy from. The selected studies include the postcricoid area, interaritenoid mucosa, vocal fold, ventricle, posterior commissure, and hypopharynx.[20,29,37,38]

The other main group of studies studied pepsin in saliva/sputum. Some studies in this group used the same diagnostic tool, the Peptest commercial kit.[2,22] The other portion of these studies used IHQ techniques such as ELISA or Western blot.[24-28,30,31] These techniques are much more expensive and unavailable in most facilities; however, they have the advantage of being quantitative tools.

Another important difference of this group is the number of samples and the time of day they were taken. In a work included in this review, Fortunato et al[25] found a wide range of pepsin values throughout the day in patients with LPR. The most common times are morning, symptomatic episodes, and night. Taking the sample upon the patient waking[24] or immediately after the reflux episode[25] seems to be the most useful. Some authors used more samples, and they found that the more samples they had, the more patients they diagnosed.[2]

Although the results of this review offer strong evidence for the use of pepsin as a marker of LPR, the role it plays in the diagnostic process remains unclear, as well as the best way to measure it. Therefore, research should be carried out to compare the Peptest with biopsies to clarify which method is better in the diagnostic process.

Conclusions

To sum up, systematic review of the current literature about pepsin suggests that it might be a reliable marker in patients with LPR, although questions remain about optimal timing, location, nature, and threshold values for pepsin testing.

Authors' Note

This work is part of the research completed by Christian Calvo-Henríquez, MD, to obtain a PhD degree.

Author Contributions

Christian Calvo-Henríquez, data analysis, drafting, final approval, accountability for all aspects of the work; **Alberto Ruano-Ravina**, data analysis, drafting, final approval, accountability for all aspects of the work; **Pedro Vaamonde**, data analysis, drafting, final approval, accountability for all aspects of the work; **Gabriel Martínez-Capoccioni**, data analysis, drafting, final approval, accountability for all aspects of the work; **Carlos Martín-Martín**, data analysis, drafting, final approval, accountability for all aspects of the work.

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