



Original Article

Combined laryngopharyngeal reflux and obstructive sleep apnea (CLOSA) – Salivary pepsin test for laryngopharyngeal reflux in obstructive sleep apnea patients

Shih-Chieh Shen^{a,b}, Yen-Ting Chiang^c, Liang-Wei Tseng^d, Chun-Ting Lu^e, Wan-Ni Lin^e, Li-Ang Lee^{e,e}, Tuan-Jen Fang^{e,e}, Wen-Nuan Cheng^f, Hsueh-Yu Li^{e,e*}

^aDepartment of Otolaryngology-Head and Neck Surgery, New Taipei Municipal Tucheng Hospital (Built and Operated by Chang Gung Medical Foundation), New Taipei, Taiwan, ^bMarco Otolaryngology Clinic, New Taipei, Taiwan, ^cDepartment of Otolaryngology-Head and Neck Surgery, Sleep Center, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan, ^dDivision of Chinese Acupuncture and Traumatology, Center of Traditional Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan, ^eSchool of Medicine, Chang Gung University, Taoyuan, Taiwan, ^fDepartment of Sports Sciences, University of Taipei, Taipei, Taiwan

ABSTRACT

Objectives: Reflux disease including gastroesophageal reflux and laryngopharyngeal reflux (LPR) is often found in obstructive sleep apnea (OSA) patients. Endoscopic examination is a gold standard diagnosis for reflux disease. However, the invasive procedure limits its widespread use. The pathophysiological characteristics of LPR are associated with refluxate components, of which pepsin is known to damage the tissues of the larynx and pharynx. Therefore, the detection of salivary pepsin to diagnose LPR becomes a potentially clinical application with noninvasiveness. In this study, we aimed to (1) validate the feasibility of salivary pepsin test for LPR in OSA patients, (2) establish the threshold of salivary pepsin in diagnosing LPR, and (3) explore the relationship between OSA and LPR. **Materials and Methods:** Seventy adult polysomnography-diagnosed OSA patients were enrolled. Reflux finding score (RFS) and salivary pepsin test were utilized to evaluate LPR. RFS is a set of eight objective laryngoscopic findings (total score: 0–26), with a total score of >7 as RFS-positive representing LPR-positive. The salivary pepsin concentration was detected by enzyme-linked immunosorbent assay with a standard protocol. **Results:** Salivary pepsin test was performed quickly and smoothly in all subjects with no discomfort or side effects. Based on RFS positive, the prevalence of LPR was up to 86% in our study population. There is a trend that the median salivary pepsin concentration in RFS-positive patients was higher than RFS-negative patients (14.9 ng/ml vs. 7.23 ng/ml). The cutoff point (2.3 ng/ml) of salivary pepsin concentration yielded a sensitivity of 93% in the diagnosis of LPR. Neither apnea/hypopnea index nor salivary pepsin concentration was different between LPR-positive versus LPR-negative groups and nonsevere versus severe OSA groups. **Conclusion:** LPR is highly prevalent in OSA patients. Salivary pepsin test could be an alternative to endoscopic findings for the diagnosis of LPR with noninvasiveness. The threshold of salivary pepsin concentration of 2.3 ng/ml offers 93% sensitivity in the diagnosis of LPR. The relationship between OSA and LPR is bidirectional and more likely to be an overlapping syndrome-combined laryngopharyngeal reflux and OSA (CLOSA). Pharmacologic therapy for LPR is needed in patients with CLOSA for comprehensive treatment.

KEYWORDS: *Combined laryngopharyngeal reflux and obstructive sleep apnea, Laryngopharyngeal reflux, Obstructive sleep apnea, Reflux finding score, Salivary pepsin test*

Submission : 11-Feb-2025
Revision : 19-Mar-2025
Acceptance : 29-Apr-2025
Web Publication : 23-Jul-2025

INTRODUCTION

Laryngopharyngeal reflux (LPR) is a clinical condition caused by the retrograde flow of gastric contents into the larynx and pharynx, affecting the upper airway and

*Address for correspondence: Dr. Hsueh-Yu Li, Department of Otolaryngology-Head and Neck Surgery, Sleep Center, Chang Gung Memorial Hospital at Linkou, 5, Fushing Street, Gueishan Shiang, Taoyuan, Taiwan. E-mail: hyli38@cgmh.org.tw

Supplementary material available online

Access this article online

Quick Response Code:



Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_55_25

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Shen SC, Chiang YT, Tseng LW, Lu CT, Lin WN, Lee LA, et al. Combined laryngopharyngeal reflux and obstructive sleep apnea (CLOSA) – Salivary pepsin test for laryngopharyngeal reflux in obstructive sleep apnea patients. Tzu Chi Med J 2025;37(4):437-43.

laryngeal structures [1]. LPR is estimated to affect around 10%–30% of the population and is becoming more common owing to increased aging and obese population [2,3]. The exact prevalence of LPR is challenging to determine due to different diagnostic criteria and assessment methods. Epidemiologic studies indicated that LPR accounted for 10% of otolaryngologic consultations and 18.8% prevalence of LPR in the Chinese population. Furthermore, an estimated 5% and 30% prevalence levels of LPR were observed in Greek and British populations, respectively [4]. In addition, underestimation of LPR prevalence is highly possible as the symptoms can be subtle and inconspicuous. Chronic intermittent symptoms of LPR include but are not limited to frequent throat clearing, hoarseness, globus sensation, sore throat, cough, and halitosis [1]. Obstructive sleep apnea (OSA) is a sleep disorder characterized by repeated episodes of upper airway obstruction during sleep, and it can have profound health implications if left untreated, including an increased risk of major cardiovascular and neurocognitive diseases. The global prevalence of OSA is estimated to be up to one billion people and is associated with a high socioeconomic burden [4].

The relationship between OSA and LPR is complex and could be bidirectional. Both OSA and LPR share common risk factors such as smoking, alcohol consumption, obesity, age, and sex. Multiple studies have been carried out to investigate the link between OSA and LPR. These studies have found that individuals with OSA are more prone to LPR than those without sleep disorders [5,6]. Regurgitated gastric irritants can lead to inflammation and edema of the upper airway tissues. This inflammation contributes to the narrowing of the airway, which increases the risk of airway obstruction during sleep. Sleep apnea-related changes in breathing patterns and increased intra-abdominal pressure can promote reflux episodes, worsening LPR symptoms. Furthermore, the disrupted sleep patterns and frequent awakenings associated with OSA can also exacerbate symptoms of LPR [7,8].

Diagnosing LPR poses a challenge due to the absence of standardized diagnostic criteria and the overlap of symptoms with other upper aerodigestive conditions. The classic diagnostic approach to LPR typically involves clinical evaluation, laryngoscopy, and impedance and pH-monitoring studies [1,9,10]. Noninvasive diagnostic methods for LPR are essential for identifying the condition without the need for invasive procedures. The initial step in diagnosing LPR often involves a thorough assessment of the patient's symptoms. Clinicians may use validated questionnaires, such as the Reflux Symptom Index (RSI), to evaluate the frequency and severity of symptoms associated with LPR [4]. The pH testing is a diagnostic tool to identify and determine the reflux events of LPR, by placing the probe in the esophagus and pharynx [11]. However, pH testing may not capture nonacidic or weakly acidic reflux events, and there was a concern regarding the accurate positioning of pharyngeal pH probes to measure the hypopharyngeal exposure to acidic refluxate [12-14]. Impedance testing, on the other hand, can detect both acidic and nonacidic reflux events by detecting impedance changes in the esophagus and pharynx [15]. Despite these two

examinations providing a more comprehensive assessment of reflux events, they are less clinically accessible, less tolerable by patients, and more expensive than clinical questionnaires and endoscopic examinations. Although endoscopic examination is a gold standard diagnostic tool for reflux disease, the invasive procedure limits its widespread use.

The pathophysiological characteristics of LPR are associated with acid and refluxate components. When acidic refluxate reaches the laryngopharyngeal area, it activates pepsin, leading to its enzymatic activity. Pepsin is known to damage the tissues of the larynx and pharynx, as it can cause inflammation, tissue damage, and disruption of mucosal barrier function [16-18]. As a result, the detection of pepsin in saliva or laryngeal secretions can serve as the indicator of reflux events of LPR, and there is increasing weight of evidence that pepsin, a biomarker of the gastric reflux, can aid in diagnosing LPR and provide additional evaluations of gastric reflux contributing to laryngeal mucosal injury [16,19-22]. To our knowledge, a few studies addressed the relationship between OSA and LPR via the salivary pepsin test. Therefore, we aimed to (1) validate the feasibility of the salivary pepsin test for LPR in OSA patients, (2) establish the threshold of salivary pepsin in diagnosing LPR, and (3) investigate the relationship between OSA and LPR.

MATERIALS AND METHODS

Ethical considerations

This study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital Ethics Committee (CMRPG1K0181). This study was conducted in accordance with the Declaration of Helsinki. The informed consent was obtained from the patients.

Study population

Seventy adult OSA patients, diagnosed by polysomnography (PSG) with an apnea–hypopnea index (AHI) larger than 5 per h, were initially enrolled into the study. Exclusion criteria were esophageal anatomical or neurological disorders, previously diagnosed gastroesophageal reflux disorder, major medical conditions involving cardiovascular and pulmonary systems, substance dependence, previous surgeries of the aerodigestive tract, and morbid obesity. Study participants who completed both endoscopic evaluations and the salivary pepsin concentration test were included. As a result, 58 patients were included in the final statistical analyses.

Outcome measurements

PSG parameters: Following the standardized protocol, OSA was classified as mild, moderate, and severe by $5 \leq \text{AHI} < 15$, $15 \leq \text{AHI} < 30$, and $\text{AHI} \geq 30$, respectively [23]. The snoring index and lowest oxygen saturation were extracted from the PSG report.

Reflux Symptom Index

A self-administered questionnaire of 9 items, consisting of hoarseness, throat clearing, excessive throat mucus or postnasal drip, difficulty in swallowing, postprandial coughing, breathing difficulties, annoying cough, lump in the throat, and heartburn, was used to evaluate the patient's reflux symptoms.

Each item is scored on a scale from 0 to 5, and the total RSI score is calculated by summing the scores of all nine items. Ranging from 0 to 45, a score ≥ 13 is suggestive of LPR [24].

Reflux finding score

A set of eight objective laryngoscopic findings, including subglottic edema, ventricular erythema, vocal fold edema, diffuse laryngeal edema, posterior commissure hypertrophy, granuloma, and thick endolaryngeal mucus, was used to evaluate tissue damages of LPR. Each finding is assigned a numerical score based on its severity, and the total reflux finding score (RFS) is calculated by summing the scores of all observed findings. An RFS score >7 indicated 95% probability of LPR and was considered RFS positive [10]. Laryngoscopic image of each patient was evaluated and scored by two experienced laryngologists with more than 10 years of experience assessing LPR endoscopically. Moreover, the evaluations of RFS were carried out without acknowledging the RSI score of the examined patient and other clinical information. An interrater reliability test was performed to ensure the consistency of RFS scores.

Salivary pepsin test (pep-test)

All enrolled patients were instructed to attend the otolaryngologic outpatient clinic at 8 am for pep-test. Salivette (Sarstedt, UK) was used to collect saliva samples. This device had a detachable tube with a sponge inside, which was placed in the mouth of the patient and chewed gently for about 45 s. Once the sponge was saturated with saliva, it was placed back into the salivette device. The salivary sample was retrieved after centrifugation at 1000 rpm for 2 min and then stored in aliquots at -80°C .

To determine the concentration of pepsin in the saliva samples, the enzyme-linked immunosorbent assay (ELISA) was performed. The Human PGA3/Pepsin A-3 ELISA Kit (RK04193, ABclonal) protocol was followed, which required loading 100 μL of standards and test samples per well, followed by serial preparations of adding 50 μL biotin conjugate antigen and 100 μL streptavidin-HRP working solution. The optical density under 450 nm was detected using the 800 TS Absorbance Reader (BioTek, USA), and quantitative analysis was achieved via a four-parameter logistic curve to calculate pepsin concentration [Figure 1]. We

designed Figure 2 to simplify and illustrate the critical steps in our study: saliva acquisition, sample preparation, ELISA test, and quantitative evaluations of salivary pepsin concentration.

Statistical analysis

Based on the findings of RSI and RFS, demographics, polysomnographic parameters, and the salivary pepsin concentration were compared between LPR-positive and LPR-negative groups. Furthermore, LPR outcome measurements, including RSI, RFS, and the pepsin concentration, were compared among different severities of OSA as per the classification by AHI of 30 per h. Student's *t*-test, Chi-squared test, and Mann-Whitney test were employed to analyze the differences between groups accordingly. In addition, the receiver operating characteristic (ROC) method was applied across various threshold settings of the salivary pepsin concentration based on the RFS-driven diagnosis of LPR. By analyzing the curve of the ROC, the performance across the spectrum of possible thresholds was presented in terms of the true positive rate (sensitivity), the false positive rate (1-specificity), and the area under the ROC curve (AUC). All statistical analyses were performed using IBM SPSS version 23 (SPSS Inc., Chicago, IL, USA), and $P < 0.05$ was considered statistically significant.

ChatGPT (GPT-4, Open AI's large-scale language-generation model) was used to generate Figures 2 and 3. The authors meticulously edited the figures to ensure the quality and take full responsibility for the content of this publication.

RESULTS

Salivary pepsin test was performed quickly and smoothly in all subjects with no discomfort or side effects. Relatively, five subjects emerged sneezing, nasal itching, or lumping throat during assessment of RFS. The interrater reliability test of RFS showed high consistency ($r = 0.89$, $P < 0.0001$) between two laryngologists in scoring the endoscopic findings reflecting LPR. Based on the RFS findings, the prevalence of LPR was up to 86% in OSA patients. Regarding the demographics of the study population, male predominance was observed in both LPR-positive and LPR-negative groups. The LPR-positive group had a higher median age, body mass index (BMI), snoring index, and ESS, whereas the LPR-negative group had

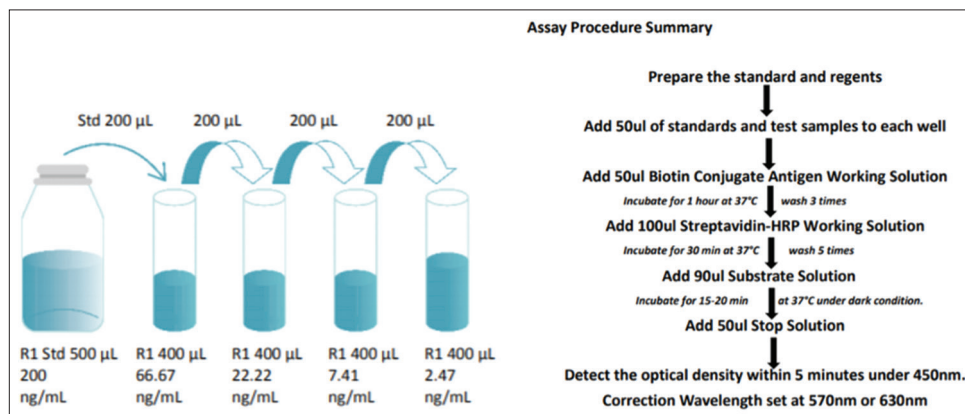


Figure 1: The Human PGA3/Pepsin A-3 ELISA Kit (RK04193, ABclonal) protocol. (From www.abclonal.com)

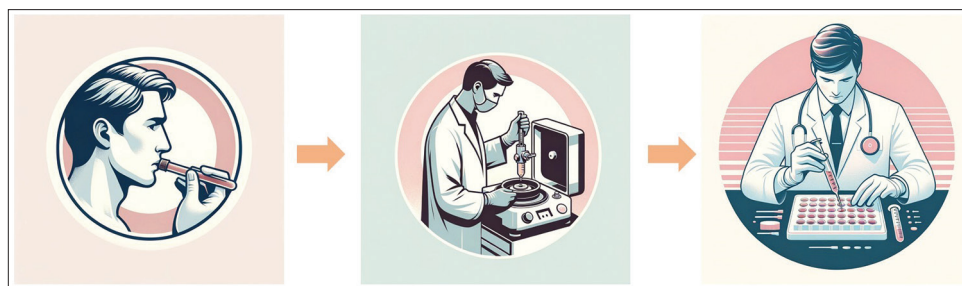


Figure 2: Simplified illustration of critical steps in the salivary pepsin test: saliva acquisition, sample preparation, ELISA test, and quantitative evaluations of salivary pepsin concentration

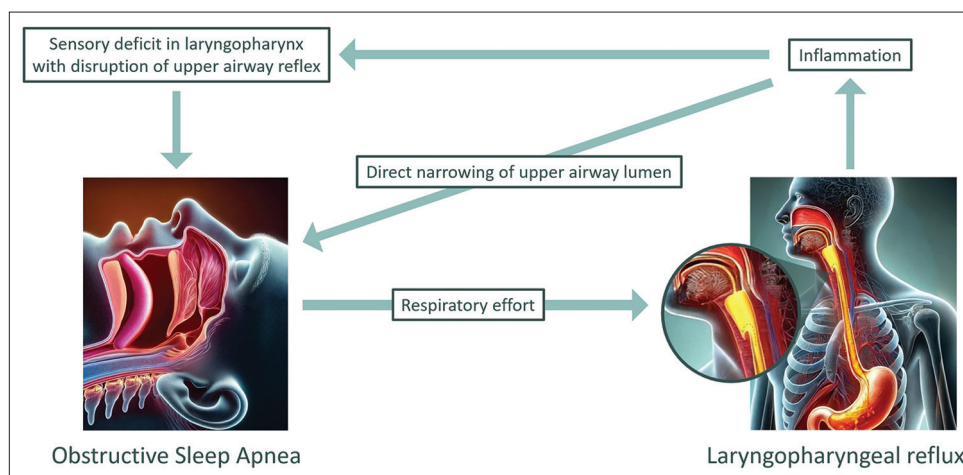


Figure 3: The mutual contributing mechanism between obstructive sleep apnea and laryngopharyngeal reflux

a marginally higher AHI. There was no significant difference between the LPR-positive and LPR-negative groups in terms of the demographics, apnea/hypopnea index, and severity of OSA as per the classification by RFS [Table 1]. The median salivary pepsin concentration in RFS-positive patients was higher than RFS-negative patients (14.9 ng/mL vs. 7.23 ng/mL), despite there was no statistical significance. Furthermore, the interaction of OSA and LRP on the salivary pepsin concentration was not significant [Supplementary Table 1]. Furthermore, the salivary pepsin concentration did not show a significant association with LRP using multivariate logistic regression after adjusting age, sex, BMI, AHI, or severity of OSA [Supplementary Table 2]. However, the threshold of salivary pepsin concentration at 2.3 ng/mL yielded a sensitivity of 93% (AUC = 0.54) in the diagnosis of LPR, whereas a specificity of 80% on the threshold of salivary pepsin concentration at 28.7 ng/mL [Figure 4].

DISCUSSION

In our study, we found that the concentration of salivary pepsin was correlated with RFS but did not show any association with the severity of OSA. This finding was also reported in other studies [25-28]. A prospective comparative study showed a significant correlation between the RFS and RSI score and the level of salivary pepsin, which was higher in LPR patients [29]. Another study measuring salivary and hypopharyngeal pepsin concentration indicated that there was an association between levels of pepsin and

RFS and RSI scores [30]. Therefore, pepsin testing can complement traditional diagnostic methods for LPR, providing additional objective evidence of refluxate reaching the upper airway and contributing to laryngopharyngeal mucosal injury [16,19-21,28-30]. In our study, the median salivary pepsin concentration in RFS-positive patients was higher than RFS-negative patients (14.9 ng/mL vs. 7.23 ng/mL), which was cohesive with previous studies.

The cutoff value of salivary pepsin could vary depending on the specific study or research findings. Barona-Lleo *et al.* used a PEP-test (RD Biomed, UK) as a diagnostic tool in LPR, which detected the salivary pepsin at a concentration ≥ 16 ng/mL as positive [19]. On the other hand, Weitzendorfer *et al.* proposed an optimal cutoff value of salivary pepsin at 216 ng/mL, which indicated a specificity of 86.2% and sensitivity of 41.5% in diagnosing LPR [31]. Based on the findings of the ROC curve in our study, we found possible thresholds of the salivary pepsin concentration at 2.3 and 28.7 ng/mL in correspondence to a sensitivity of 93.3% and a specificity of 80% in diagnosing LPR. Put simply, at the threshold of 2.3 ng/mL, the salivary pepsin showed substantial ability to detect true cases of LPR, whereas at the threshold of 28.7 ng/mL, it also correctly identified individuals without reflux. The choice of a threshold on the ROC curve is pivotal, as it balances the sensitivity and specificity of the test. However, it is general priority to consider the sensitivity threshold first in discrepant sensitivity and specificity to serve the need of timely diagnosis and treatment. For symptom-suspicious patients, we propose

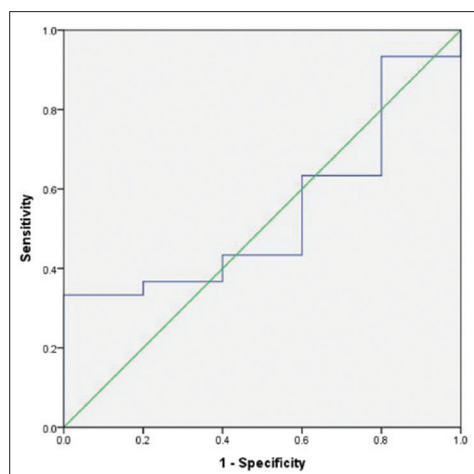


Figure 4: The receiver operating characteristic curve in sensitivity and specificity of salivary pepsin concentration

the diagnosis of LPR could be performed by either endoscopic examination or salivary pepsin test. Besides, the salivary pepsin test has the advantage of noninvasiveness and that is important in regular follow-up to adjust the treatment plan.

Since OSA and LPR share common risk factors such as smoking, alcohol consumption, obesity, and sex, the interplay between both involves a complex relationship where the effects of one condition can contribute to the development or exacerbation of the other. Our study revealed the prevalence of LPR in OSA patients with a high percentage, which went hand in hand with previous reports of frequent coexistence of LPR and OSA with a percentage ranging 20–67 [32]. However, LPR outcome measurements such as RFS, RSI, and pepsin concentration were not coherent with the severity of OSA in our study. Ing *et al.* presented that OSA patients had significantly more reflux events during sleep, and 53.4% of which were temporally related to apneas or hypopneas [33]. A meta-analysis, instead, revealed no statistical difference in AHI between LPR-positive and LPR-negative patients, which was compatible with the findings in our study [8]. In terms of the treatment effect, Eryilmaz *et al.* showed continuous positive airway pressure treatment in OSA patients improved reflux symptoms significantly, but meanwhile LPR treatments did not improve polysomnographic parameters [6]. Another study showed that multiple-level surgical intervention for OSA significantly reduced the RSI score of patients [34]. That said, OSA and LPR do not necessarily correlate in terms of clinical presentations of disease severity or treatment effects.

The contributing mechanism between OSA and LPR is complex [Figure 3]. LPR-related laryngopharyngeal mucosal inflammation and hypertrophic tissue contribute to the narrowing of the upper airway and sensory dysfunction as well as possible laryngospasm hinders reflexes maintaining the patency of the upper airway at sleep [7]. Conversely, anatomical barriers can be altered during sleep to potentiate reflux, and mechanical and physiologic changes in OSA may contribute to LPR symptoms. For example, the basal upper esophageal sphincter tone (UES) was reduced from 60 mmHg at stage wake to 4 mmHg at stage slow wave [35].

Table 1: Demographics and severity of obstructive sleep apnea by reflux finding score

	RFS negative	RFS positive	P
Number of patients	8	50	
Demographic data			
Sex			
Male	6	49	0.05
Female	2	1	
Age	37 (27–47)	39 (31–45)	0.60
BMI	23.8 (22.4–26.1)	26.3 (24.1–28.7)	0.08
OSA measurement			
OSA severity			
Mild	2	10	0.77
Moderate	1	12	
Severe	5	28	
AHI	34.5 (13.2–47.6)	32.9 (17.1–50.1)	0.77
Snoring index	105.8 (62.2–142.8)	215.5 (77.1–432.2)	0.03*
Lowest saturation	82.5 (78.3–83.0)	76.0 (66.5–81.5)	0.10
ESS	10.5 (7–16)	11 (8–16)	0.61
VAS	8 (7–10)	8 (7–10)	0.94
LPR measurement			
Pepsin concentration (ng/mL)	7.23 (4.9–47.5)	14.9 (4.3–29.7)	0.80

* $P=0.05$ as threshold of statistical significance. Statistics were reported by the median and its IQR. RFS-negative: RFS score ≤ 7 , RFS positive: RFS score >7 . RFS: Reflux finding score, OSA: Obstructive sleep apnea, IQR: Interquartile values, AHI: Apnea-hypopnea index, ESS: Epworth Sleepiness Scale, VAS: Visual analog score

Furthermore, primary and secondary esophageal peristalsis and saliva production were reduced. At the same time, inspiratory effort was notably increased during apnea and hypopnea episodes in OSA, which led to higher negative intrathoracic pressure [36,37]. Eventually, the lower esophageal sphincter was passively pulled open. As a result, OSA, along with sleep-related alterations, could increase the propensity of refluxate breaching the UES and intensified tissue damage by prolonged acidic exposure in the laryngopharynx. The mechanisms underlying the link between OSA and LPR are multifactorial and bidirectional. The relationship between OSA and LPR is interconnected but not necessarily a causality, which leads to the new comorbidity entitled combined laryngopharyngeal reflux and OSA (CLOSA).

Limitations

The weaknesses of this study included: (1) Patient selection bias: Tertiary medical center as in our study often attracted OSA patients with more severe severity that might contribute to imbalanced datasets with more endoscopically LPR-positive patients and might not represent the general OSA patient population. (2) Lack of the reference level of salivary pepsin concentration: Without knowing the reference level of salivary pepsin, it was struggling to accurately assess the significance of the salivary pepsin concentration observed in the study population, and posed challenges in distinguishing between normal variations and potential abnormalities in pepsin levels. (3) Lack of baseline information regarding antireflux treatment: Not knowing the baseline antireflux management status might interfere the level of salivary pepsin concentration and hinder the ability to draw meaningful conclusions about

the relationship between LPR and OSA. (4) LPR classification bias: In our study, the classification of LPR was made based on the findings of RFS, which was subjective rather than objective diagnostic methods such as impedance and pH-monitoring studies. At the same time, given that the RFS focused on glottic/subglottic, posterior commissure, and locoregional pharyngeal area findings, it might overlook inflammatory signs at oro-pharyngeal or other hypo-pharyngeal structures. Meanwhile, the RFS might not reflect the actual severity of LPR due to its analogic assessment and potential poor intrarater reliability. (5) Lack of longitudinal data: Our study was conducted in a cross-sectional fashion, which could not provide information about changes or patterns of salivary pepsin concentration over time in OSA patients regarding treatments of LPR. (6) Another limitation of this study is that the sample size did not meet the requirements of the statistical power calculation. As a result, the study may have been underpowered to detect small to moderate effects, which could affect the generalizability and robustness of the findings.

CONCLUSION

LPR is highly prevalent in OSA patients. For symptom-suspicious patients, salivary pepsin test is feasible in the diagnosis of LPR and could be an alternative to endoscopic findings with noninvasiveness and facilitation in long-term follow-up. The relationship between OSA and LPR is more likely to be an overlapping syndrome, and the new term of combined CLOSA is proposed. For CLOSA patients, it is worthwhile to take an integrated approach by thoroughly evaluating reflux symptoms and intervening reflux with behavior modifications or pharmaceutical treatments such as proton pump inhibitors and H2-receptor antagonists. Future research, including but not limited to a more robust longitudinal study design, will be needed to understand the interplay between OSA and LPR.

Data availability statement

The datasets generated during and/or analyzed during the current study are not publicly available due to the institute's regulation to protect individual patient's privacy but are available from the corresponding author on reasonable request.

Financial support and sponsorship

The authors would like to thank Chang Gung Memorial Foundation for supporting this study (CMRPG1K0181 and CMRPG1K0182).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Koufman J, Sataloff RT, Toohill R. Laryngopharyngeal reflux: Consensus conference report. *J Voice* 1996;10:215-6.
- Hammer HF. Reflux-associated laryngitis and laryngopharyngeal reflux: A gastroenterologist's point of view. *Dig Dis* 2009;27:14-7.
- El-Serag HB. Time trends of gastroesophageal reflux disease: A systematic review. *Clin Gastroenterol Hepatol* 2007;5:17-26.
- Lechien JR, Mouawad F, Bobin F, Bartaire E, Crevier-Buchman L, Saussez S. Review of management of laryngopharyngeal reflux disease. *Eur Ann Otorhinolaryngol Head Neck Dis* 2021;138:257-67.
- El-Serag HB, Graham DY, Satia JA, Rabeneck L. Obesity is an independent risk factor for GERD symptoms and erosive esophagitis. *Am J Gastroenterol* 2005;100:1243-50.
- Eryilmaz A, Erişen L, Demir UL, Kasapoglu F, Ozmen OA, Ursavas A, et al. Management of patients with coexisting obstructive sleep apnea and laryngopharyngeal reflux disease. *Eur Arch Otorhinolaryngol* 2012;269:2575-80.
- Lim KG, Morgenthaler TI, Katzka DA. Sleep and nocturnal gastroesophageal reflux: An update. *Chest* 2018;154:963-71.
- Magliulo G, Iannella G, Polimeni A, De Vincentiis M, Meccariello G, Gulotta G, et al. Laryngopharyngeal reflux in obstructive sleep apnoea patients: Literature review and meta-analysis. *Am J Otolaryngol* 2018;39:776-80.
- Ford CN. Evaluation and management of laryngopharyngeal reflux. *JAMA* 2005;294:1534-40.
- Belafsky PC, Postma GN, Koufman JA. The validity and reliability of the reflux finding score (RFS). *Laryngoscope* 2001;111:1313-7.
- Kawamura O, Aslam M, Rittmann T, Hofmann C, Shaker R. Physical and pH properties of gastroesophagopharyngeal refluxate: A 24-hour simultaneous ambulatory impedance and pH monitoring study. *Am J Gastroenterol* 2004;99:1000-10.
- Koufman JA, Aviv JE, Casiano RR, Shaw GY. Laryngopharyngeal reflux: Position statement of the committee on speech, voice, and swallowing disorders of the American Academy of Otolaryngology-Head and Neck Surgery. *Otolaryngol Head Neck Surg* 2002;127:32-5.
- Harrell SP, Koopman J, Woosley S, Wo JM. Exclusion of pH artifacts is essential for hypopharyngeal pH monitoring. *Laryngoscope* 2007;117:470-4.
- Muderris T, Gokcan MK, Yorulmaz I. The clinical value of pharyngeal pH monitoring using a double-probe, triple-sensor catheter in patients with laryngopharyngeal reflux. *Arch Otolaryngol Head Neck Surg* 2009;135:163-7.
- Srinivasan R, Vela MF, Katz PO, Tutuian R, Castell JA, Castell DO. Esophageal function testing using multichannel intraluminal impedance. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G457-62.
- Johnston N, Knight J, Dettmar PW, Lively MO, Koufman J. Pepsin and carbonic anhydrase isoenzyme III as diagnostic markers for laryngopharyngeal reflux disease. *Laryngoscope* 2004;114:2129-34.
- Johnston N, Wells CW, Blumin JH, Toohill RJ, Merati AL. Receptor-mediated uptake of pepsin by laryngeal epithelial cells. *Ann Otol Rhinol Laryngol* 2007;116:934-8.
- Johnston N, Bulmer D, Gill GA, Panetti M, Ross PE, Pearson JP, et al. Cell biology of laryngeal epithelial defenses in health and disease: Further studies. *Ann Otol Rhinol Laryngol* 2003;112:481-91.
- Barona-Lleo L, Barona-De Guzman R, Krstulovic C. The diagnostic usefulness of the salivary pepsin test in symptomatic laryngopharyngeal reflux. *J Voice* 2019;33:923-8.
- Knight J, Lively MO, Johnston N, Dettmar PW, Koufman JA. Sensitive pepsin immunoassay for detection of laryngopharyngeal reflux. *Laryngoscope* 2005;115:1473-8.
- Samuels TL, Johnston N. Pepsin as a marker of extraesophageal reflux. *Ann Otol Rhinol Laryngol* 2010;119:203-8.
- Wood JM, Hussey DJ, Woods CM, Watson DI, Carney AS. Biomarkers and laryngopharyngeal reflux. *J Laryngol Otol* 2011;125:1218-24.
- Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, et al. Rules for scoring respiratory events in sleep: Update of the 2007 AASM manual for the scoring of sleep and associated events. Deliberations of the sleep apnea definitions task force of the American Academy of Sleep Medicine. *J Clin Sleep Med* 2012;8:597-619.
- Belafsky PC, Postma GN, Koufman JA. Validity and reliability of the reflux symptom index (RSI). *J Voice* 2002;16:274-7.
- Lee JS, Heo SJ, Kim JS, Ahn D, Sohn JH, Kim H. Relationship between the severity of laryngopharyngeal reflux and sleep apnea:

- Using drug-induced sleep endoscopy (DISE). *Eur Arch Otorhinolaryngol* 2018;275:219-24.
26. Caparroz FA, Campanholo MA, Regina CG, Park SW, Haddad L, Gregório LC, et al. Clinical and polysomnographic predictors of laryngopharyngeal reflux in obstructive sleep apnea syndrome. *Braz J Otorhinolaryngol* 2019;85:408-15.
 27. Koufman JA. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): A clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. *Laryngoscope* 1991;101:1-78.
 28. Iannella G, Vicini C, Polimeni A, Greco A, Gobbi R, Montevecchi F, et al. Laryngopharyngeal reflux diagnosis in obstructive sleep apnea patients using the pepsin salivary test. *Int J Environ Res Public Health* 2019;16:2056.
 29. Sereg-Bahar M, Jerin A, Jansa R, Stabuc B, Hocevar-Boltezar I. Pepsin and bile acids in saliva in patients with laryngopharyngeal reflux – A prospective comparative study. *Clin Otolaryngol* 2015;40:234-9.
 30. Wang L, Liu X, Liu YL, Zeng FF, Wu T, Yang CL, et al. Correlation of pepsin-measured laryngopharyngeal reflux disease with symptoms and signs. *Otolaryngol Head Neck Surg* 2010;143:765-71.
 31. Weitzendorfer M, Antoniou SA, Schredl P, Witzel K, Weitzendorfer IC, Majerus A, et al. Pepsin and oropharyngeal pH monitoring to diagnose patients with laryngopharyngeal reflux. *Laryngoscope* 2020;130:1780-6.
 32. Zanation AM, Senior BA. The relationship between extraesophageal reflux (EER) and obstructive sleep apnea (OSA). *Sleep Med Rev* 2005;9:453-8.
 33. Ing AJ, Ngu MC, Breslin AB. Obstructive sleep apnea and gastroesophageal reflux. *Am J Med* 2000;108(Suppl 4a):120S-5S.
 34. Kim SJ, Kim HY, Jeong JI, Hong SD, Chung SK, Dhong HJ. Changes in the reflux symptom index after multilevel surgery for obstructive sleep apnea. *Clin Exp Otorhinolaryngol* 2017;10:259-64.
 35. Bajaj JS, Bajaj S, Dua KS, Jaradeh S, Rittmann T, Hofmann C, et al. Influence of sleep stages on esophago-upper esophageal sphincter contractile reflex and secondary esophageal peristalsis. *Gastroenterology* 2006;130:17-25.
 36. Freidin N, Fisher MJ, Taylor W, Boyd D, Surratt P, McCallum RW, et al. Sleep and nocturnal acid reflux in normal subjects and patients with reflux oesophagitis. *Gut* 1991;32:1275-9.
 37. Shepherd K, Hillman D, Holloway R, Eastwood P. Mechanisms of nocturnal gastroesophageal reflux events in obstructive sleep apnea. *Sleep Breath* 2011;15:561-70.

SUPPLEMENTARY MATERIAL

Supplementary Table 1: The interaction of obstructive sleep apnea and laryngopharyngeal reflux on the salivary pepsin concentration

Pepsin concentration (ng/mL)	RFS-negative	RFS-positive	<i>P</i>
OSA severity			
Mild	26.3	8.2 (5.1–27.5)	1.00
Moderate	0.3	7.3 (5.1–31.1)	1.00
Severe	7.0 (4.9–14.3)	10.3 (4.6–62.1)	0.84
OSA severity			
Mild	26.3	8.2 (5.1–27.5)	1.00
Moderate-severe	7.0 (0.3–14.3)	10.3 (4.6–62.1)	0.69

P=0.05 as threshold of statistical significance. Statistics were reported by the median and range. RFS negative: RFS score ≤7, RFS positive: RFS score >7. RFS: Reflux finding score, OSA: Obstructive sleep apnea

Supplementary Table 2: The effects of salivary pepsin concentration on laryngopharyngeal reflux using multivariate logistic regression

Clinical factor	Coefficient	<i>P</i>
Age	0.079	0.78
Sex	12.325	0.00
BMI	0.696	0.40
AHI	0.003	0.95
Pepsin concentration	0.759	0.38

P=0.05 as threshold of statistical significance

Clinical factor	Coefficient	<i>P</i>
Age	0.079	0.78
Sex	12.325	0.00
BMI	0.696	0.40
Grade of OSA	0.163	0.69
Pepsin concentration	0.759	0.38

P=0.05 as threshold of statistical significance. Grade of OSA by AHI: Mild, moderate, and severe. OSA: Obstructive sleep apnea, BMI: Body mass index, AHI: Apnea–hypopnea index