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ORIGINAL RESEARCH

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Multitime point pepsin testing can double the rate of the diagnosis of laryngopharyngeal reflux

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Abstract

Objective: To study the value of multitime point salivary pepsin testing (MTPSPT) for the diagnosis of laryngopharyngeal reflux (LPR).

Study Design: Prospective noncontrolled.

Methods: For patients who met the enrollment criteria, the reflux symptom index (RSI) and reflux finding score (RFS) were calculated and salivary pepsin testing was performed. The pepsin test was performed every hour from 7:00 a.m. to 6:00 p.m. by collecting fresh saliva samples. A single positive test result was needed for the diagnosis of LPR. The consistency in the diagnosis of LPR between the two methods was compared with the weighted Cohen's kappa statistic.

Results: A total of 204 patients were included. The kappa value between the two methods was 0.566 ($p = .00$). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MTPSPT were 76.43%, 85.94%, 92.24%, and 62.5%, respectively. We also compared a single pepsin measure at 7 a.m. with the screening results based on the RSI and RFS, and found a much lower kappa agreement value (0.223, $p = .00$). The sensitivity, specificity, PPV, NPV, and false-negative rate of pepsin testing at 7 a.m. (fasting) were 37.86%, 92.18%, 91.38%, 40.41%, and 58.57%, respectively.

Conclusion: The use of the result of a single salivary pepsin test in the morning yields a relatively higher rate of missed diagnosis of LPR, and multitime point testing through a day increased the accuracy and sensitivity of detection of LPR twofold compared to a single morning fasting sample.

Level of Evidence: 3

KEYWORDS
consistency, laryngopharyngeal reflux, pepsin, saliva

1 | INTRODUCTION

Laryngopharyngeal reflux (LPR) is an inflammatory condition of the upper aerodigestive tract tissues related to direct and indirect effect of gastroduodenal content reflux, which induces morphological changes in the upper aerodigestive tract.[1] LPR is not a disease until it becomes symptomatic. The major symptoms of LPR are hoarseness of voice, globus sensation, throat irritation, dysphagia, frequent throat clearing, dryness of throat, chronic cough, and voice fatigue.[2] However, many people with LPR have other reasons for their symptoms such as glottic insufficiency,[3] allergy,[4] and so on.

In 1990, Koufman first reported that LPR had an incidence of 4%–10% in otorhinolaryngology patients,[5] and then he reported that at least 50% of patients in the Department of Pharyngology, Laryngology & Phonosurgery had LPR symptoms.[6] Chinese researchers found that the incidence of LPR among otolaryngology and head and neck surgery outpatients was 10.15% in China.[7] However, the true incidence of LPR in the general population is still unknown.

Currently, there is still no recognized gold standard for the diagnosis of LPR. The current diagnostic methods mainly include clinical evaluation (reflux symptom index [RSI] and reflux finding score [RFS]), 24-h multichannel intraluminal impedance and pH monitoring (24 h MII-pH), empirical treatment, and salivary pepsin detection. The RSI and RFS are noninvasive and convenient, and they are currently the most commonly used screening method. The 24 h MII-pH method is regarded by many researchers as the gold standard for diagnosing LPR,[1,8] but because it is invasive, expensive, and not accepted by some patients, it is only used in a few hospitals.[9,10] Empirical therapy commonly involves the administration of proton pump inhibitors (PPIs) for 3–6 months and the assessment of the treatment response.[11] Salivary pepsin detection is a novel, convenient, and patient-accepted diagnostic method[12] that has been applied in some clinical studies. At present, the main methods for the detection of pepsin are enzyme-linked immunosorbent assay (ELISA) and Peptest (RD Biomed, Hull, East Yorkshire, United Kingdom). ELISA specifically detects only Pepsin A (PGA3), and its detection threshold is 1.56 ng/ml. However, ELISA must be performed in a specific laboratory, which is a major disadvantage in clinical practice.[13] Peptest is a simple, convenient, and patient-accepted method,[14] with a threshold of 16 ng/ml. However, some researchers have found that the concentration of salivary pepsin in healthy Chinese people is 24.22 ± 6.94 ng/ml.[15] Therefore, the application of Peptest in the Chinese population could lead to misdiagnoses and missed diagnoses. Some researchers have suggested that only the morning (fasting) salivary pepsin level should be tested[16,17]; however, the use of this single value may lead to missed diagnoses and misdiagnoses in some patients. We developed an inexpensive pepsin test strip[18] that makes multitime point pepsin detection possible. The aim of this study is to investigate the value of multitime point salivary pepsin testing (MTPSPT) for the diagnosis of LPR and find the possibly suitable time for collecting saliva samples and testing.

2 | MATERIALS AND METHODS

This study was approved by the Ethics Review Committee of the Sixth Medical Center of the PLA General Hospital and The Eighth Medical Center of Chinese PLA General Hospital, and the ethics review batch numbers are HZQX-PJ-2019-7 and 2019-05-03-01. All enrolled patients signed an informed consent form, and the entire trial process was supervised by a special regulatory agency.

2.1 | Patient recruitment

The enrolled patients were hospitalized in the Departments of Otorhinolaryngology and Head and Neck Surgery at the Sixth Medical Center and Eighth Medical Center of the PLA General Hospital. The inclusion criteria were as follows: (1) age 18–65 years old and lack of pregnancy and (2) the need to undergo laryngoscopy due to throat discomfort, foreign body sensation, hoarseness, and other throat symptoms. The exclusion criteria were as follows: (1) the use of proton pump inhibitors or gastrointestinal motility drugs within 1 week; (2) participation in other clinical trials within 3 months; (3) the need for long-term oral corticosteroid drugs or diagnoses of other diseases and the need for hormone therapy after diagnosis; (4) diagnoses of Zollinger-Ellison syndrome, achalasia, esophageal spasm, esophageal stricture, and other esophageal lesions or a history of major chronic diseases and neurological diseases; and (5) any conditions assessed by the doctor that can cause bleeding in the mouth, including blood diseases, oral trauma or ulcers, and mental illness cannot cooperate with the collection of samples.

2.2 | Diagnostic methods

2.2.1 | Clinical screening

The RSI[19] and the RFS,[20] proposed by Belafsky et al., were used to quantitatively score the symptoms and the degree of severity of the laryngeal lesions related to reflux, respectively. The doctor instructed the patient to complete the RSI, and then the patient was examined with fiberoptic laryngoscopy performed by experienced laryngologists. The results of the laryngoscopy were blindly evaluated by three experienced doctors, in which a RSI score > 13 or a RFS > 7 was used to suspect LPR.

2.2.2 | Salivary pepsin testing

We used the salivary pepsin test strip produced by Jiangxi Nord Medical Equipment Co. Ltd. This test strip uses the colloidal gold lateral chromatography method. In a previous study,[15] we prepared five types of test strips with different thresholds of 30, 45, 60, 75, and 90 ng/ml and collected saliva samples from 50 normal people for testing. The results showed that 45 ng/ml was the most suitable

threshold for detection. When the concentration of pepsin in the saliva is higher than 45 ng/ml, a red band (T) will be formed in the detection area, indicating that LPR may be occurring. Fresh saliva or sputum samples were collected every hour from 7 a.m. (fasting) to 6 p.m., for a total of 12 times. Saliva (0.1 ml) was collected with an eyedropper each time and added to the sample area of the test card. The results were observed 8 min later. If the test result only showed the quality control line (C), the result was negative, indicating the patient did not have LPR; if the test result showed two red bands, namely, the quality control line (C) and the test line (T), the result was positive, indicating that the patient had LPR (see Figure 1). If the quality control line did not appear in the test result, the result was invalid and needed to be retested. A positive result on any of the 12 tests led to the diagnosis of LPR. The hourly results were recorded by two trained doctors. The entire test process was carried out at room temperature, each sampling and the amount used for testing was constant, and we did our best to maintain the consistency of other possible influencing factors.

2.3 | Statistical methods

Statistical Package for the Social Sciences for Windows (SPSS version 26.0; IBM, Armonk, NY) was used for the statistical analysis. The consistency between the results of the MTPSPT and the RSI and RFS was compared with the weighted Cohen's kappa statistic. The value of diagnostic test was performed with Likelihood Ratio test. A level of significance of $p < .05$ was used.

3 | RESULTS

Between July 2019 and July 2020, a total of 204 patients were recruited, including 165 males and 39 females, and the average age was 38.75 ± 12.60 years. A total of 140 cases of LPR were suspected based on the RSI and RFS, whereas 64 patients were negative; 116 cases of LPR were diagnosed based on MTPSPT, and 88 patients were negative (see Table 1). The number of tests and the positive rates collected at each time point seen Table 2. Using the RSI and RFS scores as the referent, we calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and false-negative rate.

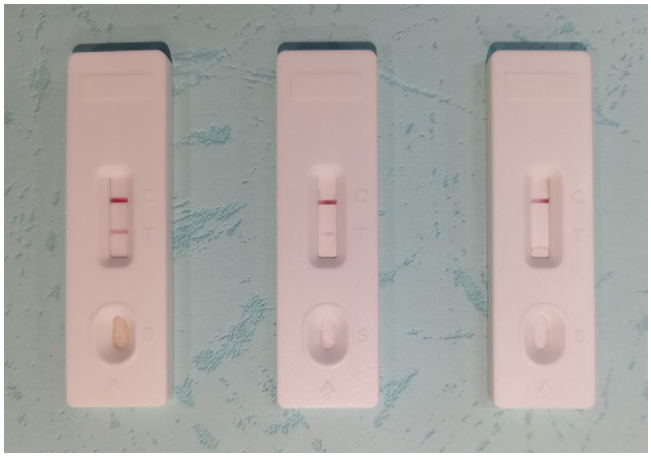

FIGURE 1 The example of the results of salivary pepsin testing. Left: The control line is visible and the test line is more apparent than middle, and the result was strong positive. Middle: the control line and the test line are visible, and the result was weak positive. Right: Only the control line is visible, and the result was negative

3.1 | Consistency of the RSI and RFS with MTPSPT

The weighted kappa value between the RSI and RFS and MTPSPT was 0.566, the likelihood ratio was 6.059 ($p = .014$) and the total rate of consistency was 79.41% (162/204). The sensitivity, specificity, PPV, NPV, and false-negative rate of MTPSPT for the diagnosis of LPR were 76.43%, 85.94%, 92.24%, 62.5%, and 17.10%, respectively.

3.2 | Consistency of the RSI and RFS and the pepsin test results at 7 a.m. (fasting)

It has been reported that the pepsin test result after waking up in the morning (fasting) could be the best for use in the diagnosis of LPR. Therefore, we also compared the diagnosis based on the result of a single pepsin test at 7:00 a.m. (fasting) with the screening results based on the RSI and RFS. According to the weighted Cohen's kappa statistic and found that the consistency between them was very poor (Kappa value = 0.223, $p = .00$). The sensitivity, specificity, rate of consistency, PPV, and NPV of the result of the pepsin test were 37.86% (53/140), 92.18% (59/64), 54.90% (112/204), 91.38% (53/58), and 40.41% (59/146), respectively, but the false-negative rate of the single pepsin test at 7:00 a.m. was 58.57%. The weighted kappa values of the two methods from 8 a.m. to 6 p.m. were 0.136 ($p = .00$), 0.084 ($p = .02$), 0.100 ($p = .00$), 0.060 ($p = .01$), 0.115 ($p = .00$), 0.002 ($p = .96$), 0.157 ($p = .00$), 0.074 ($p = .03$), 0.090 ($p = .00$), 0.075 ($p = .00$), and 0.094 ($p = .00$). We found that the consistency between the results of the other time points and the RSI and RFS was poor. However, we also calculated the sensitivity, specificity, PPV, NPV, and false-negative rate based on the detection of pepsin at each time point (see Figure 2),

TABLE 1 RSI and RFS versus MTPSPT

		MTPSPT		
Item	Category	Positive (*n*)	Negative (*n*)	Total (*n*)
RSI/RFS	Positive (*n*)	107	33	140
	Negative (*n*)	9	55	64
Total (*n*)		116	88	204

Abbreviations: MTPSPT, Multitime Point Salivary Pepsin Testing; RSI and RFS, Reflux Symptom Index and Reflux Finding Score.

TABLE 2 The number of tests collected at each time point

Time	7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12 a.m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.
+(*n*)	58	37	30	21	19	30	29	35	28	19	16	29
−(*n*)	146	167	174	183	185	174	175	169	176	185	188	175
Positive rate	28.43%	18.14%	14.71%	10.29%	9.31%	14.71%	14.22%	17.16%	13.73%	9.31%	7.84%	14.22%

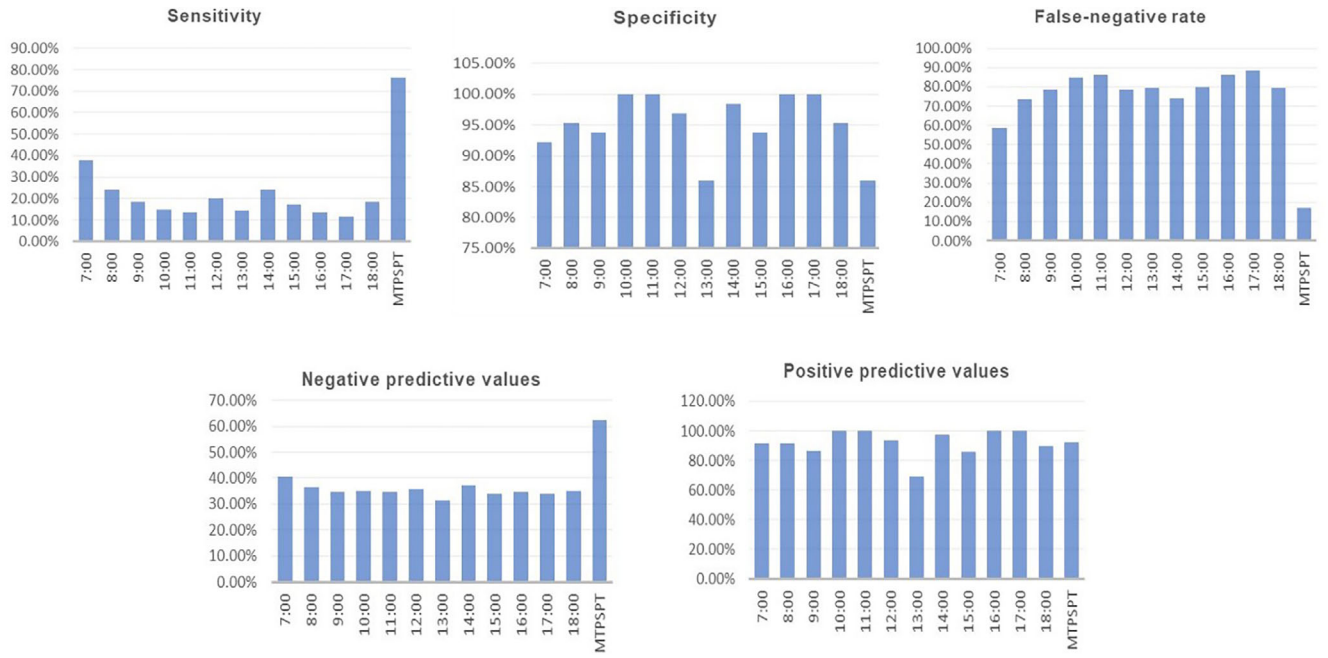

FIGURE 2 The sensitivity, specificity, PPV, NPV and false-negative rate based on the detection of pepsin at each time point. Abbreviations: MTPSPT, multi-time point salivary pepsin testing

the results showed that the highest sensitivity and NPV were associated with MTPSPT; the highest specificity and PPV were associated with the tests performed at 10 a.m., 11 a.m., 4 p.m., and 5 p.m.; and the lowest false-negative rate was associated with MTPSPT.

4 | DISCUSSION

In recent years, the value of salivary pepsin testing for the diagnosis of LPR has been extensively studied, and an increasing number of studies have shown that salivary pepsin can be used as a specific diagnostic marker for LPR.[8,21-23] Our research adopted a new method of detecting pepsin in saliva,[18] and we compared the consistency between the results of MTPSPT with those obtained with the RSI and RFS. The results showed that there was a moderate degree of consistency between the two methods (kappa value = 0.566, $p = .00$). In addition, we found that the use of multiple pepsin samples through a day increased the accuracy and sensitivity of detection of LPR twofold compared to a single morning fasting sample.

At present, there is no consensus on the best time at which to detect salivary pepsin levels. Klimara et al.[17] and Na et al.[16] reported that the detection of pepsin before eating and brushing one's teeth in the morning could be used as the best option for the diagnosis of LPR. However, Kim et al.[24] and Wang et al.[25] believed that the detection of pepsin when reflux symptoms occurred was the most sensitive diagnostic method. Lu et al.[26] reported that there was no significant difference among the saliva samples collected at different times from patients with OSHAS. Our results show that among the single tests of salivary pepsin during the day, the sample taken at 7:00 a.m. had the highest positivity rate (28.43%), with a sensitivity of 37.86% and a specificity of 92.18%. However, the positivity rate of salivary pepsin level detection at multiple time points during the day was 56.86%, with a sensitivity of 76.43% and a specificity of 85.94%. This shows that the best way to improve the rate and sensitivity of the diagnosis of LPR is to test the salivary pepsin level many times during the day. The reasons for these differences may be as follows: (1) The normal value of salivary pepsin in the Chinese population was found to be 24.22 ± 6.94 ng/ml. Some studies used ELISA, which has a low threshold (1.56 ng/ml) and higher sensitivity than our method. The Peptest threshold adopted by Wang et al. was 16 ng/ml. (2) The numbers of samples were different. Our study used 2448 saliva samples, whereas the other studies had fewer than 200 samples. (3) The time

of sample collection was different. Most studies collected saliva samples in the morning, 1 h after meals, before bed and during symptomatic periods. Johnston et al.[27] reported that pepsin was found to be taken up by laryngeal epithelial cells, so the symptoms are probably not from new pepsin being added to the throat, and likely, further acidic events from diet reactivate the pepsin already there. However, many patients may not experience obvious paroxysmal symptoms. Therefore, in order to reduce missed diagnosis, we adopted MTPSPT.

Although the measurement of pepsin for the diagnosis of LPR has been accepted by many researchers, there are still different views on whether salivary pepsin levels can be used as a sensitive diagnostic method for LPR. Wang et al.[25] showed that there was poor consistency between the use of the salivary pepsin test and the RSI and RFS for the diagnosis of LPR (kappa value was 0.002). Alberto et al.[28] and Yadlapati et al.[29] reported that the salivary pepsin test alone could not be used as a diagnostic method for LPR because there was no difference in the positivity rate of salivary pepsin testing between patients with LPR symptoms and normal people. Fei et al.[30] reported a low sensitivity of salivary pepsin detection in children. The results of all these studies are contrary to our findings, and the reasons may be as follows: (1) The detection thresholds of Peptest and ELISA are lower. (2) The numbers of saliva samples were insufficient in previous studies, and the results were not representative of the entire population. (3) The timing of detection may not had been appropriate. (4) The detection of salivary pepsin can be interfered with by other components, such as blood. (5) There may have been a certain number of patients with silent reflux who were included in the group of normal people. (6) Children secrete an abundant amount of saliva and swallow more frequently, which leads to the faster clearance of pepsin.

At present, the most widely used clinical screening methods for LPR are the RSI and RFS. However, to date, there is has been no research on the consistency of the screening results obtained with the RSI and RFS with those obtained with MTPSPT for the diagnosis of LPR. In our study, the Kappa value between the two methods was 0.566, indicating moderate consistency between the two methods. The sensitivity, specificity, PPV, and NPV of MTPSPT for the diagnosis of LPR were 76.43%, 85.94%, 92.24%, and 62.5%, respectively. Jiang et al.[31] used immunohistochemical staining (IHC) to detect pepsin in the epithelium of the laryngeal mucosa, and the results showed that the sensitivity and specificity of pepsin detection were 80% and 85.7%, respectively, which are consistent with our results. For IHC, However, laryngeal mucosal tissue samples were needed, and patients had to endure some pain. This method was time consuming and expensive, so it was not conducive to routine clinical testing. Knight et al.[21] reported that the sensitivity and specificity of sputum pepsin testing were 100% and 89%, respectively. The reason for the higher sensitivity than our results may be that they used ELISA, and its detection threshold is lower. Barona et al.[14] reported that the positivity rate for salivary pepsin in patients detected by Peptest was 52.82%. The authors conducted a second test in patients whose first tests were negative, and the positivity rate increased to 73.94%. This also showed that multiple tests could improve the diagnosis rate of LPR. A meta-analysis[32] showed that the sensitivity and specificity of pepsin testing were 64% and 68%, respectively, and proposed that multiple tests could improve the sensitivity of the diagnosis. At the same time, Ocak et al.[33] reported that the sensitivity and specificity of salivary pepsin testing were 33% and 100%; their results were likely affected by the fact that they only collected saliva when the patients' reflux symptoms were the most obvious, thereby excluding many patients with no obvious symptoms. However, they also proposed that increasing the frequency of testing saliva samples during the day could improve the sensitivity. Our study confirms these hypotheses.

Interestingly, with the increase in the number of tests performed, the sensitivity and NPV for the diagnosis of LPR increased, reaching the highest values of 76.43% and 62.5%, respectively, but the specificity decreased (85.94%), and the false-negative rate decreased to a lowest value of 17.10%). This shows that MTPSPT can improve the rate and accuracy of the diagnosis of LPR. Furthermore, MPTSPT has the lowest rate of missed diagnoses. Compared with MPTSPT, the tests performed at 10 a.m., 11 a.m., 4 p.m., and 5 p.m. had the highest specificity (100%) and PPV (100%). However, at the same time, the false-negative rate at these four time points was also the highest (86.42%). This indicates that LPR can be diagnosed if pepsin is detected at these time points, but detection at these four points alone will lead to a high missed diagnosis rate. For patients who test negative at these four time points, further methods are needed to rule out the diagnosis of LPR.

The limitations of this study mainly lie in the choice of diagnostic methods. We did not use 24 h MII-pH monitoring to verify the diagnosis of LPR, and our salivary pepsin detection method has the following limitations: (1) it is limited to the qualitative and not quantitative detection of pepsin and (2) if the saliva contains blood, the results will be false. In addition, we have not identified the optimum timing of detection or the best threshold. Besides, the test has not been validated by multicenter research. In the future, we should perform more research in these areas to improve the rate and accuracy of the diagnosis of LPR.

5 | CONCLUSION

There was a moderate degree of consistency between the two methods. Salivary pepsin detection is a convenient, economic and accepted diagnostic method for LPR, but the accuracy of a single test in the morning (fasting) is not satisfactory. The use of multiple pepsin samples through a day increased the accuracy and sensitivity of detection of LPR twofold compared to a single morning fasting sample. And further invasive examinations, such as 24 h MII-pH, may be needed to rule out diagnosis in patients with negative MPTSPT results.

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