Research

Salivary pepsin detection for laryngopharyngeal reflux diagnosis: sensitivity and specificity comparison with scoring system

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ABSTRACT

Background: Laryngopharyngeal reflux (LPR) is characterized by the backflow of stomach contents into the larynx and pharynx. Gastroesophageal reflux disease (GERD) is a similar condition often mistaken for LPR. Detection of oral salivary pepsin has been developed as an alternative diagnostic modality for LPR. Additionally, there are diagnostic aids for LPR utilizing scoring systems, namely Reflux Symptoms Score (RSS) and Reflux Sign Assessment (RSA). **Purpose:** To assess the sensitivity and specificity of saliva pepsin testing compared to RSS and RSA in diagnosing LPR. **Method:** A prospective cross-sectional study involving 30 subjects with LPR symptoms was conducted to evaluate the diagnostic accuracy of saliva pepsin levels compared to RSS and RSA scores in LPR patients. Diagnostic tests performed included sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). **Result:** The male-to-female ratio was 1:1.3. Saliva pepsin testing with a cutoff value of ≥16 ng/mL, demonstrated a sensitivity of 100%, specificity of 90%, PPV of 95.24%, and NPV of 100% against the RSS questionnaire. Meanwhile, when assessed against the RSA questionnaire, saliva pepsin testing showed a sensitivity of 95.24%, specificity of 88.89%, PPV of 95.24%, and NPV of 88.89%. The results indicated that saliva pepsin testing had good sensitivity and specificity, with values of 100% and 90%, respectively, against the RSS questionnaire; and 95.24% and 88.98%, respectively, against the RSA questionnaire. **Conculsion:** Saliva pepsin testing could be used as a primary diagnostic modality in the future due to its non-invasive nature, ease of administration, and good patient tolerance.

Keywords: laryngopharyngeal reflux, pepsin, reflux symptoms score, reflux sign assessment, saliva

ABSTRAK

Latar belakang: Laryngopharyngeal reflux (LPR) *ditandai dengan adanya aliran balik isi lambung ke laring dan faring.* Gastroesophageal reflux disease (GERD) *merupakan penyakit serupa yang sering disalah-artikan sebagai* LPR*. Deteksi pepsin saliva oral telah dikembangkan sebagai modalitas diagnosis alternatif untuk* LPR*. Selain itu, terdapat alat bantu diagnosis* LPR *dengan menggunakan sistem skoring, yakni* Reflux Symptoms Score (RSS) *dan* Reflux Sign Assessment (RSA)*.* ***Tujuan:*** *Untuk mengetahui sensitivitas dan spesifisitas pemeriksaan pepsin saliva dibandingkan dengan RSS dan* RSA *pada diagnosis* LPR*.* ***Metode:*** *Studi korelatif dengan desain potong lintang prospektif yang melibatkan 30 subyek dengan gejala* LPR*, dilakukan untuk menilai uji diagnostik kadar pepsin saliva dibandingkan dengan skor* RSS *dan* RSA *pada pasien* LPR*. Uji diagnostik yang dilakukan meliputi uji sensitivitas, spesifisitas,* Positive Predictive Value (PPV)*, dan* Negative Predictive Value (NPV)*.* ***Hasil:*** *Rasio perbandingan jenis kelamin pria dan wanita sebesar 1:1,3. Pemeriksaan pepsin saliva dengan nilai* cut-off *≥16 ng/mL memiliki sensitivitas 100%, spesifisitas 90%,* PPV *95.24%, dan* NPV *100% terhadap kuesioner* RSS*. Sementara itu, ketika dinilai terhadap kuisioner* RSA*, pemeriksaan pepsin saliva memiliki sensitivitas 95.24%, spesifitias 88.89%,* PPV *95.24%, dan* NPV *88.89%. Penelitian ini menunjukkan bahwa pemeriksaan pepsin saliva memiliki nilai sensitivitas dan spesifitias yang baik, masing-masing sebesar 100% dan 90% terhadap kuesioner* RSS*, serta 95.24% dan 88.98% terhadap kuesioner* RSA*.* ***Kesimpulan:*** *Pemeriksaan pepsin saliva kedepannya dapat digunakan sebagai modalitas diagnostik utama karena sifatnya yang non-invasif, mudah dilakukan, dan dapat ditolerir dengan baik oleh pasien.*

Kata kunci: laryngopharyngeal reflux*, pepsin,* reflux symptoms score, reflux sign assessment, *saliva*

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INTRODUCTION

Laryngopharyngeal reflux (LPR) is a disease characterized by the backflow of gastric contents into the larynx and pharynx, which then comes into contact with the upper gastrointestinal tract.[1] The most common symptoms of LPR are dysphonia, globus pharyngeus, mild dysphagia, chronic cough, throat itching, and excessive throat mucus production. Most patients are relatively unaware of LPR with only 35% reporting heartburn.[2] Gastroesophageal reflux disease (GERD) is a similar disease that is often mistaken for LPR. In GERD there is reflux of gastric acid into the esophagus and not into the larynx and pharynx, as occurs in LPR. Reflux of gastric acid containing pepsin, bile salts, hydrochloric acid (HCl), and various other substances in the gastric juice, can irritate the mucosa of the larynx and pharynx, and even the entire mucosa of the other digestive tract.[3]

Direct exposure to gastric acid can damage the laryngeal epithelium. Ciliary flow will be inhibited under pH conditions below 5.0 and completely stopped at pH 2.0. With decreased ciliary flow, there is also a decrease in resistance to infection. The risk factors for LPR are almost the same as GERD such as eating foods that contain a lot of acid and fat, consuming caffeine or alcohol, eating large portions before bed, obesity, and smoking.[4] The main difference between LPR and GERD is the manifestation and underlying anatomical defect, where the disturbance of lower esophageal sphincter can be found in GERD, and the upper esophageal sphincter in LPR. As many as 10% of patients who visit the ENT clinic have symptoms caused by LPR. LPR also contributes to the onset of hoarseness in up to 55% of patients with dysphonia. In patients with LPR, almost 100% will complain of hoarseness on presentation, despite the absence of other classic reflux-related symptoms. The prevalence of GERD and LPR has increased by 4% annually since 1976, and data from the US National Cancer Institute showed a 600% increase in esophageal cancer prevalence since 1975. Altman et al. qouted by Campagnolo et al.[1] reported a 500% increase in visits to ENT specialists due to LPR between 1990 and 2001.

The diagnosis of LPR is based on evidence of gastric acid reflux into the laryngopharynx. Oral salivary pepsin detection has been developed as an alternative diagnostic modality. Pepsin is only synthesized by chief cells in the gastric mucosa, so its presence in saliva can be concrete evidence, and the diagnosis of LPR can be established. Apart from supporting examinations such as salivary pepsin detection, there are LPR diagnosis tools using a scoring system. Some of these tools include the Reflux Symptoms Score (RSS) and Reflux Sign Assessment (RSA). These tools could also be used to follow changes in LPR symptoms during the treatment period.[5,6] The Reflux Symptoms Score was first developed at the World Ear Nose Throat (ENT) Congress of IFOS in Paris in 2016. The RSS content consisting of symptoms, structure, and presentation, had been compiled based on expert opinion and systematic reviews that described the symptoms of LPR based on current literature.[5] Meanwhile, the RSA was developed by the Young Otolaryngologists of the International Federation of OtoRhino-Laryngological

Societies (YO-IFOS). The RSA content consists of an assessment of the oral cavity, pharyngeal cavity, and larynx.[7] The use of this tool is due to the non-specificity of LPR symptoms, and the limitations of supporting examinations that can be performed in clinical practice. Based on the above background, the researchers designed this study to determine the sensitivity and specificity of salivary pepsin examination compared to Reflux Symptoms Score (RSS) and Reflux Sign Assessment (RSA), in the diagnosis of LPR.

METHOD

This was a correlative study with a prospective cross sectional design that assessed the diagnostic test of salivary pepsin levels compared to RSS and RSA scores in LPR patients. The study was conducted at Dr. Moewardi Surakarta Regional General Hospital (DMS-RGH), specifically at the Ear, Nose, and Throat Head and Neck Surgery Polyclinic, in the period of May-July 2023. A total of 30 samples were taken using consecutive sampling technique.

Saliva was collected in the morning before any oral activity. Patients were instructed not to brush or rinse prior to providing 2 mL of sali. Submitted saliva was then analyzed using ELISA.

In this study, the dependent variables were RSS and RSA scores, while the independent variable was salivary pepsin levels. The use of the Indonesian RSS and RSA questionnaires has been previously validated with Cronbach's alpha values of 0.734-0.831 and 0.743-0.809 respectively. The reliability test obtained RSS and RSA r values of 0.930 ($p<0.001$) and 0.842 ($p<0.001$) respectively.[8] The RSS score is assessed from 3 main parameters, namely ear, nose, and throat disorders; stomach disorders; and chest/respiratory disorders. The three parameters are interpreted in the frequency of disorders (score 0-5), severity of disorders (score 0-5), and impact on quality of life (score 0-5). The RSA score is assessed by evaluation of the oral cavity, pharynx, and larynx which are interpreted in a score that interprets "present" or "absent". The level of pepsin enzyme contained in oral saliva was detected by ELISA method. Salivary pepsin value≥16 ng/mL was suggestive of LPR classified as indicative of LPR based on Zhang et al.[5]. RSS is an LPR symptom scoring system consisting of 3 component questions. RSS score >13 was suggestive of LPR. RSA is an LPR symptom scoring system consisting of 3 components with a maximum score of 72. RSA score >14 was suggestive of LPR.

This study collected data from oral saliva and questionnaires from the RSS and RSA forms. Data analysis was performed with the help of IBM SPSS version 25 (Chicago, USA). The numeric variable was presented by descriptive presentation. Diagnostic tests performed consisted of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) tests expressed as a percentage. A p value of <0.05 was considered statistically significant.

Prior to research implementation, a research approval letter was prepared from the Head of the ENT-HN Department of DMS-RGH, and forwarded to the Standing Committee for Medical Research Ethics of the Faculty of Medicine, Sebelas Maret University / DMS-RGH, Surakarta.

RESULT

The mean age of the subjects in this study was 47.57 years, with more women (56.67%) than men (43.33%). The sex ratio of male and female was found to be 1:1.3. The most common comorbidity in this study was Diabetes Mellitus (DM). The mean RSS score was 16.5, while the mean RSA score was 17.73. The mean salivary pepsin level was 19.4 ng/mL. Subject characteristics could be seen in Table 1.

Sensitivity and specificity tests of salivary pepsin were performed against RSS and RSA questionnaires. Salivary pepsin levels >16 ng/mL were positive for LPR and vice versa, while RSS>13 and RSA>14 scores were suggestive of LPR diagnosis.

Table 1. Characteristics of research subjects

Characteristics	N (%) Mean ± SD
Age	47.57±4.55
Gender	
Male	13 (43.33)
Female	17 (56.67)
Comorbid*	
Diabetes mellitus	8 (26.67)
Dyslipidemia	2 (6.67)
Asthma	5 (16.67)
Chronic kidney disease	2 (6.67)
Heart disease	13 (43.33)
None	-
RSS Score	16.63±5.35
RSA Score	17.73±5.87
Salivary pepsin (ng/mL)	19.4±6.4

*Subjects could have more than one comorbidity so the grand total is >100%.

Table 2. Analysis of salivary pepsin examination against RSS and RSA Scores

Salivary pepsin	RSS		Total	RSA		Total
	Positive	Negative		Positive	Negative	
≥16 ng/mL	19	1	20	20	1	20
<16 ng/mL	2	8	10	1	8	10
Total	21	9	30	21	9	30

Table 3. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Salivary Pepsin Screening against RSS and RSA

Parameters	Value	95% CI	
		Lower	Upper
Salivary Pepsin vs RSS			
Sensitivity	100%	83.16	100
Specificity	90%	55.5	99.75
PPV	95.24%	75.7	99.23
NPV	100%	66.37	100
Salivary Pepsin vs RSA			
Sensitivity	95.24%	76.18	99.88
Specificity	88,89%	51.75	99.72
PPV	95.24%	75.87	99.22
NPV	88.89%	53.18	99.18

DISCUSSION

In this study, 30 subjects had an average age of 47.5 years, and were more dominated by women than men. These results were in line with research by Lechien et al.[8] who also found that patients with LPR symptoms had an average age of 50 years. Research by Divakaran et al. [9] reported that patients with LPR were dominated by women compared to men. Laryngopharyngeal reflux is mostly found in middle aged to the elderly, because the main defense mechanisms against reflux such as esophageal motility, bicarbonate secretion, and tonicity of the lower and upper esophageal sphincters decreased with age. Older patients have decreased salivary flow and bicarbonate secretion, which is associated with decreased neutralization of acid reflux, and increased pepsin activity.[10] Women are known to be more prone to LPR than men, as their shorter and thinner vocal cords are more susceptible to gastric fluid damage.[11]

In this study, it was found that more than half of the subjects had comorbid diseases, which the most common found was DM. These results were in line with research by Massawe et al.[12] who also found that DM mellitus as the most common comorbidities found in LPR patients. Hamdan et al.[13] reported that DM patients had a higher mean RS score than healthy people. This is because the condition of hyperglycemia experienced by DM patients will cause neuropathy, which has an impact on weakening the esophageal sphincter. This will then have an impact on the onset of LPR symptoms.

The results of this study showed that the salivary pepsin test with a cut-off value of ≥16 ng/mL had a sensitivity of 100%, specificity of 90%, PPV of 95.24%, and NPV of 100% against the RSS questionnaire. Meanwhile, when assessed against the RSA questionnaire, the salivary pepsin test had a sensitivity of 95.24%, specificity of 88.89%, PPV of 95.24%, and NPV of 88.89%. These results indicated that salivary examination with a cut-off value of ≥16 ng/mL had good sensitivity and specificity. Sensitivity indicates the ability of a test to detect disease. The more sensitive a test is, the fewer false negative results. If the sensitivity of a test is high, the detected disease can be excluded. Specificity refers to the percentage of people who do not actually have a disease and test negative. Therefore, a diagnostic test with high specificity has few false positive results. High specificity tests are suitable for disease screening.[14,15]

Pepsin is only produced in the stomach, so it is a specific biomarker for gastric reflux and can be detected in saliva, sputum, otitis media secretions, and tears. Salivary pepsin examination is a non-invasive and more practical diagnostic tool compared to endoscopy and 24-hour MII-pH monitoring.[16,17] Salivary pepsin examination has been proposed as a simpler, cost-effective, and less invasive alternative to other diagnostic modalities in LPR such as MII-pH and endoscopic examination.[5]

Pepsin has been recognized to play an important role in the pathogenesis of LPR. Pepsin is the active form of pepsinogen, a peptidase enzyme excreted by chief cells in the stomach. Pepsin digests proteins through hydrolysis of peptide bonds. As it exits the stomach along with other gastric contents, pepsin damages the mucosal lining of the structures with which it comes into contact. By digesting gap-junctions, pepsin can damage the epithelial barrier. Pepsin reaches peak activity in a low pH environment in the range of 2-3.2. Successive reflux episodes, especially acid reflux, can activate pepsin located in the structures of the larynx and nasopharynx. Some pepsin activity has also been observed at pH 6-7.2. Thus, mixed or slightly alkaline reflux can also cause pepsin activation. pH in the range of 6.4-7.2 is the physiological pH of the oral cavity and upper respiratory tract. Upon penetration of pepsin into these structures, damage to the mucosa of the upper aerodigestive tract can occur

not only during episodes of slightly alkaline reflux, but can persist due to pepsin activation at that pH.[5]

The limitation of this study was the finding of an imbalance in quantity between the number of LPR and non-LPR research subjects based on RSS and RSA which causes non-comparability. The smaller non-LPR group caused the risk of overestimating specificity because random variation was more influential. The researchers realized that this finding was due to consecutive sampling, which had an impact on the selection of subjects who were dominated by patients who came to the ENT polyclinic, so that there was a risk of selection bias. In addition, there was a representativeness that does not reflect LPR in the community, such as asymptomatic patients. This method also had difficulty controlling comorbidities. Further research could be carried out using a sampling method with stratified random sampling with age, gender, and comorbidity stratification up to proportional allocation (1:1). The balance of research subjects could be added with strict exclusion criteria.

In conclusion, the results of this study indicated that salivary pepsin examination had good sensitivity and specificity values, with values of 100% and 90% respectively on the Reflux Symptoms Score (RSS) questionnaire, and 95.24% and 88.98% on the Reflux Sign Assessment (RSA) questionnaire. Salivary pepsin test can be used as the main diagnostic modality in the future, because it is non-invasive, easy to perform, and well tolerated by patients.

Future research is expected to increase the number of research samples, so that it can describe more broadly related to the results of the study. Future research is also recommended to be able to take saliva samples at several different times, so that the best time to take saliva samples can be known.

ACKNOWLEDMENT

This study was funded by Dr. Moewardi General Hospital in Surakarta.

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