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# Significance of the Pepsin from the Saliva in the Diagnosis and Treatment of Laryngopharyngeal Reflux Disease

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## ABSTRACT

*Etiopathogenesis of the laryngopharyngeal reflux has not been sufficiently clarified. It is believed that damage to the lining of laryngopharynx in laryngopharyngeal disease occurs in the use of acid and pepsin. The diagnosis of reflux acidic 24-hour Dual probe pH-metry is considered the gold standard. However, since the laryngopharyngeal reflux is often non-acidic new diagnostic methods are being explored, safe diagnosis of the disease according to symptoms of this uncharacteristic disease. In our study on 45 patients with laryngopharyngeal disease, we have proved that tracking the value of pepsin in the saliva of a valuable diagnostic indicator of laryngopharyngeal reflux and a valuable indicator of the success of the treatment.*

**Key words:** biological markers, diagnostic test, gastroesophageal reflux, laryngopharyngeal reflux, laryngitis, pepsin

## Introduction

Pathogenesis and molecular mechanisms of laryngopharyngeal diseases (LPRD) are relatively unknown. It is believed that in the development of laryngopharyngeal reflux have a crucial role: the dysfunction of the upper esophageal sphincter<sup>1</sup>, prolonged contact with gastric contents and laryngopharyngeal mucus vagal reflex activity in the distal esophagus. According to recent studies<sup>2-5</sup> after exposure of laryngeal mucus of stomach contents containing acid and pepsin, pepsin activity leads to depletion of the enzyme carbonic anhydrase III (CAIII), inhibiting the expression of protective protein mucin 2,3,5 A, 5B, Sep70 and Sep53 and E-cadherin who have a vital role in maintaining cellular integrity of the epithelium<sup>3</sup>. The most recent research stresses the importance of the so-called nonacidic or low acidity reflux in the etiology of the LPRD<sup>6</sup>. It is the cause of this problem in about 20% of patients with persistent symptoms despite therapy laryngopharyngeal reflux (LPR) medication to combat acidity and pepsin. They are held responsible for the development of mucus injury laryngopharynx. The former gold standard in diagnosing reflux laryngopharyngeal

multichannel 24-hour pH-metry is slightly useful in non-acidic laryngopharyngeal reflux and it is necessary to combine with multi intraluminal impedance, which is used in everyday practice and often unavailable and very invasive for patients. Several recent studies have investigated the potential use of pepsin as a diagnostic marker LPR<sup>2,6-9</sup>. The aim of this study show that the values of pepsin in saliva combined with clinical symptoms (»reflux symptom index« – the Belafsky RSI) and clinical findings videolaryngoscopy (»reflux finding score« – the RFS by Belafsky) are significant diagnostic indicator of disease.

## Patients and Methods

In a prospective clinical study included 45 patients with clinical symptoms and signs LPR, and treated at the Department for Otorhinolaryngology and Head and Neck Surgery in the period from September 2010 until May 2011, and 30 subjects in the control group without clinical symptoms and signs laryngopharyngeal reflux.

All subjects on the basis of clinical symptoms of disease specified »reflux symptom index« – RSI by Belafsky for suspicion of laryngopharyngeal disease had to be greater than the seven. All respondents were based on clinical findings identified videolaryngoscopy »reflux finding score« – RFS by Belafsky for suspicion of laryngopharyngeal disease had to be greater than 10. All subjects underwent esophagogastroduodenoscopy (Olympus). During the performance of esophagogastroduodenoscopy there were taken two biopsies from the antrum and two biopsies from the corpus of the stomach. Biopsies from the antrum and corpus of the stomach were fixed in formalin for histopathologic analysis. Also, all respondents were sampled saliva for analysis of the value of pepsin in saliva at baseline and eight weeks after treatment with proton pump inhibitors. Proton pump inhibitors have ordained the first four weeks at a dose of 2x40 mg, and then at a dose of 2x20 mg next four weeks. Eight weeks after therapy, we again found the RSI and RFS by Belafsky. Samples of saliva were centrifuged for 15 minutes at 1000 rpm and then frozen and stored at  $-70^{\circ}\text{C}$  until analysis. After we collected all saliva samples, the samples were dissolved at room temperature. ELISA (USCN Life Science Inc. Wuhan, China) according to manufacturer's instructions, we determined the value of pepsin in saliva samples. From the studies excluded patients who did not regularly take treatment, which had previously been taking proton pump inhibitors, patients who had previously performed radiation of head and neck and patients with previously demonstrated salivary gland diseases. In support of data processing applications used the Microsoft Excel 2003 and SPSS statistical package (SPSS Statistics 17.0, SPSS Inc.) And Statistica (Statistica 8.0, StatSoft, Inc.). The study considered significant differences confirmed the significance level  $p < 0.05$ .

During the research, ethical principles are respected. Subjects were given oral and written all information regarding participation in the proposed study and gave voluntary consent to participate in the study. Confirmation of the Ethics Commission – an explanation that the research in accordance with ethical principles – issued by the Ethics Committee of the Clinical Hospital Osijek, in which research is conducted. Also, the Ethics Committee for Medical Research, University of Josip Juraj Strossmayer gave the opinion and consent of the multidisciplinary research ethically acceptable.

## Results

In a prospective clinical study included 45 patients with clinical symptoms and signs LPR (28 women 62.22% 37.78% 17 men) average age of 51.69 years. The control group consisted of 30 patients without clinical symptoms and signs laryngopharyngeal reflux (19 women and 11 63.33% 36.67% men) with an average age of 38.87 years. The average value of pepsin in patients before treatment was 91.97 ng / ml, while the average value of pepsin after treatment was 34.96 ng / ml. Table 1. Test results show that it can accept the hypothesis of the existence of statistically significant differences in the levels of pepsin be-

**TABLE 1**  
DESCRIPTIVE STATISTICAL INDICATOR OF PEPSIN LEVELS  
IN PATIENTS WITH SUSPECTED LPR BEFORE AND AFTER  
THERAPY

STATISTICAL INDICATORS	PEPSIN	
	before therapy	after therapy
Number of date	45	45
Arithmetic mean	91.97	34.96
Median	17.90	6.40
Minimal value	3.20	1.60
Maximum value	345.10	280.10
Bottom quartile	10.60	3.00
Upper quartile	200.50	23.50
Range of variation	341.90	278.50
Interquartile	189.90	20.50
Standard deviation	110.42	63.85
Coefficient of variation	120.06	182.65
Skewness	0.89	2.55
Kurtosis	-0.75	6.28

fore and after therapy ( $Z = -5842$ ,  $p = 0.0000$ ). Comparing the values of pepsin before and after treatment with the finding of gastroscopy showed a statistically significant difference in the amount of pepsin in patients with chronic gastritis ( $Z = -4703$ ,  $p = 0.000$ ), and that there is a significant difference in subjects in which the diagnosed with chronic gastritis and other gastric diseases (Hiatus hernia, GERD) ( $Z = -3\ 517$ ,  $p = 0.000$ ). Based on the results obtained by the average value of pepsin in the saliva of subjects in the control group 10.76 ng / ml, with a standard deviation of 10.16 ng / ml. Based on the median, the levels of pepsin in the saliva of half of the respondents was 6.65 ng / ml or less. Comparing the level of pepsin before therapy in patients with suspected reflux laryngopharyngeal and pepsin in the saliva of the control group confirmed a statistically significant difference in the amount of pepsin before therapy and pepsin in the saliva of the control group (Table 2). Average value of pepsin after treatment determined by the respondents was for a 24.2 ng / ml higher than the levels of pepsin in the saliva

**TABLE 2**  
MEAN RANGE, THE SUM OF RANKS AND MANN-WHITNEY  
RESULTS OF THE TEST

GROUP	MEAN RANGE	SUMA OF RANKS	MANN-WHITNEY TEST
PATIENTS	45.71	2057.00	$Z = -3.753$
CONTROL	26.43	793.00	$p = 0.000$

of the control group, but these values were not statistically significant (Table 3). The average value of the RFS (Reflux Finding Score) before therapy was 11.96 average value of the RFS after therapy was 5.78. The average value of RSI (Reflux Symptom Index) before treatment

**TABLE 3**  
MEAN RANGE, THE SUM OF RANKS AND MANN-WHITNEY  
RESULTS OF THE TEST

GROUP	MEAN RANGE	SUMA OF RANKS	MANN-WHITNEY TEST
PATIENTS	38.04	1712.00	Z=-0.022
CONTROL	37.93	1138.00	p=0.983

was 18:02, while the average value of RSI after treatment was 6.78. Based on Spearman rank correlation coefficient, we can conclude that the RFS and the RSI before therapy there is a positive relationship that was statistically significant ( $p=0.0397$ ,  $p=0.007$ ). Comparing the RFS and the RSI values with pepsin prior to therapy there is no statistically significant association ( $p=0.0056$ ,  $p=0.714$ ), and after treatment ( $p=0.105$ ,  $p=0.49$ ).

### Discussion

LPR is a disease that occurs in nearly 10% of patients visiting the ENT and about 50% of the hoarseness is caused by them. Since laryngopharyngeal nonspecific symptoms of reflux disease and a number of diagnostic dilemmas certain diagnosis of laryngopharyngeal reflux is not easy to set up. So far, multi-channel 24-hour pH-Geometry was considered the gold standard, a method which is invasive, expensive and limited in application only with acidic reflux. Since LPR disease often non-acidic it is necessary to set a new gold standard for diagnosis of this common ease. As a diagnostic method, a proton pump inhibitor therapy is indicated, except for good and adverse effects such as diarrhea, atrophic gastritis, changes in the absorption of iron and vitamin B12, possible liver damage. Therefore, if possible, long-term use and over-prescribing of these drugs is certainly desirable to avoid<sup>11</sup>. So far, several studies have shown that pepsin plays an important role in the development of mucus damage of laryngopharynx with interaction of acidic and non-acidic reflux<sup>3,4,10</sup>. In our study we determined the value of pepsin in the saliva of subjects with symptoms of LPR. We found that pepsin is present in the saliva of pa-

tients with laryngopharyngeal reflux and that the value of pepsin in saliva prior to treatment increased and the difference was statistically significant compared with the values of pepsin in the saliva of subjects in the control group. In 23 subjects (31%) with clinically clear signs of LPR, the value of pepsin before therapy was comparable with the levels of pepsin in the control group which raises doubts about the reliability of the sample. After completing therapy, proton pump inhibitors, there is no statistically significant difference in the values of pepsin in the saliva of the test group and control subjects, which indicates the contribution to the success of a proton pump inhibitor therapy. In 5 patients (11%) levels of pepsin in saliva after completion of therapy still showed a significantly increased value of 100.3–280.1 ng / ml, suggesting that in these patients must continue therapy with inhibitors of the IPP instructions of the American Academy of Otolaryngology and head and neck surgery. According to the findings esophagogastroduodenoscopy all respondents had pathological changes in the gastric mucus, but the value of pepsin did not show a statistically significant difference with the type of clinical findings in the gastric mucus, we confirmed that LPR and gastroesophageal reflux are two different diseases. Average values of RFS and RSI by Belafsky before and after therapy showed a statistically significant difference.

### Conclusion

In this study we have shown that the amount of pepsin in the saliva of patients with reflux laryngofaringeal-nim significantly higher than the value of pepsin in the saliva of subjects in the control group. We have also shown that the subjects were diagnosed as gastroesophageal reflux disease have significantly higher levels of pepsin in saliva in relation to the subjects were diagnosed as chronic gastritis, suggesting that gastroesophageal reflux and laryngopharyngeal reflux two the etiopathogenesis of various diseases. We expect that in the near future in everyday practice as a diagnostic method LPR will be introduced in a simple, reliable, sensitive and inexpensive analysis of pepsin in saliva ELISA test.

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## **ZNAČENJE PEPSINA IZ SLINE U DIJAGNOSTICI I LIJEČENJU LARINGOFARINGEALNE REFLUKSNE BOLESTI**

### **S A Ž E T A K**

Etiopatogeneza laringofaringealnoga refluksa do danas nije dovoljno razjašnjena. Smatra se da oštećenje sluznice laringofarinksa u laringofaringealnoj bolesti nastaje djelovanjem kiseline i pepsina. U dijagnostici aciditetnog refluksa 24-satna pH-metrija smatra se zlatnim standardom. Međutim, budući je laringofaringealni refluks često neaciditetan istražuju se nove dijagnostičke metode sigurnog postavljanja dijagnoze ove po simptomima nekarakteristične bolesti. U našem istraživanju na 45 ispitanika s laringofaringealnom bolešću dokazali smo da je praćenje vrijednosti pepsina u slini vrijedan dijagnostički pokazatelj laringofaringealnoga refluksa i vrijedan pokazatelj uspješnosti primijenjene terapije.