LETTER TO THE EDITOR

LARYNGOPHARYNGEAL REFLUX DISEASE IN ADULT PATIENTS: TEARS AND PEPSIN

G. MAGLIULO[1], A. PACE[1], R. PLATEROTI[1], A.M. PLATEROTI[1], R. CASCELLA[2,3], C. SOLITO[1], V. ROSSETTI[1] and G. IANNELLA[1]

[1]Sense Organs Department, Sapienza University of Rome; [2]Laboratory of Genomic Medicine UILDM, IRCCS Santa Lucia Foundation, Rome, Italy; [3]Department of Chemical Pharmaceutical and Biomolecular Technologies, Catholic University "Our Lady of Good Counsel" Laprakë, RrugaDritanHoxha, Tirana, Albania

Received October 17, 2019 – Accepted March 18, 2020

To the Editor,

Laryngopharyngeal reflux disease (LPRD) is defined as the retrograde flow of stomach content to the upper aero-digestive structures, with an estimate incidence about 4-10% of the general population. (1). LPRD is used to describe the pathological condition caused by the reflux of gastric and/or duodenal juices to the larynx, oropharynx and/or nasopharynx, through the upper oesophagus sphincter (UES). The most frequent symptoms are dysphonia, chronic cough, sore throat, excess throat mucus and pharyngeus globous. Endoscopy evidence related to this pathology could be laryngeal oedema, laryngeal hyperaemiagranulomatosis and polypoid lesions of the larynx (2).

Pepsin is a protein produced by the gastric mucosa. In recent years, several studies have shown the existence of a relationship between salivary pepsin and LPRD signs and symptoms (3). Salivary pepsin has therefore been proposed as a biomarker for the diagnosis of LPRD. Some authors reported the positive pepsin expression in middle ear effusion of patients with effusive otitis media and in the nasal cavity of patients with chronic rhinosinusitis. In 2014, Luo et al. investigated the relationship between pepsin and pepsinogen in children affected by otitis media with effusion (OME). The results showed high levels of pepsinogen protein expressed in cytoplasm of epithelial cells in adenoid specimens of the children with OME (4). Magliulo et al., in 2013, hypothesized that, in patients with LPRD, pepsin might reach the tear film through the lacrimal ways eliciting irritation and edema phenomena on the tear ducts (5). Iannella et al., in 2015, were the first to confirm this hypothesis by demonstrating the presence of pepsin in the tears of 20% of children with LPRD enrolled in a prospective study (6).

The aim of this study is to confirm this evidence, evaluating the presence/absence of pepsin in tears of adult patients affected by LPRD. Pepsin was evaluated in tears of healthy patients without LPRD in order to compare the two groups in terms of pepsin expression in tears.

MATERIALS AND METHODS

All patients who came to our Department with signs and symptoms of LPRD between February 2018 and June 2019 were evaluated for possible enrolment in this prospective study. Exclusion criteria was: age <18 years old, pregnancy,

Key words: LPRD; pepsin; tears; larynx

Corresponding Author:
Giuseppe Magliulo, M.D.,
Sapienza University of Rome, Policlinico Umberto I,
Viale dell'Università 33, Rome 00185, Italy
Tel.: +393388622344 - Fax: +390649976817
e-mail: giuseppe.magliulo@uniroma1.it

0393-974X (2020)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

the presence of ocular diseases and treatment with proton pump inhibitors (PPIs) or other drugs used for the treatment of LPRD at the time of the study. All patients gave their written consent for all the tests and for their enrolment in the study. This research was performed in accordance with the principle of the Declaration of Helsinki and approved by the local ethics committee of the University "Sapienza", Rome (RIF.CE.4841).

Initially, all patients underwent anamnestic evaluation, Reflux Symptom Index (RSI) evaluation and a fiber optic laryngoscopic evaluation with Reflux Finding Score (RFS). RSI is a self-conducted questionnaire, developed by Belafskyet al 2002, based on nine questions, with a maximum of 5 points for each question, giving a total of 45 points. The score was considered pathological when the score was ≥13 (Table I) (7). The RFS evaluates the presence of 8 laryngoscopic findings with a scale that goes from 0 (normal) to 26 (strongly pathological). RFS ≥ 7 is considered pathological and indicative of LPRD (Table I) (8). Using both these scores it is possible to use them as a guide for the clinical diagnosis of LPRD.

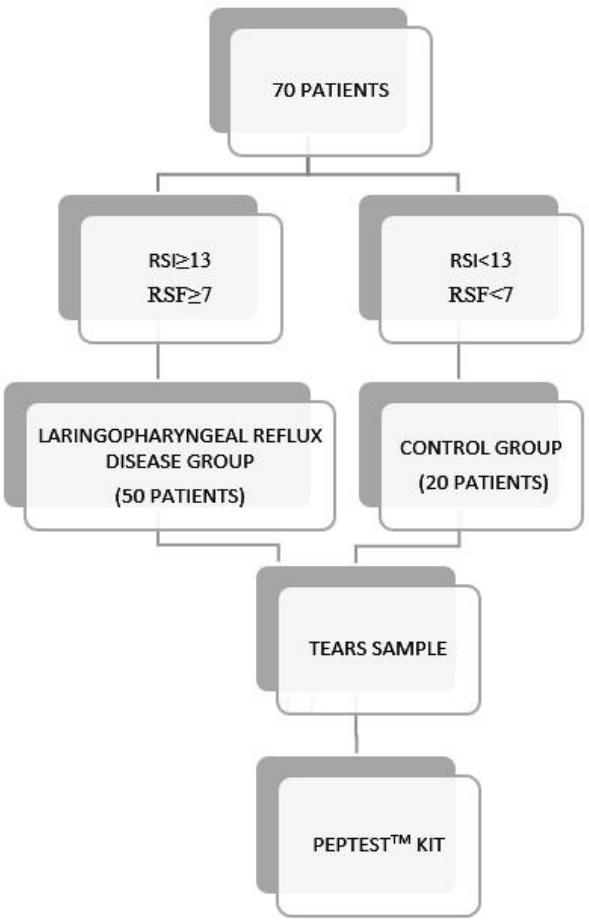

Fig. 1. *Flow chart of the study.*

Fifty adult patients (21 males, 29 females; 19-75 years of age, average 44.1 years) in our study showed pathological values in both questionnaires and they were enrolled in the study group as considered suffering from the symptoms of LPRD. The control group consisted of 20 patients (9 males, 11 females; 26-64 years of age, average 41.8) considered negative for LPRD, because negative to both RSF and RSI questionnaires. A flow-chart of the study is shown in Fig. 1.

A tear sample was taken from all patients in the study in order to estimate the presence of pepsin and its concentration. The tear samples were collected in the early hours of the morning with a micropipette, a silicone tube with a diameter of 0.3 cm, 2 cm long, cut obliquely at 45°, siliconized to a small tank (diameter of 0,5 cm, 2 cm long), provided with a suction tube. The micropipette works by pipetting the tear fluid from the tear lake, located on the bulbar conjunctiva, at the level of the inner chant of the eyelid and depositing the liquid on a glove slide. The collection takes place through the micropipette with a rapid movement, in order to avoid any subsequent tearing. The tears of both eyes were harvested and carried in a single test tube.

The collected tears were analysed by Peptest™ kit (BIOHIT HealthCare) that is a qualitative and quantitative test to determine the pepsin concentration in body fluids. The test required 100 µl of tears with the addition of 100 µl of 0.01M citric acid. Each sample was centrifuged at 4000 rpm for 5 min. Subsequently, 80 µl of supernatant was collected and was added to 240 µl of migration buffer and the mixture was vortexed for 10 s: 80 µl of this mixture was pipetted into the well of the Peptest™ Lateral Flow Device (LFD) and the results were ready after 15 min. The test is based on a chemical reaction antigen-antibody utilizing a monoclonal anti-pepsin antibody (T band reveals the pepsin presence). In addition, the system involves an inner reaction control useful for estimating the system's integrity (C band). The test is valid when it obtains a reaction related to the Internal Control (IC, C band). The existence of T band indicates that pepsin is present in the tested sample and, furthermore, the intensity of the T band is directly proportional to the pepsin quantity. The concentration of pepsin level was accurately measured by Peptest Cube that displays the result directly in ng/ml in just three seconds. The reader is able to detect a minimum amount of pepsin equal to 16ng/ml. The device

Table I. *Reflux Symptoms Index (Belafsky PC et al 2002); Reflux Finding Score (Belafsky PC et al 2001).*

REFLUX SYMPTOMS INDEX	
Hoarsness or a problem with voice	0 1 2 3 4 5
Cleaning your throat	0 1 2 3 4 5
Excess throat mucus or postnasal drip	0 1 2 3 4 5
Difficulty swallowing food, liquids, or pills	0 1 2 3 4 5
Coughing after you ate or after lying down	0 1 2 3 4 5
Breathing difficulties or choking episodes	0 1 2 3 4 5
Troublesome or annoying cough	0 1 2 3 4 5
Sensations of something sticking in your throat or lump in your throat	0 1 2 3 4 5
Heartburn, chest pain, indigestion, or stomach acid coming up	0 1 2 3 4 5
REFLUX FINDING SCORE	
Subglottic edema	2=present 0= absent
Ventricular obliteration	2= partial 4= complete
Erythema/ hyperemia	2= arytenoids only 4=diffuse
Vocal fold edema	1= mild 2= moderate 3= severe 4= polypoid
Diffuse laryngeal edema	1= mild 2= moderate 3= severe 4= obstruction
Posterior commissure hypertrophy	1= mild 2= moderate 3= severe 4= obstruction
Granuloma/ granulation	2=present 0= absent
Thick endolaryngeal mucus	2=present 0= absent

is able to conduct a colorimetric tests based on reflectance measurements that capture the optical density. The test can provide three possible results: negative (only the IC is present), positive (the T and C bands are present), null (absence of IC signal). (9-10)

Statistical analysis was performed using Statview statistical software version 8.0. Student's *t*-test was employed to compare the data (age, RSI, RSF, pepsin) of the study. A value of $p<0.05$ was considered statistically significant.

RESULTS

No statistical significative difference emerged between the LPRD and control groups regarding age ($p=0.6$). In the study group, the average RSI value

Table II. *AGE. Reflux index symptoms and Reflux finding score of study and control groups.*

	AVERAGE VALUE		p-value
	LARYNGOPHARYNGEAL REFLUX DISEASE GROUP	CONTROL GROUP	
AGE	44.1 (range 19-75)	41.8 (range 26- 64)	p=0.6
Reflux index symptoms	21 (range13-36)	10 (range 9-12)	p=0.0001
Reflux finding score	11.4 (range 8-17)	5.6 (range 4-6)	p=0.0001

Table III. *Values of AGE. RSI. RSF and Pepsin concentration level in 32 patients of LPRD group positive to Pep-test.*

PATIENTS	AGE	RSI	RFS	PEPSIN ng/ml
1	35	25	11	179.9
2	33	32	8	43.9
3	39	26	12	65.7
4	19	24	10	58.6
5	34	16	14	200.9
6	63	29	16	58
7	44	28	14	32.2
8	75	28	16	59
9	65	24	10	91.1
10	33	13	11	107.3
11	52	13	11	39.9
12	60	13	11	247.6
13	24	28	8	177.9
14	33	25	10	20.5
15	33	19	17	20.5
16	43	13	8	26.3
17	32	17	12	56.4
18	53	33	12	120.1
19	27	13	8	20.5
20	25	18	8	148.3
21	32	25	12	20.5
22	57	21	8	90.7
23	24	26	14	20.5
24	67	19	9	20.5
25	60	22	12	128
26	26	28	12	20.5
27	39	13	8	189.9
28	41	15	8	96.8
29	34	13	8	129
30	40	15	10	65
31	25	13	9	20.5
32	27	24	12	193.1

was 21 (range 13-36) and the average RFS value was 11.4 (range 8-17). In the control group, the average RSI value was 10 (range 9-12). Statistical difference emerged between the two groups regarding RSI value (p=0.0001). The average RFS value was 5.6 (range 4-6) with a statistically significant difference with respect the LPRD group (p=0,0001) (Table II).

The results of pepsin evaluation in tears showed that 32 of the 50 patients, affected by LPRD, presented a concentration of pepsin in the tear samples obtained. Therefore, the percentage of patients with LPRD positive to pepsin was estimated at 64%. The average level of pepsin concentration in the positive patients of the study group was 86.6 μg/ml (range20.5- 247.6 μg/ml) (Table III).

Regression analysis showed no correlation between the values of RSI and pepsin dosage (p=0.4); the same no correlation emerged between RFS values and pepsin concentration (p=0.3). None of the patients in the control group presented pepsin in the tear samples obtained.

DISCUSSION

Laryngopharyngeal reflux disease is defined as the reflux of gastric contents in the larynx, oropharynx and/or nasopharynx through the upper esophageal sphincter (UES) (1). The present investigation showed that most of the LPRD selected patients presented high levels of pepsin concentration in their eyes, while no patients in the control group presented pepsin in the tear samples obtained.

It has been hypothesized that pepsin arrives in the pre-corneal tear film through the nasolacrimal duct after reaching the nasopharynx during reflux attacks (5-6). However, other mechanisms should be taken into consideration such as arrival of pepsin from blood and pepsin production of lacrimal glands cells. The present study presents some limitations. The first one is the limited number of enrolled patients; other studies on larger series of patients are underway. Another criticism regards the diagnosis of LPRD based on RSI and RSF. These tests are the most used in LPRD diagnosis in clinical practice (11). These tests are also economical and easy to administer. However, the gold standard for LPRD diagnosing remains the multichannel intraluminal impedance (MII) and 24-hour dual probe Phmetry, but they are invasive and more expensive methods with a poor reproducibility due to the position of the proximal sensor (in case of suspected LPRD), and without a clear pH cutoff level (12). In our study no correlation was observed between the values of pepsin concentration in tear samples with those of RSI and RFS using regression analysis. Further studies withMII and 24-hour dual probe Phmetry would be useful to confirm the relationship between pepsin level concentration in tears and the number or type of reflux episodes.

Some patients affected by LPRD in this study also complained of ocular symptoms. They described sensations of burning or the presence of foreign bodies in their eyes (like sand granules). Probably these irritating symptoms may be related to the involvement of the tear film from the LPRD pathology due to the presence of gastric substances in the lacrimal film. How and how much the presence of these gastric substances can cause irritation of the conjunctiva and ocular pathologies will be the subject of further experimental studies.

REFERENCES

1. Hayat JO, Yazaki E, Moore AT, Hickiln L, Dettmar P, Kang JY, Sifrim D. Objective detenction of esophagolaryngeal reflux in patients with hoarsness and endoscopic signs of laryngeal inflammation. J Clin Gastroenterol 2014; 48:318-27.
2. Saritas Yuksel E, Vaezi MF. Extraesophageal manifestations of gastroesophageal reflux disease: cough, asthma, laryngitis, chest pain. Swiss Med Wkly 2012; 142:w13544
3. Iannella G, Vicini C, Polimeni A et al. Laryngopharyngeal reflux diagnosis in obstructive sleep apnoea patients using the pepsin salivary test. Int J Environ Res Public Health 2019; 16(11).
4. Luo HN, Yang QM, Sheng Y, et al. Role of pepsin and pepsinogen: linking laryngopharyngeal reflux with otitis media with effusion in children. Laryngoscope 2014; 124(7):E294-300.
5. Magliulo G, Plateroti R, Plateroti AM. Gastroesophageal reflux disease and the presence of pepsin in the tears.

Med Hypotheses 2013; 80(2):129-30.
6. Iannella G, Di Nardo G, Plateroti R, et al. Investigation of pepsin in tears of children with laryngoesophageal reflux disease. Int J Pediatr Otorhinolaryngol 2015; 79(12):2312-15.
7. Belafsky PC, Postma GN, Koufman JA. Validity and Reliability of the Reflux Symptom Index (RSI). J Voice 2002; 16(2):274-77.
8. Belafsky PC, Postma GN, Koufman JA. Laryngopharingeal reflux symptoms improve before changes in physical findings. Laringoscope 2001; 11:979-81.
9. Wang L, Liu X, Liu YL, et al. Correlation of pepsin-measured laryngopharyngeal reflux disease with symptoms and sign. Otolaryngol Head Neck Surg 2010; 143(6):765-71.
10. Barona Lleo L, Barona- De Guzman R, Krstulovic C, The diagnostic usefulness of salivary pepsin test in symptomatic laryngopharyngeal reflux. J Voice 2018.
11. Park KH, Choi SM, Kwon SU, Yoon SW, Kim SU. Diagnosis of laryngopharigeal reflux among globus patients. Otorinolaryngol Head Neck Surg 2006; 134:81-85.
12. Reichel O, Issing WJ. Impact of different Ph thresholds for 24-Hour dual probe Ph monitoring in patients with suspected laryngopharingeal reflux. J Laryngol Otol 2008 122:485-89.