The Role of Peptest™ inLaryngopharyngeal Reflux

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Abstract

Laryngo Pharyngeal Reflux (LPR) is a term recently described, classified among the "extra-esophageal disorders", caused by another pathology called "Gastro-Esophageal Disease" (GED). Pepsin has been recognized as the true marker and responsible factor of mucosal damage.

The clinical-instrumental diagnosis of LPR is especially based upon video laryngoscopy exam, pH-measuring, pH-impedance measuring, using "score" questionnaires, designed to establish the presence of specific symptoms (Reflux Symptom Index – RSI) and endoscopic signs (Reflux Finding Score – RFS). The evidence of the role of pepsin associated to the LPR helped to develop new technologies, able to detect the presence of that enzyme within the saliva; this device is called Peptest™.

The aim of this study was to correlate the qualitative quantitative values of salivary pepsin (using Peptest™) with symptoms and signs obtained from RSI and RFS. The ultimate goal is to validate this enzyme immunoassay method using LPR diagnostic tools.

Seventy-five patients were recruited (41 females, 34 males) aged between 12 and 71 years old, (average age 45 years). All patients performed anamnesis, including food habits analysis, RSI evaluation, rhinofibrolaryngoscopy exam with RFS calculation and Peptest™.

Highly comparable results were obtained in all of the 75 patients undergoing double detection of Peptest™, with LFD measure (T band intensity) and pepsin concentration (ng/ml).

Statistically significant correlation concerning symptoms and saliva pepsin concentration was demonstrated because the relationship between objective signs and saliva pepsin concentration.

The Peptest™ could become the "Gold standard" in all cases LPR pathology suspicion and may allow revealing the symptoms of that pathology at its early onset, avoiding dealing with more invasive methods and helping to adopt the correct therapeutic follow-up as soon as possible.

Keywords: Peptest™, Laryngopharyngeal Reflux, Gastroesophageal Reflux, Therapy

Introduction

Laryngo Pharyngeal Reflux (LPR) is a described, classified among the "extra-esophageal disorders" caused by another well-known pathology called "Gastro-Esophageal Disease" (GED). The primary insight that can be found in literature, concerning the possible involvement of ENT anatomical structures in the reflux pathology, goes back to 1903; actually, Coffin speculated "gas reflux from the stomach" as well as the "hyper-acidity" might be considered liable for hoarseness and back rhinorrhea. Starting from the '80s, besides the relatively "old" knowledge of GED, a replacement concept arose; it proposed LPR could have been caused by "acid vapors" action, coming from the stomach. Those vapors, after being in contact with the esophageal mucosa and having passed through the Upper Esophageal Sphincter

(UES), reach the upper respiratory tract: larynx, laryngopharynx, and finally the nose sinus area and therefore the tympanic cavity through the auditory tubes.

Over time, several Authors have proposed various etiopathogenetic models in order to justify the damage of upper airways and alimentary canal done by LPR. Pepsin has been recognized as the true marker and therefore the responsible factor of mucosal damage. Pepsin is a proteolytic enzyme originated within the stomach; it is present within the acid vapors. Once in contact with the laryngopharyngeal region, it can undergo an endocytosis process thanks to a specific cellular receptor located upon the mucosal epithelial surface; subsequently, the enzyme could be activated within the Golgi apparatus, causing a sequence of biochemical reactions finally leading to a crucial cell suffering.

Nowadays the clinical-instrumental diagnosis of LPR is mainly based upon video laryngoscopy exam, pH measuring (esophageal, esophagopharyngeal, oropharyngeal), pH impedance measuring, besides the utilization of "score" questionnaires designed to determine the presence of specific symptoms (Reflux Symptom Index – RSI) as well as endoscopic signs (Reflux Finding Score – RFS). However, the growing evidence of the role of pepsin associated to the LPR pathology has encouraged the development of new technologies, able to detect the presence of that enzyme within the saliva; thus leading to a device called Peptest™. This is an innovative diagnostic device, based on immune-enzymatic test (i.e. using monoclonal antibodies) able to detect the presence of pepsin in a saliva sample with a sensitivity and specificity of, respectively, 88 and 87%. It is a rapid and non-invasive test. Pepsin, thanks to this device (Peptest™), becomes a highly predictive marker of reflux pathology, if detected outside the gastric tract.

Evaluation of Reflux Symptom Index (RSI)

During the anamnestic evaluation, each patient had to fill "RSI" questionnaire as designed by Belafsky, in order to evaluate the possibility and/or the seriousness of LPR occurrence. The RSI questionnaire may be a widely used and approved, self-administered, nine - item questionnaire for the assessment of symptoms in LPR patients. The questionnaire consists of questions regarding hoarseness, throat clearing, postnasal drip, difficulty with swallowing, coughing, breathing difficulty, troublesome cough, lump sensation, and heartburn. The patients were requested to attain each symptom from 0 to 5 (0 meant no symptom, 5 very frequent symptoms). Although the authors considered positive for LPR a complete score ≥ 13, we thought it was more meaningful to decrease the value down to 10 (RSI cut-off), consistent with our previous findings (we have notice a positive Peptest™ result at this value). According to our experience, we finally included other significant symptoms for LPR diagnosis, such as: muffled ear feeling, burping; hiccup, nostril burning, conjunctiva disorders (burning and tearing). Rhinofibrolaryngoscopy exam and RFS evaluation.

The endoscopic examination of upper air and digestive ways has been carried out using a flexible rhinofibrolaryngoscope Vision Science (diameter 3.6). No patients required anaesthesiological procedures. Important alterations of RFS calculation are supported endoscopic evaluation of each patient. At this stage, the endoscopist assigned the score (0 to 4). An RFS value > 7 has been assumed as pathologic. Similarly, as far because the objective examination was concerned, we searched for the presence of further signs deemed important in sight of an LPR diagnosis, e.g.: hyperemia of the nasopharynx and of the tube torus, hyperemia of the tonsil pillars and hyperemia of the uvula. Peptest™ All patients performed Peptest™ using their saliva samples. So as to ensure the reliability of the ultimate result, patients didn't take PPI (proton pump inhibitors) or alginate based drugs, during 48h before the test. Furthermore, they didn't get coffee or carbonated soft drinks and didn't smoke: these substances, in fact, have a reflux action. Two separate saliva samples (1 ml each, roughly) were collected during two successive days into graduated test tubes, within 1 hour after the most meal. Those samples not processed, were stored, refrigerated at 4°C with addition of 0.5 ml of 0.01M acid, for not than 3 days. Each saliva sample was processed as follow: 0.5 ml of the sample was centrifuged at 5000 rpm for five minutes for obtaining supernatant; 80 µl of supernatant was added to 240 µl of migration buffer. After vortexing for 10 seconds, 80 µl of the answer was pipetted into the well of the Peptest™ Lateral Flow Device (LFD). The test was read after quarter-hour. The presence of a C band (control) indicates

that the Peptest™ was correctly performed. The looks of a second band (T) means pepsin was present within the test sample.

Since the intensity of the T band is directly proportional to the number of pepsin, the result provided by PeptestTM was both qualitative and quantitative. It's possible to urge a quantitative value using the reader LFDR101®. It's an instrument, whose readout provides the pepsin concentration in ng/ml. because of a special conversion table, we were ready to determine the various pepsin levels originally present within the saliva samples. Finally, so as to see the reliability of the test, two readings were recorded for Peptest™ compared to "ELISA human pepsin detection test" is more specific for LPR. In fact, it's ready to detect pepsin concentration using saliva sample, unlike ELISA rates, in blood sample, precursor protein of this enzyme: type I and II pepsinogen. Peptest™ is additionally considered the most cost effective and therefore the most usable test for LPR, available at the instant.