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Clinical Trial Paper

Detecting laryngopharyngeal reflux in patients with upper airways symptoms: Symptoms, signs or salivary pepsin?

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ABSTRACT

Background: Laryngopharyngeal reflux (LPR) can induce laryngeal hyper-responsiveness, a unifying feature underlying chronic cough and vocal cord dysfunction. The diagnosis of LPR currently relies on invasive oesophageal pH impedance testing. We compared symptoms, laryngeal signs and salivary pepsin as potential diagnostic methods for identifying LPR in patients with upper airway symptoms.
Methods: Symptoms were assessed using the Reflux Symptom Index (RSI) and signs of laryngeal inflammation quantified using the Reflux Finding Score (RFS) during laryngoscopy. Saliva samples were analysed for the presence of pepsin. A sub-group of patients with severe symptoms and signs of LPR were investigated with oesophageal pH monitoring and impedance study.
Results: Seventy eight patients with chronic cough and/or suspected vocal cord dysfunction were recruited, mean (SD) age, 54.6 (15.6) years. The majority (87%) had significant symptoms of reflux (RSI > 13). There were clinical signs of LPR (RFS > 7) in 51% of cases. Pepsin was detected in the saliva of 63% of subjects and 78% of those with a high RFS. Salivary pepsin had a sensitivity of 78% and specificity of 53% for predicting a high RFS. There was a correlation between the RSI and RFS ($r = 0.51$, $p < 0.001$) and between the severity of laryngeal inflammation and the concentration of pepsin ($r = 0.28$, $p = 0.01$). All cases investigated with pH-impedance study had objective evidence of proximal reflux.
Conclusion: Salivary pepsin may be used as a screening adjunct to supplement the RFS in the clinical workup of patients with extra-oesophageal symptoms and upper respiratory tract presentations of reflux.

1. Introduction

Reflux is a commonly reported finding in patients with respiratory disease, with a prevalence as high as 50% in patients with chronic cough, severe COPD and difficult to control asthma [1–3]. Despite increasing evidence for an association between reflux and respiratory symptoms [4–6] the pathological mechanism for this has not yet been established [7]. Laryngeal hyper-responsiveness is one feature proposed to underlie conditions such as chronic cough and vocal cord dysfunction [7] that may be induced by laryngopharyngeal reflux (LPR).

Laryngopharyngeal reflux describes the retrograde flow of gastric refluxate into the laryngopharynx, an area which is highly susceptible to both the acidic and non-acidic components of reflux, where as few as three episodes a week may induce laryngeal inflammation [8]. The symptoms associated with LPR include persistent throat irritation and tightness, dysphonia, globus, excessive mucus production as well as dyspnoea and stridor, with the typical symptoms of dyspepsia or heartburn being less common [9]. Current methods employed in the diagnosis of LPR have inherent limitations. The Reflux Symptom Index (RSI) questionnaire is useful in quantifying severity of symptoms and variance with treatment [10] but may not differentiate LPR amongst other causes of upper respiratory tract symptoms [11]. The Reflux Finding Score (RFS) quantifies the severity of laryngoscopic findings of inflammation [12] but may be exaggerated in the presence of chronic laryngeal irritation from other sources. Twenty-four hour oesophageal pH monitoring detects changes in pH associated with reflux however it is invasive and labour intensive, and simultaneous intraluminal impedance monitoring is required to detect episodes of weakly- or non-acidic reflux [13,14].

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Studies in patients with persistent symptoms despite maximal acid suppressive therapy have established a positive symptom and temporal association between non-acid reflux episodes and symptoms such as chronic cough [15,16]. The presence of pepsin in the laryngopharynx has also been shown to correlate with reflux events [17] and has been found in the laryngeal epithelial mucosa of patients with reflux-attributed laryngeal disease [18,19]. Pepsin has been identified in the sputum and bronchoalveolar lavage fluid of patients with chronic cough and LPR [6,20], and it has been used to indicate aspiration in patients with lung allografts [21] and the effectiveness of anti-reflux surgery [22]. Salivary pepsin has a moderate sensitivity and specificity for the diagnosis of gastro-oesophageal reflux disease in patients with heartburn [23], and its association with scores obtained by clinical diagnostic tools such as the RSI and RFS has been investigated only in smaller studies to date [24,25].

The presence of pepsin in the upper airway is therefore indicative of reflux suggesting that it may be used as a biomarker for the objective assessment of LPR. The aim of this study was to evaluate the correlation and performance of the RSI, the RFS and the presence of pepsin in saliva and examine their role as diagnostic adjuncts in the investigation of patients with predominantly upper respiratory tract symptoms.

2. Methods

2.1. Subjects

Participants were recruited from the Airways Clinic of the Royal Preston Hospital, UK. Patients who had at least eight weeks' history of upper respiratory tract symptoms suggestive of laryngeal hyperresponsiveness, (for example persistent throat irritation or clearing, dysphonia, globus, episodic choking or breathlessness, chronic cough or vocal cord dysfunction) due to be investigated with fibre-optic laryngoscopy, were invited to participate in the study. Patients on treatment for obstructive airways disease or on anti-reflux treatment were included in the cohort. The protocol was approved by the Greater Manchester Research Ethics Committee, (12/NW/0016) and all patients provided written, informed consent.

2.2. Measurements

A flow diagram outlining the study procedures is shown in Fig. 1. Participants completed the self-administered RSI questionnaire, a validated tool which quantifies the nature and severity of symptoms over the previous month. The RSI is made up of nine components each of which is rated on a scale of 0–5, with a score of more than 13/45 considered suggestive of LPR [10]. They were then asked to produce a sample of saliva from their throat. Following this, subjects underwent flexible fibre-optic laryngoscopy to assess for any evidence of laryngeal mucosal inflammation or structural changes, which were quantified using the RFS. The RFS is an assessment tool that quantifies the severity of inflammation and other structural changes seen during laryngoscopy and is based on eight components, with a score of more than 7/26 being suggestive of LPR [12]. Where clinically indicated, patients with symptoms and/or signs of laryngeal inflammation, usually despite previous anti-reflux treatment, were also referred for oesophageal pH studies.

Salivary sample collection: Saliva samples were all collected by one researcher explaining the same technique; subjects were encouraged to produce a deep salivary sample through a throat clearing manoeuvre into a collecting tube containing 0.5 ml of 0.01 M citric acid preserving medium. Samples were collected within four hours of participants' last meal. Samples were anonymised and analysed for the presence of pepsin by an independent investigator blinded to subjects' symptom scores and laryngoscopy findings. All samples were analysed within 36 h of collection.

Pepsin assay: The pepsin assay used in the study was a lateral flow device, the Peptest (RDBiomed, Hull, UK) [26]. Analysis involved extraction of 0.5 mls of each clinical sample, micro-centrifugation and separation of a clear supernatant layer which was then extracted and mixed with a migration buffer and added to the test well of the lateral flow device. The detection monoclonal antibody (specific to pepsin-3) labelled with blue latex beads was solubilised by the clinical sample and carried across a nitrocellulose membrane, sandwiched to the capture monoclonal antibody. A positive result resented as a blue test line showing on the device after an analysis time of 15 min. A control line formed by a third antibody in the presence of pepsin confirmed the validity of the result. Pepsin concentration was quantitated in ng/ml by measuring the intensity of the test line against the standard curve of human pepsin-3, with a lower limit of detection of 25 ng/ml [26].

Flexible fibre optic laryngoscopy: This was performed using the Pentax FNL-10RP3 flexible fibre-optic laryngoscope (Pentax Medical, Hamburg, Germany) and laryngeal inflammation and structural changes quantified using the RFS by a specialist respiratory consultant blinded to the results of the pepsin assay.

Oesophageal pH study: 24 h oesophageal pH-monitoring was performed using a dual channel antimony catheter. The distal pH probe was positioned 5 cm above the lower oesophageal sphincter (LOS) and the upper probe was placed in the proximal oesophagus 2 cm below the upper oesophageal sphincter using manometry. Multichannel intraluminal impedance pH study (Impedance catheter and Bioview analysis; Sleuth system, Sandhill Scientific Inc, Oxon, UK) was performed using an antimony catheter with six paired impedance rings positioned 3, 5, 7, 9, 15 and 17 cm above the LOS and pH electrodes positioned at 10 cm below and 5 cm above the LOS as determined by manometry. Patients were asked to record medication and meal times, symptoms and periods they were supine and upright but otherwise to maintain normal activities. Reflux events were identified as acidic when pH < 4 and weakly acidic when pH > 4 with an associated rapid drop in intraluminal impedance progressing proximally; gas events were identified when impedance increased in all channels, and gas/liquid events when there was a combination of both. Proximal reflux events were identified when the signal reached the proximal two electrode rings. Patients were maintained on anti-reflux treatment during the study.

2.3. Statistical analysis

Performance characteristics were calculated for the pepsin assay versus a clinical diagnosis of LPR based on an RSI > 13, RFS > 7 and impedance–pH study results. Mann–Whitney U test was used to test for significant between group differences in symptoms, signs of inflammation and pepsin concentrations for anti-reflux treatment and quantified pepsin levels; chi-squared test was used for categorical parameter. Correlations between symptoms, signs, pepsin concentrations and reflux events were assessed using Spearman's rank correlation in StatsDirect (version 2.7.9, StatsDirect Ltd, Cheshire, UK).

3. Results

Seventy eight subjects were recruited into this study (mean [+/−SD] age, 54.6 [+/− 15.6 years]), 24% male. In 68 patients the indication was for assessment of possible vocal cord dysfunction, which was confirmed in 45 subjects, and the remaining 10 for chronic cough. Thirty patients had concomitant asthma, and 42 were taking at least one form of anti-reflux therapy.