Diagnosis of Reflux Disease using Pepsin as a Marker

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Introduction: Laryngopharyngeal reflux (LPR) is the reflux of gastric contents above the upper oesophageal sphincter and into the larynx, pharynx and upper airways. The symptoms include vocal dysfunction, hoarseness, chronic cough and dysphagia. Reflux is implicated in a range of airway disorders including otitis media with effusion¹, sinusitis, lung allograft rejection, asthma ², sudden infant death syndrome ³ and laryngeal cancer ⁴.

The current ‘gold standard’ for diagnosis of reflux disease is 24 hour double probe ambulatory pH monitoring. This is an invasive technique that detects changes in pH to below 4 in the oesophagus or laryngopharynx. Evidence shows that acid is not the damaging component of the refluxate. Pepsin has been shown in many studies to cause significant damage to the oesophageal ⁵-⁸ and laryngeal mucosae ⁹, ¹⁰.

Objectives: The aim of this project is to develop a range of non-invasive, commercially viable, diagnostic tests for reflux disease. These tests will detect pepsin, a more appropriate marker for reflux disease than pH.

Methods: Samples of sputum/saliva were collected (into 0.5 ml 100 mM citric acid) from patients with chronic cough suspected of being caused by reflux disease when attending clinic.

Pepsin activity was assessed using a modified colorimetric assay to detect newly-formed N-terminals from proteolytic digestion of a protein substrate ¹¹ using microtitre plates for high through-put.

Levels of pepsin protein were assessed using an indirect sandwich ELISA with specific antibodies to human pepsin 3b. This method detects the presence of active and inactive pepsin.

Results: In an initial study 10 out of 19 samples (53%) from chronic cough patients were significantly positive for pepsin activity ranging from 0.4 -4.2 µg/ml with a mean (±SE) of 1.6 (±0.4) µg/ml.

In a larger study 23 out of 107 samples (21.5%) were significantly positive for pepsin activity ranging from 0.1 - 3.7 µg/ml with a mean (±SE) of 1.0 (±0.2) µg/ml.

84 samples were tested for pepsin protein using the more sensitive pepsin ELISA method. 17 samples were positive for pepsin (20.2%).
**Conclusions:** Pepsin can be successfully measured and quantitated in human sputum/saliva samples on a lab scale with high throughput. Using two simple methodologies it is possible to detect both active (and damaging) pepsin and the presence of pepsin protein (previously damaging). Samples are collected non-invasively and the results are more relevant to reflux disease pathology than measuring pH with the invasive pHmetry technique. It is anticipated that if samples are obtained when reflux symptoms occur that more pepsin positive samples will be seen thus providing an effective diagnostic test.

**References:**


