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Validation of Peptest™ in Patients with Gastro-Esophageal Reflux Disease and Laryngopharyngeal Reflux Undergoing Impedance Testing

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ABSTRACT

Background & Aims: Pepsin in the gastric refluxate is a marker for a prior reflux event and rapid detection might be achieved using the Peptest™, an *in vitro* diagnostic medical device. The aim of this study was to validate the use of Peptest™ to reliably diagnose reflux in patients with gastro-esophageal reflux disease (GERD) and laryngopharyngeal reflux (LPR) disease diagnosed with multichannel intraluminal impedance/pHmetry (MII-pH).

Methods: 20 reflux patients were recruited of whom 10 had classical GERD and 10 had LPR. All patients underwent MII-pH and provided expectorated saliva samples when a MII-pH reflux event was observed, or reflux symptoms were experienced, and all were tested for the presence of pepsin using the Peptest™.

Results: Pepsin was detected in 31 out of 45 samples (68.9%). At least 1 positive pepsin result was seen in 16 patients (80%) and this was the same, irrespective of the GERD or LPR diagnosis. Peptest™ had a positive predictive value of 69% to detect MII-pH reflux events.

Conclusions: Peptest™ is a good first-line diagnostic procedure to use in reflux sufferers to confirm the presence of reflux.

Key words: gastro-esophageal reflux disease – laryngopharyngeal reflux – multichannel intraluminal impedance – pepsin – Peptest™.

Abbreviations: GERD: gastro-esophageal reflux disease; GI: gastrointestinal; LPR: laryngopharyngeal reflux; MII-pH: pH-monitoring combined with multichannel intraluminal impedance; PPV: positive predictive value; RFS: reflux finding score; RSI: reflux symptom index.

INTRODUCTION

The diagnosis of gastro-esophageal reflux disease (GERD) is usually made by invasive tests including upper gastrointestinal (GI) endoscopy, 24-hour ambulatory pH-monitoring and pH-monitoring combined with multichannel intraluminal impedance (MII-pH) [1-3]. Gold standard methodology does not exist. In the case of laryngopharyngeal reflux (LPR) the diagnosis is difficult, the first line diagnostic strategy of the otolaryngologist being the visualisation of the larynx using fibreoptic laryngeal examination. Quantification of the lesions can be assessed using the Reflux Finding Score (RFS) [4]. The use of specific questionnaires is very common in case of ear, nose, throat (ENT) symptoms [5-7]. Use of pHmetry and MII-pH testing is useful in the diagnosis of LPR [8, 9], pharyngeal pHmetry being also available [10]. However, their value is questionable, and studies have not been able to prove diagnostic capability.

Pepsin, secreted into the gastric juice, is an excellent marker of reflux [11]. There is a range of techniques available to detect pepsin in refluxates including enzymatic assays, immunohistochemistry, Western blot and ELISA, all of which are time consuming and require technical knowledge. Rapid lateral flow tests to detect pepsin are the most recent addition to the diagnostic portfolio. Peptest™ is an *in vitro* diagnostic medical device in the form of a lateral flow test that uses two unique monoclonal antibodies to human pepsin. Peptest™ enables the rapid detection of pepsin in a clinical sample of refluxate (e.g. expectorated saliva). It has been validated in LPR patients compared to healthy controls [12-15] and its sensitivity and specificity are established [16].

The aim of the study was to validate the reliability of Peptest™ to measure pepsin in expectorated saliva samples of GERD and LPR sufferers against reflux events documented by on-line MII-pH when reflux occurs.

METHODS

Study cohort

Twenty consecutive patients 18 years or older were prospectively recruited into the study. Ten patients presented with GERD (heartburn and/or regurgitation at least once a week) and were referred for reflux testing by 24-hour multichannel intraluminal impedance pH-monitoring (MII-pH). Patients with esophageal motility disorders, malignancy, previous upper GI surgery or pregnancy were excluded. Ten patients with ENT symptoms including LPR symptoms or chronic cough were included, all without associated classical GERD symptoms. They were diagnosed with LPR if they had >13 Reflux Symptom Index (RSI) and >7 RFS. All patients were recruited from Ege University Medical School, Reflux Outpatient Clinic, Izmir, Turkey. The study was conducted in accordance to the ICH GCP guidelines under the Declaration of Helsinki. All subjects provided informed written consent and ethical approval was obtained from Ege University clinical research Ethics Committee (reference number 12-12/11).

Peptest™

The patients were asked to clear their throat, to cough up the resultant saliva and to spit it into a 30 ml universal collection tube containing 0.5 ml of 0.01 M citric acid (pH 2.2-2.8). Saliva samples were collected approximately 5 minutes after experiencing a reflux event or identifying a reflux event on the impedance trace. Saliva samples were centrifuged (4000-7000rpm / 2500g for 5 minutes) and 80 µl of supernatant were mixed with 240 µl of migration buffer. The presence of pepsin was determined by applying a further 80 µl of the saliva: buffer mixture to the well of the Peptest™ lateral flow device (Peptest™, RD Biomed Ltd, Hull, UK). After 15 minutes, the presence of a discreet blue band at the Test (T) line indicated the presence of pepsin and the intensity of the band was proportional to the concentration of pepsin (from 16 ng/ml to 500 ng/ml). The appearance of a blue band at the 'C' (control) line within the window of the lateral flow device indicated that the test had been successful [17].

24 hours MII-pH

Patients were required to stop acid suppression medication for 7-10 days prior to MII-pH testing. Esophageal manometry was performed in fasting patients to locate the lower esophageal sphincter (LES). The impedance-pH catheter was inserted intranasally under local anaesthetic. The lower esophageal pH sensor was placed 5 cm above the LES. There were 8 impedance sensors positioned at 2, 4, 6, 8, 10, 14, 16 and 18 cm above the pH sensor at the tip. Data was recorded onto a portable recording system (Ohmega, MMS Inc., The Netherlands) and the hardware was connected to a computer for online evaluation. Patients remained in the clinic and stayed on-line for the duration of the sample collection procedure. For each expectorated saliva sample the presence of reflux symptoms and the presence of a reflux event detected by MII-pH within the previous 5 minutes were noted. A subset of patients who were fully conversant with the saliva collection procedure provided their samples at home within 5 minutes of experiencing a reflux symptom. These saliva samples were stored at 4°C in a fridge prior to returning them for laboratory analysis within 48 hours of collection. Data was extracted regarding the pH and height of any reflux event preceding collection of the saliva sample. Standard MII-pH analysis was performed including % time pH < 4 and DeMeester score (>14.72 indicates pathological reflux). The reflux events were classified as acidic (pH<4), weak acidic (pH 4-7) or non-acidic (pH>7).

Questionnaires

Patients completed the GERD outpatient clinic routine questionnaire (demographic data) and the Reflux Symptom Score (RSS) devised by Locke et al. in 1994 [18] and translated and validated into Turkish [19]. The RSS questionnaire of Locke et al. [18] is specifically designed to measure the level of clinical psychosomatic research and has been fully validated in the English language. The questionnaire covers the frequency, duration and severity of heartburn and regurgitation and produces a definitive scale with a score ranging from 0 – 24 . A RSS value greater than 13 indicating a positive symptomatic response.

Statistical analysis

2 x 2 contingency tables were analysed by Fisher's exact test. All other contingency tables were analysed by the Chi-squared test. Data was considered statistically significant if $p<0.05$. Sensitivity and positive predictive value (PPV) were calculated. Sensitivity corresponded to the fraction of those with the disease/condition correctly identified as positive by Peptest™. PPV defined the fraction of people with positive Peptest™ that actually have the condition/disease.

RESULTS

There were 20 patients recruited into the study (10 GERD, 10 LPR) with a mean (SD, range) age of 40.3±13.2 (19-65) years of whom 8 were female and 12 were male. There were 9 non-smokers, 5 current smokers and 6 ex-smokers and all either did not drink alcohol or were minimal (< 10 units of alcohol per week) users. The mean (SD, range) BMI was 25.8±4.6 (17.7-34.6). The mean (SD, range) RSS was 16.1±3.7 (9-24). *Helicobacter pylori* status was known for 15 patients (10 were negative, 3 positive and 2 previously eradicated).

Upper GI endoscopy detected esophagitis in 3 patients and gastritis in 11. Six were endoscopically normal (one with a hiatal hernia, which was surprisingly low in frequency when considering this group of patients). Based on assessment by MII-pH, 6 patients were considered normal and 14 considered to have pathological reflux (8 LPR and 6 GERD patients, pH<4 for >5% of the time or DeMeester score >14.72) and the outcomes of the MII-pH testing are described in Table I. Although the patient numbers are low, a greater number of the LPR patients presented with pathological reflux compared to the GERD group. On further breakdown 8 out of 10 patients in both the GERD and LPR groups presented with a RSS greater

Table I. Results from 24-hour MII-pH testing of reflux patients.

	Percentage time of acid reflux episodes (pH < 4)			DeMeester score			Number of non-acid reflux episodes			Number of weak acid reflux episodes		
	GERD	LPR	Overall	GERD	LPR	Overall	GERD	LPR	Overall	GERD	LPR	Overall
Mean	9.7	16.9	13.3	31.1	55.5	43.3	33.5	18.6	26.1	1.9	2.7	2.1
SD	7.9	22.2	16.6	25.2	71.4	53.6	20.1	9	17	1.5	2.3	1.6
Range	1-21.2	1.2-77.3	1-77.3	4.2-70	4.43-248	4.2-248	15-82	2-33	2-82	0-5	0-4	0-5

GERD: gastro-esophageal reflux disease; LPR: laryngopharyngeal reflux

than 13, suggestive of reflux symptoms. On further investigation 75% of the patients in each group were pepsin positive.

Peptest™

The number of expectorated saliva samples provided for pepsin analysis by Peptest™ ranged from 1 to 7 samples (mean 2.35). The total number of samples in the final analysis was 45 and the results are described in Table II. Out of the 45 samples, pepsin was detected in 31 samples (68.9%). There was no difference in the number of samples that were positive for pepsin when grouped as LPR (65.2%) or GERD sufferers (72.7%).

Table II. Peptest™ results.

Patients	Number of samples	Positive pepsin samples	Negative pepsin samples
GERD (n=10)	22	16	2
LPR (n=10)	23	15	2
Total	45	31	4

Data was analysed according to the patient and 11 patients had only 1 sample, but 9 patients had between 2-7 samples. At least 1 positive pepsin result was seen in 16 patients (80%) and this was the same irrespective of diagnosis (80% pepsin positive for GERD and 80% pepsin positive for LPR).

Three patients collected saliva samples 5, 10 and 15 minutes after a substantial acid reflux event. In two cases, a positive pepsin result was seen in all three samples indicating that pepsin may linger in the esophagus / pharynx / larynx for valid testing by Peptest™ up to 15 minutes after a reflux event. In the third case, a negative pepsin result was seen in all three samples.

Peptest™ association with symptoms and MII-pH

A saliva sample was provided shortly after experiencing symptoms in 41/45 (91.1%) and a recent MII-pH reflux event was noted in 40/45 (88.9%) samples.

Data was collated for Peptest™ positive or negative samples according to the presence of symptoms (heartburn, regurgitation or cough) (Table III) and to the detection of an MII-pH reflux event (Table IV). If a patient had reflux symptoms then the Peptest™ was 68.3% more likely to objectively identify them and therefore excluded the need for an expensive and invasive test such as a MII-pH or endoscopy. Likewise, if someone had an objective reflux event, then the Peptest™ was 67.5% more likely to identify it. MII-pH had a sensitivity of 89.7% to record a reflux event when the patient had a symptom (not shown); the PPV was 87.5%. Number of tests should also be taken into consideration.

Table III. Contingency table of Peptest™ results and reflux symptoms.

		Peptest™		
		Positive	Negative	Total
Reflux symptoms	Yes	28	13	41 (91.1%)
	No	3	1	4
	Total	31 (68.9%)	14	45

Sensitivity 90.3%, PPV = 68.3% p=1.000

Table IV. Contingency table of Peptest™ results and a MII-pH noted reflux event.

		Peptest™		
		Positive	Negative	Total
MII-pH reflux event	Yes	27	13	40 (88.9%)
	No	4	1	5
	Total	31	14	45

Sensitivity 87.1%, PPV = 67.5% p=1.000

The 24-hour MII-pH testing detected 14 patients with pathological reflux (pH<4 for >5% of the time or DeMeester score >14.72). Positive pepsin was seen in 78.5% of the patients with pathological reflux and 83.3% of those classified as normal by MII-pH (Table V). When subdivided by a diagnosis of LPR (87.5% pathological positive) or GERD (66.7% pathological positive) similar results were obtained. There was no association between the height of the reflux event and the presence of pepsin (p=0.6282). There was no association between the RSS symptom score and the presence of pepsin (p=0.1184).

Table V. Contingency table of Peptest™ results and 24-hour MII-pH testing for individual patients.

		Peptest™		
		Positive	Negative	Total
MII-pH	Pathological	11 (78.5%)	3	14
	Normal	5 (83.3%)	1	6
	Total	16	4	20

Sensitivity 68.8%, PPV = 78.6% p =1.000

DISCUSSION

This study aimed to validate pepsin detection in expectorated saliva by the Peptest™ in 20 reflux patients; the reference method was MII-pH impedance. Reflux events within the previous 5 minutes were objectively identified by MII-pH in 90% of samples and in the remainder, a reflux symptom was

noted prior to their saliva sample being collected. There were 10 classical GERD patients and 10 LPR patients in the study population providing 45 saliva samples for pepsin detection. There were 69% positive samples for pepsin; 80% of the reflux patients having at least one single pepsin positive sample.

Interestingly, there was no difference in the proportion of pepsin positive samples in the subgroup of patients with classical GERD or LPR (with the limitation of small size of investigated groups, n=10). It could be presumed that reflux events that were not identified by Peptest™ were likely to be classical GERD symptoms (heartburn and regurgitation) determined by a distal refluxate that could not be identified by Peptest™ in expectorated saliva. But in this small study there was no association between pepsin detection and the height of the reflux event. It is important to note that MII-pH is considered one of the best diagnostic tools for GERD but not for LPR (especially when using GERD-based pathological parameters). The positive predictive value for Peptest™ to detect pepsin in samples of those with reflux symptoms was 68% and the same in those samples with a MII-pH documented reflux event.

Lack of symptoms does not mean lack of reflux or pepsin in the saliva. An awareness of 'silent reflux' needs to be taken into consideration and those with LPR may be unlikely to have discrete symptoms that can be noted [20]. Therefore, a population with negative symptoms/positive pepsin is not improbable. Pepsin was detected in 3 out of 4 samples that did not report a reflux event with a similar proportion in MII-pH negative samples (4/5).

Spyridoulias et al. [21] evaluated with different diagnostic tests a heterogeneous group of patients with a chronic cough and possible vocal cord dysfunction. Patients with a high reflux finding score exhibited more pepsin in their saliva (78%). Pepsin was positive in the saliva of 63% of subjects. They calculated that salivary pepsin had a sensitivity of 78% and specificity of 53% for predicting a high RFS with a significant correlation between the RSI and RFS ($p < 0.001$).

Few studies have reported the use of 24 hours MII pH monitoring in patients with LPR [22]. Sereg-Bahar et al. [23] compared 24 LPR patients with 48 controls. They measured pepsin with two different methods, immunologic and enzymatic, and found a significantly higher level of total pepsin in LPR group, but pepsin enzymatic activity was not different [23]. Hayat et al. [24] evaluated different diagnostic methodologies including Peptest™ in patients presenting with GERD, hypersensitive oesophagus and functional heartburn. High levels of pepsin were found in the GERD patients and patients with hypersensitive oesophagus; in contrast, low pepsin levels were observed in the functional heartburn patients and in a low number of the healthy controls. The overall sensitivity was shown to be of 77.6% with a specificity of 63.2%.

There are some limitations in our study. First, there was a lack of 'true controls', thereby the specificity based on low numbers of putative control patients (i.e. 4 without reflux symptoms or 6 without MII-pH event) could not be determined. Other limitations are the small sample size of patients and saliva samples. In this study, we did not quantify pepsin concentration, but this is achievable with Peptest™ when using a lateral flow device reader calibrated with a pepsin standard curve. A positive pepsin result by Peptest™ in this study was related to a concentration of greater than 16 ng/ml pepsin. More recently, Peptest™ was validated in a large multicentre study in China consisting of 1032 patients. The overall sensitivity value was 85% with a specificity of 60% [25].

Based on these studies, the Peptest™ might be used as a first line diagnostic tool for reflux patients without the need to undergo invasive diagnostic tests in the majority of patients. Also it might be indicated in symptomatic patients with negative diagnostic tests. This remains the subject of on-going validation studies.

CONCLUSION

Pepsin detection by Peptest™ and validated in our GERD and LPR patients undergoing MII-pH for the reliability to detect a reflux event demonstrated that Peptest™ is a good first-line diagnostic procedure to use in reflux sufferers to confirm the presence of reflux.

Conflicts of interest: P.W.D. is a director of RD Biomed Limited. A.D.W. and J.F. are employed by RD Biomed Limited.

Authors' contributions: S.B., R.V., P.W.D.: concept and design; D.C, J.F.: administrative support; D.C., R.V, S.B.: provision of study materials and/or patients; A.D.W., J.F.: collection and assembly of data; A.D.W., J.F., PWD: data analysis and interpretation; S.B., P.W.D., J.F.: manuscript writing. All authors approved the final version of the manuscript.

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