A pilot study demonstrating the evidence for reflux disease in patients presenting with non-allergic rhinitis (NAR)—reflux disease in association with non-allergic rhinitis

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Background: To overcome the misdiagnosis of reflux disease and provide subjects with the appropriate treatment determine the link between non-allergic rhinitis (NAR) and reflux disease through analyzing pepsin concentration in patients presenting with NAR using the reflux diagnostic test, Peptest.

Methods: Patients with negative allergen skin prick tests and CT scans of the sinuses were recruited from ENT outpatient visits. All patients provided an eosinophil count and underwent nasal endoscopy with all participants completing the Reflux Disease Questionnaire. Participants (31 patients and 42 healthy) provided one postprandial saliva sample and patients with NAR provided nasal lavage and additional saliva on waking and post-symptom for the determination of pepsin concentration by Peptest. Pepsin concentrations >25 ng/mL were considered positive and eosinophil counts <5% normal.

Results: A positive pepsin result was detected in all patients (100%), with the most frequent detection being in the postprandial sample (70.79%). A significant difference for pepsin concentration was determined in all NAR samples when compared to nasal lavage (P values; 0.0324, <0.001 and 0.0015). A lack of significant difference (P=0.9450) displayed the eosinophil count has no true effect associating NAR with reflux disease. A high sensitivity (87.1%), specificity (61.9%), positive predictive value (62.8%) and negative predictive value (86.7%) were determined using Peptest.

Conclusions: Patients with NAR displayed positive for reflux disease due to the significant concentration of pepsin present, specifically in the postprandial sample. The ease of Peptest provided quick and cost effective results and provided a clear link between NAR and reflux-disease.

Keywords: Non-allergic rhinitis (NAR); pepsin; Peptest; pilot study; reflux

Introduction

Non-allergic rhinitis (NAR) is a growing, adult onset disease (1) most common between patients aged 30–60 years (2). The disease affects up to 30% of individuals in the Western population (3). Thirty percent of all rhinitis cases are diagnosed to be NAR despite being poorly understood due to the convoluted, non-recognizable mechanisms which trigger the reaction (4). NAR is often described through a series of chronic nasal symptoms including obstruction, rhinorrhoea and postnasal drip (4-6). These heterogeneous symptoms occur in relation to non-allergic and non-infectious triggers such as environmental changes including the weather, exposure to caustic odours, cigarette smoke and barometric pressure differences (1).

The backflow of gastric contents including acid and pepsin into the esophagus is termed gastroesophageal reflux (GER) (6-8). Today, we recognize that reflux reaches beyond the esophagus causing extra-esophageal symptoms such as laryngitis, asthma and chronic cough, defined as extra-esophageal reflux (EER) or laryngopharyngeal reflux (LPR) (9). LPR is viewed as a subtype of gastroesophageal reflux disease (GERD) by gastroenterologists or a very different disease amongst ear nose and throat (ENT) and respiratory specialists. It has been found that many reflux induced extra-esophageal symptoms including hoarseness, throat clearing and postnasal drip (10) are very similar to those of patient's suffering from NAR. Due to these similarities in symptoms, it is believed that LPR is an aetiology and increasing contribution towards diagnosis of NAR (4), however to date there is no data present about the role of reflux in this condition.

We aim to confirm the hypothesis and to evaluate the possible association between GERD and NAR with a non-invasive in-vitro diagnostic tool, Peptest. This study will be performed using clinical samples of patient's saliva and nasal lavage.

Methods

Recruitment

Thirty-one patients (22 males and 9 females) were recruited during their ENT outpatient visits to The Affiliated Jinling Hospital of Nanjing University Medical School and The Affiliated BenQ Hospital of Nanjing Medical University upon their consent. All patients were presenting typical NAR clinical symptoms and underwent nasal endoscopy. An allergen skin prick test and CT scan of the sinuses were conducted to rule out allergic rhinitis and sinusitis respectively. Demographical characteristics were taken for 16 of the 31 patients (*Table 1*).

A healthy control group (n=42, 11 males and 31 females) was recruited from Tongji Hospital at Tongji University School of Medicine, providing they achieved a score of 0 on completion of the Reflux Disease Questionnaire (RDQ). The age ranged between 22–66 years and the mean and median ages were 37 years and 34 years respectively (*Table 2*).

Questionnaire

All participants were asked to complete the Reflux Disease Questionnaire (RDQ) (11,12).

Salivary pepsin

Collection

All participants (patients and control group) provided one postprandial saliva sample, with the patients suspected of NAR providing a further three samples. These included two additional saliva samples taken in the morning and after any patient self-reported symptoms and one nasal lavage sample collected on attendance to the clinic. Participants were informed not to take any medication during the study period and not to consume caffeinated or carbonated drinks 60 minutes prior to providing a sample.

Any morning samples were collected on waking before eating and cleaning teeth, provided postprandial; (pp) 60 minutes after finishing a meal and finally the third sample was provided 15 minutes after experiencing symptoms.

All the saliva and nasal samples were stored and refrigerated at 4 ℃ before being analyzed for the presence of pepsin using Peptest (RD Biomed Limited, UK).

Each sample was collected in a 30 mL collection tube containing 0.5 mL, 0.01 M citric acid, which acted as an antibacterial agent and stabilized the sample at an acidic pH to prevent pepsin auto-digestion (13).

Analysis

Collection tubes were centrifuged at 4,000 rpm for 5 minutes until a clear supernatant layer was visible. If not, the samples were centrifuged again, and 80 µL from the surface layer of the supernatant sample was drawn up into an automated pipette. The 80 µL sample was transferred to a screw-top microtube containing 240 µL of migration buffer solution. This sample was mixed on a vortex mixer for 10 seconds. A second pipette was used to transfer

Table 1 Demographical characteristics of all patients

Parameter	N=31
Male/female	22/9
Age range	21–60
Average age	32
Height range (cm)	155–182
Weight range (kg)	50–85
Eosinophil count range	0–90
Participants with a history of smoking	3
Participants with a history of drinking	1
Participants that prefer spicy cuisine	8
Failure to provide additional information	13

Table 2 Demographic characteristics of all healthy control subjects

Parameter	N=42
Male/female	11/31
Age range	22–66
Average age	37
Participants with a history of smoking	6

80 μL of the sample to the circular well of a lateral flow device (LFD) (*Figure 1*) containing two unique human monoclonal antibodies; one to detect and the other to capture pepsin in the saliva or nasal lavage samples (Peptest, RD Biomed Limited, UK).

Fifteen minutes after introducing the clinical sample for pepsin analysis into the well of the Peptest, the lateral flow device was placed into the Peptest reader to determine the intensity of the pepsin test line. Pepsin concentrations >25 ng/mL were considered positive.

Eosinophil count

Collection
A clinician gently pushed a swab through the nose of each NAR presenting patient into the nasopharynx whilst rotating; ensuring an adequate amount of nasal lavage was collected.

Analysis
Swabs were rolled onto glass microscope slides transferring the specimen. The specimen slide was dried and dipped into CAMCO STAIN for 10 seconds before being transferred into distilled water for 20 seconds.

The specimen slide was placed under a microscope and observed through a high dry lens and morphology confirmed using a high oil lens before referring to Clinical Haematology Atlas (14).

Eosinophil counts less than 5% were considered normal.

Statistical analysis

Data was determined as mean and standard deviation (SD). Statistics were calculated using GraphPad Prism 7. For comparisons, an unpaired t test was carried out to determine P values (*Figure 2*) with a Pearson test of correlation and a Chi-square test performed where appropriate.

Results

Pepsin was detected in all 31 NAR patients in at least one sample provided. A higher level of pepsin was found in the postprandial sample (mean 142 ng/mL) compared to the morning sample (mean 67 ng/mL), P=0.0126. Pepsin levels greater than 25 ng/mL, were detected in 22 subjects (70.97%) in the postprandial test and 13 (41.94%) in the morning sample, suggesting that food has an effect on inducing reflux and increasing pepsin levels within the body. Of all the subjects, 1 patient (3.23%) had positive Peptest results for all four samples, 11 patients (35.48%) had positive results for 3 out of 4 samples, a further 11 patients (35.48%) were positive in two of the samples and 8 (25.81%) had tested positive in only one sample.

Pathological concentrations of pepsin were considered to be greater than 75 ng/mL. A total of 39/93 (41.94%) saliva samples and 4/31 nasal lavage samples (12.9%) were greater than this concentration in the patient samples. In the healthy control group there were 16 out of 42 positive postprandial saliva samples (38.1%). Fifteen of these positive saliva samples (35.7%) had a concentration greater than the 75 ng/mL cut off.

Not one NAR patient scored negatively for pepsin detection in all 4 of the samples. The average age for those who were determined positive in at least three of the samples was slightly lower than those who obtained positive in a maximum of two samples (29 versus 39 years) proving that age was not an essential cofactor in this study.

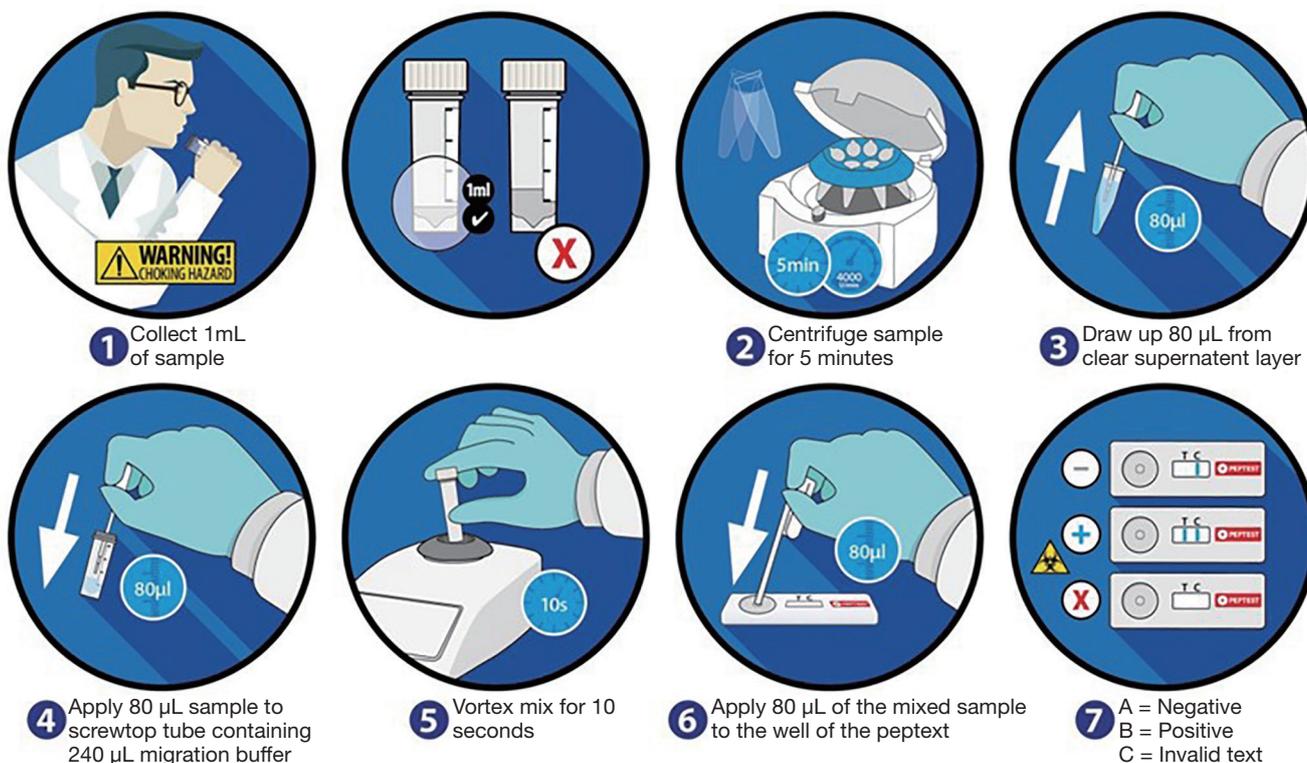The mean RDQ score for NAR patients was 5.16 with only two subjects with a score equal to or greater than

Figure 1 Collection of saliva sample and testing procedure for the use of Peptest.

Table 3 Average response scored from self-evaluation Reflux Disease Questionnaires

Average response ranges	Total number of responses
0–2	10
3–5	8
6–8	6
9–12	6
13+	1
Mean response	5.16

12—the cut-off point for GERD diagnosis, *Table 3*.

Fifteen of thirty-one (48.39%) nasal irrigation fluid samples analyzed for the presence of pepsin were positive. The mean pepsin concentration for the nasal lavage sample group was low at 25 ng/mL. The mean pepsin concentrations in the three saliva samples were all significantly different (P=0.0324, <0.0001 and 0.0015) to the mean pepsin concentration seen in the nasal irrigation fluid sample (*Table 4*). *Figure 2* is a graphical representation of the pepsin concentration found in NAR patients present in this study.

An eosinophil count was taken for all 31 NAR patients and their score recorded. The mean response for the number of eosinophils was high with an abnormal value of 23.48; however 18 of 31 subjects (58.06%) had a count within the normal, healthy range (*Table 5*).

The comparison between the healthy control group and NAR patient postprandial saliva samples displayed visual differences. Greater quantities of positive samples were detected in postprandial patient samples (22/31) compared to the healthy control group (n=15). Similarly as predicted, the healthy control group had 27 negative samples whereas the patient population had 9.

The mean concentration in the postprandial samples of the NAR patient group compared to the healthy control group is shown in *Table 6*.

A Chi-square test was performed and sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were determined, *Table 7*.

Discussion

As the link between NAR and reflux disease is yet to

Table 4 Finalized test results and statistical comparison between saliva and nasal lavage samples

Parameters	Number of participants with samples within the concentration range			
	Morning	Postprandial	After symptom	Nasal irrigation
Concentration ranges (ng/mL)				
0–50	17	10	15	26
51–100	5	5	4	4
101–150	4	2	4	0
151–200	0	4	3	1
201–250	2	3	1	0
251–300	1	3	1	0
301–350	0	2	1	0
351–400	1	1	0	0
401+	0	1	2	0
Test failure	1	0	0	0
Mean concentration (ng/mL)	67	142	105	25
Standard deviation	99	126	129	38
P values*	0.0324	<0.0001	0.0015	N/A

*, in cases where the subject did not provide additional demographical data, they were recorded as a neutral value (0), with the amount of failure to complete subjects stated.
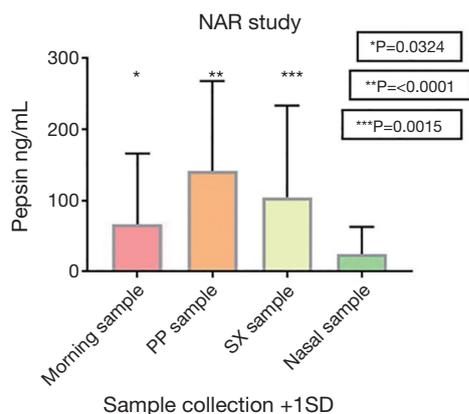

Figure 2 Statistical evaluation displaying pepsin concentrations over a range of samples taken at specific different, time intervals. All data presented to 1 standard deviation (SD).

Table 5 Average number of eosinophils present in patients presenting with non-allergic rhinitis (n=31)

Average response ranges	Total number of responses
0	1
1–4	18
5–10	2
11–30	2
31+	8
Mean response	23.48

be confirmed, a key indicator whether there is a strong association is through the detection of pepsin. As pepsin is a digestive enzyme that is only produced in the stomach, it is detectable due to its large size (6,7). As a result of pepsin being generated in one location within the body (12), concentrations found within nasal and saliva samples is strong evidence that reflux is taking place, potentially resulting in GERD, LPR and respiratory diseases.

By using Peptest, a lateral flow device designed to detect the presence of pepsin, it displayed many of the test samples contained the enzyme pepsin (*Table 4*). This suggested that reflux and pepsin may be the contributing factor for a common clinical condition like NAR where the exact cause is still unknown. Therefore, the data gathered in the study shows the benefit of testing NAR patients for the presence of GERD and LPR.

Table 6 The mean pepsin concentration (ng/mL) in the postprandial samples in NAR patients (n=31) compared to the postprandial pepsin concentration in the healthy control group (n=42)

Groups	Mean response (ng/mL)
NAR patients (n=31)	142
Healthy control (n=42)	45

Table 7 Recorded sensitivity, specificity, positive predictive values and negative predictive values for patients presenting with non-allergic rhinitis (n=31)

Variables	%
Sensitivity	87.1
Specificity	61.9
PPV	62.8
NPV	86.7

As LPR is often the underlining cause for all rhinitis cases, the progression and development of NAR implies the patient has being suffering from LPR related diseases for an extensive period of time. This may potentially cause unnecessary distress due to the incorrect treatment being used (2,15).

Due to pepsin being a digestive enzyme, it is expected that food intake will cause an increased effect in its concentration, during the digestion process (16). This expectation is emphasized by the results from patient's postprandial samples which are calculated to be significantly different to their morning samples (*Figure 2*), with a P value of 0.0126. Furthermore, 16 out of 42 healthy control samples had a positive result for pepsin, with 15 of these samples having a positive result >75 ng/mL. This further demonstrates food intake and diet can have an increased effect towards the levels of pepsin found within the body following a reflux event. Research has found the level of pepsin present within a sample is dependent on the time of collection and the symptoms experienced prior to the sample being taken (17), potentially affecting the results generated causing the significant difference in NAR patient samples to be present.

As eosinophils are normally found within the lamina propria, their presence in the epithelia of the GI tract implies a pathological condition (GERD) is present (18). Through analyzing the percentage of eosinophils in the NAR subjects, it will imply whether the patient is experiencing GERD due to the abnormal eosinophil count detected.

As there is not a visual correlation between those with an abnormal eosinophil count and those with a high pepsin concentration in the nasal lavage sample, a Pearson correlation was performed to further demonstrate the absence of correlation (r=−0.015).

Twenty one of 36 (58.33%) abnormal eosinophil samples were positive for pathological pepsin levels in comparison to 18 of 56 (32.14%) normal eosinophil levels. Despite this difference, they were not deemed to be significantly different (P=0.9450).

High pepsin sensitivity (87.1%) and specificity (61.9%) was determined using Peptest. This demonstrates the benefits and ease of the non-invasive, lateral flow device, compared to the Reflux Disease Questionnaire used in this study. The RDQ results displayed the classic GERD syndrome-based diagnostic method is not a sensitive method to diagnose LPR with the mean score achieved (5.16) being considerably lower than the limit to determine GERD (≥12), resulting in 29 NAR patients potentially being misdiagnosed for GERD and LPR.

As there was an absence of a clear visual difference between NAR patients who used alcohol and those who smoke in any of the samples provided, statistical data was not performed on these categories.

The data generated in the study demonstrated that there was a high level of pepsin in patient saliva samples compared to the healthy control population, providing a clear link and association of reflux disease in patients presenting with NAR. The effects of age, diet including spicy cuisine and eosinophil count were not contributing factors in this study.

The ease of using Peptest provided a rapid and efficient method with all subjects displaying positive for reflux disease due to the significant concentration of pepsin present, specifically in the postprandial saliva sample.

Physicians treating patients with NAR should consider reflux treatment for patients presenting with high pepsin concentrations.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.

org/10.21037/aoe.2019.03.02). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (Ethical Approval ID: 2017NZKY-008-02). Informed consent was obtained from all individual participants included in the study.

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