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Fasting salivary pepsin level as a reliable non-invasive method of screening for laryngopharyngeal reflux in Egyptian patients

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Abstract

Background: Laryngopharyngeal reflux (LPR) is caused by the regurgitation of gastric contents above the upper esophageal sphincter. Diagnostic gold standard tests like multichannel intraluminal impedance (MII) and 24-h dual-probe pH-metry are invasive and expensive which limits their accessibility especially in resource-limited settings. Since pepsin is only produced in the stomach, detecting pepsin in the laryngopharynx would make it a specific marker for reflux.

Therefore, in this study, we measured fasting salivary pepsin in patients with symptoms suggestive of LPR. We aimed to confirm the role of fasting salivary pepsin as a non-invasive diagnostic tool of LPR, to detect a cut-off value for it in Egyptian patients and to study predictors of changes in its level.

Methods: We conducted a prospective case control study at the gastroenterology clinic in Ain Shams University Hospitals. After testing with esophageal pH-metry, 25 symptomatic patients with confirmed LPR and 25 healthy controls were enrolled in the study. Patients diagnosed with organic upper gastrointestinal disorders, autoimmune diseases, diabetes, malignancy or organ failure were excluded. Patients on PPI were advised to stop 2 weeks before testing. All patients were tested for fasting salivary pepsin levels, esophageal pH-metry, and indirect laryngoscopy in addition to routine laboratory parameters.

Results: Out of the 25 LPR patients, 16% of patients had laryngoscope abnormality in the form of mucosal hyperemia and inflammation, and the average percentage of time pH < 4 in esophageal pH-metry testing was 29.14 ± 39.5%.

Comparative study between the 2 groups revealed a significant increase in salivary pepsin in LPR group compared to control group ($p < 0.001$). By using ROC-curve analysis, salivary pepsin at a cut-off point > 5 ng/ml diagnosed patients with LPR, with fair (77.9%) accuracy, sensitivity = 100% and specificity = 56% ($p = 0.0001$) while pH-metry (% Time pH < 4) at a cut-off point > 14% diagnosed patients with LPR, with good (87%) accuracy, sensitivity = 80%, and specificity = 100% ($p < 0.0001$)

Conclusion: Fasting salivary pepsin level at a cut-off value of > 5 ng/ml is a reliable, non-invasive method for detection of LPR especially in resource-limited settings.

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Keywords: Salivary pepsin, Laryngopharyngeal reflux, pH-metry

Introduction

Laryngopharyngeal reflux (LPR) is caused by the regurgitation of gastric contents above the upper esophageal sphincter [1]. Owing to its non-specific symptoms, LPR is usually seen in otolaryngology or gastroenterology clinics. With an estimated incidence of 5 to 30% of the general population [2], methods of diagnosis with high level of accuracy, availability and safety are thoroughly sought. Diagnostic gold standard tests like multichannel intraluminal impedance (MII) and 24-h dual-probe pH-metry are invasive and expensive which limits their accessibility especially in resource-limited settings. Whereas clinical questionnaires and proton-pump inhibitor (PPI) therapeutic trials are of limited value in diagnosis [2, 3].

Pepsin is known to be an exclusive product of gastric chief cells. It is produced as pepsinogen, and is transformed into active pepsin by gastric HCL. It was found that pepsinogen was not detected in laryngeal tissue specimens positive for pepsin protein [4]. This verified that pepsin is not a product of the laryngopharyngeal mucosa and that presumably pepsin detected in patients within the laryngeal or pharyngeal mucosa resulted from reflux. Since pepsin is only produced in the stomach, it was hypothesized that detecting pepsin in the laryngopharynx would make it a specific marker for reflux [5, 6].

Therefore, in this study we measured fasting salivary pepsin in patients with symptoms suggestive of LPR. We aimed to confirm the role of fasting salivary pepsin as a non-invasive diagnostic tool of LPR, to detect a cut-off value for it in Egyptian patients and to study predictors of changes in its level.

Patients and methods

We conducted a prospective case control study at the gastroenterology clinic in Ain Shams University Hospitals. A total of 50 subjects were enrolled in the study. They were divided into two groups:

Group 1

Twenty-five patients with confirmed laryngopharyngeal reflux by esophageal pH-metry. Patients complained of symptoms suggestive of LPR (e.g., dysphonia/hoarseness, mild dysphagia, globus pharyngeus, chronic cough, and heart burn).

Group 2

Twenty-five apparently healthy volunteers matched for age and sex, who deny any symptoms suggestive of any upper GIT disorder and served as a control group for measurement of salivary pepsin in normal persons.

Patients with isolated gastritis or duodenitis, previous gastric surgery, diabetes mellitus, autoimmune diseases, organ failure (renal, hepatic or heart failure), malignancies, or chronic otolaryngological disorder (e.g., sinusitis, allergic rhinitis etc.) were excluded. All patients were non-smokers. Proton pump inhibitors (PPI) were stopped 2 weeks before testing.

All patients and controls were subjected to full history taking and complete clinical examination with special emphasis on dysphonia or hoarseness, chronic cough, globus pharyngeus (a persistent or intermittent non-painful sensation of a lump or foreign body in the throat), non-productive throat clearing, mild cervical dysphagia, excessive throat mucus, sialorrhea, sensation of postnasal drainage, dysgeusia, halitosis, throat pain as well as symptoms suggestive of any chest, otolaryngological, cardiac, or upper gastrointestinal disease.

Routine investigations including complete blood count (CBC), random blood sugar (RBS), urea, creatinine, sodium, and potassium (Na and K), and abdominal ultrasound were done to all participants in addition to indirect laryngoscopy, pepsin level in saliva by ELISA and oesophageal pH-metry.

Salivary collection and pepsin measurement

Saliva was collected upon awakening before mouth washing. Patients on proton pump inhibitors were assured to stop them 2 weeks before doing salivary pepsin test. One milliliter of saliva was collected from each participant in a standard transport tube. All samples were centrifuged 3000 rpm for 20 min on the same day it was collected. The supernatant was stored at − 20 °C. The human pepsin ELISA kit (Catalog No. BYEK2634; Chongqing Biospes Co., Ltd, China) was used to detect salivary pepsin concentrations. We were blinded to whether subjects were healthy controls or patients with LPR when testing for salivary pepsin.

Esophageal pH-metry

Restech pH probe was used for oropharyngeal pH testing to help insertion of the sensor in the correct position. Probes were calibrated in pH 4 buffer solutions and were

placed trans-nasally to reach 1 cm below the uvula. The study participants were advised to perform their usual daily activities with no changes to their lifestyle during the period of the testing. Subjects were told to record the times they were supine and their oral intake of foods and fluids. They carried a wireless receiver to note their symptoms (cough, throat clearing, heartburn) by pressing the appropriate buttons on the transponder and in a hand-written diary. The probe was removed 24 h later, and the data from the digital recorder were analyzed with Data View software by AEMC Instruments, Foxborough, MA. A graphical tracing of all events was plotted and the data retrieved from the electronic transponder were modified according to paper diary recordings. Mealtimes with 5-min pre- and post-prandial intervals were excluded. All data collected were reviewed by the study team.

Ethical considerations

An informed consent was signed by all patients after full explanation of the study.

Before starting the study, the Faculty of Medicine, Ain Shams University Ethical Committee approved the study protocol. The study had been performed in accordance with the ethical guidelines of the 2013 Declaration of Helsinki [7].

Statistical methods

Data entry, processing and statistical analysis was carried out using MedCalc ver. 18.11.3 (MedCalc, Ostend, Belgium). Tests of significance (Mann-Whitney's, chi-square tests, logistic and multiple regression analysis, and ROC Curve analysis) were used. Data were presented and suitable analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. *P* values less than 0.05 (5%) was considered to be statistically significant.

Results

This prospective comparative study was conducted on 50 subjects to evaluate the salivary pepsin as a non-invasive rapid test for diagnosis of LPR in comparison to esophageal pH-metry. The 50 subjects were classified according to the presence of LPR into 2 independent groups; LPR group (25 patients) and control group (25 patients). Patients and controls were matched for age and sex.

The mean age of all LPR patients was 38.56 years (27–48 years), 80% ($n = 20$) of the patients were males; while 20% ($n = 5$) were females. Their most prevalent clinical symptoms were chronic cough and halitosis in 76%, sensation of postnasal drainage in 56%, and throat pain in 40% of patients. Dysphonia, hoarseness, non-productive throat cleaning, mild cervical dysphagia, excessive throat mucus, and sialorrhea were noted in less patients.

Out of the 25 LPR patients, 16% of patients had abnormal laryngoscopic findings in the form of mucosal hyperemia and inflammation. On esophageal pH-metry testing, the average percentage of time with pH < 4 was 29.14 ± 39.5% in this group.

Comparative study between both groups revealed a significant increase in salivary pepsin in LPR group compared to control group ($p < 0.001$); however, other studied laboratory parameters did not show significant differences ($p > 0.05$) (Table 1, Fig. 1). In addition, the percentage of time with pH < 4 on pH-metry testing and presence of abnormal laryngoscopic findings were significantly increased in LPR group ($p < 0.0001$ and $p < 0.01$ respectively) compared to control group (Table 2).

Correlation studies between salivary pepsin as a diagnostic method and its relative independent predictors (clinical findings, laboratory, and diagnostic

Table 1 Comparison between the 2 groups as regards laboratory data using Mann-Whitney's *U* test

Variable	Control group (25) Median (IQR)	LPR group (25) Median (IQR)	Mann-Whitney's *U* test *P* value
Hb (g/dL)	12.5 (12–13.5)	13.2 (12.5–13.7)	= 0.1567
PLT (10^3/μL)	239 (219–319)	278 (229–332)	= 0.4667
TLC (10^3/μL)	7.5 (5.4–8.5)	6.5 (5–7.6)	= 0.2888
RBS (mg/dL)	125 (120–135)	130 (119.7–135)	= 0.8148
Urea (mg/dL)	16 (14–21)	18 (16–19)	= 0.8074
Creatinine (mg/dL)	1 (0.7–1.1)	0.9 (0.8–1)	= 0.6934
Na (mEq/L)	140 (139–141)	139 (137–141)	= 0.1902
K (mEq/L)	3.6 (3.5–4)	3.7 (3.6–3.9)	= 0.6373
Pepsin in saliva (ng/ml)	**5 (4.7–8.2)**	**9 (7–11.2)**	**= 0.00065****

Hb Hemoglobin, *PLT* Platelets, *TLC* Total leucocytic count, *RBS* Random blood sugar, *Na* Sodium, *K* Potassium

** statistically significant

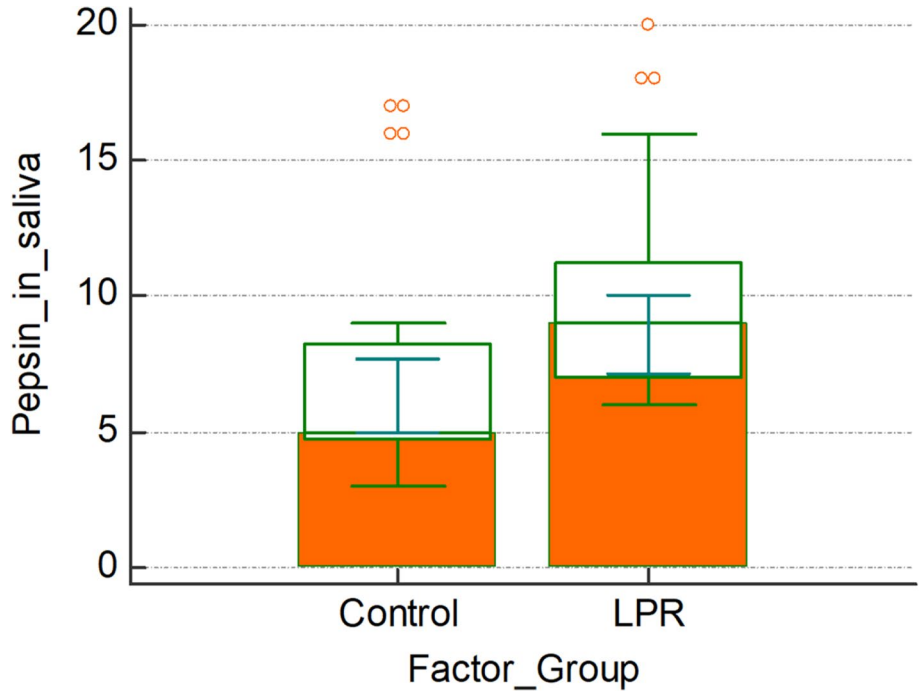

Fig. 1 Comparison between the 2 groups as regards salivary pepsin

Table 2 Comparison between the 2 groups as regards laryngoscopic and pH-metry data using Mann-Whitney's *U* and chi-square tests

Variable		Control group (25)	LPR group (25)	Mann-Whitney's *U* test
		Median (IQR)	Median (IQR)	*P* value
% Time pH < 4 (total) (%)		12 (10–13)	26 (16–67.5)	< 0.0001**
Variable		Control group (25)	LPR group (25)	Chi square test
				P value
Laryngoscope abnormality	+ve	0 (0%)	4 (16%)	= 0.039*

*Percentage of column total

**statistically significant

Table 3 Multiple regression model for the factors affecting salivary pepsin using forward method

Predictor factor	β	SE	*P*
(Constant)	− 7.8263		
Age	0.2229	0.07945	0.0074**
Urea	0.3929	0.1597	0.017*
Laryngoscope abnormality	5.6049	2.0799	0.0099**
pH-metry (% Time pH < 4)	0.03024	0.01448	0.042*

Other factors excluded from the model as (p value > 0.1), β Regression coefficient, *SE* Standard error

** high statistical significance, *statistically significant

tests variables) were conducted with multiple logistic regression analysis. Multiple regression analysis showed that the increase in age, urea, presence of laryngoscope abnormality, and percent of time with pH < 4 were independent predictors of increased salivary pepsin with significant statistical difference (Table 3, Fig. 2).

Logistic regression analysis shows that, after applying Forward method, the increase in the percentage of time with pH < 4 by pH-metry had an independent effect on increasing the probability of LPR occurrence with significant statistical difference ($p < 0.005$) (Table 4).

By using ROC-curve analysis, salivary pepsin at a cut-off point > 5 ng/ml diagnosed patients with LPR, with fair (77.9%) accuracy, sensitivity of 100%, and specificity of 56% ($p = 0.0001$) (Fig. 3). In addition, pH-metry (% Time pH < 4) at a cut-off point > 14% diagnosed patients with LPR, with good (87%) accuracy, sensitivity of 80%, and specificity of 100% ($p < 0.0001$) (Fig. 4).

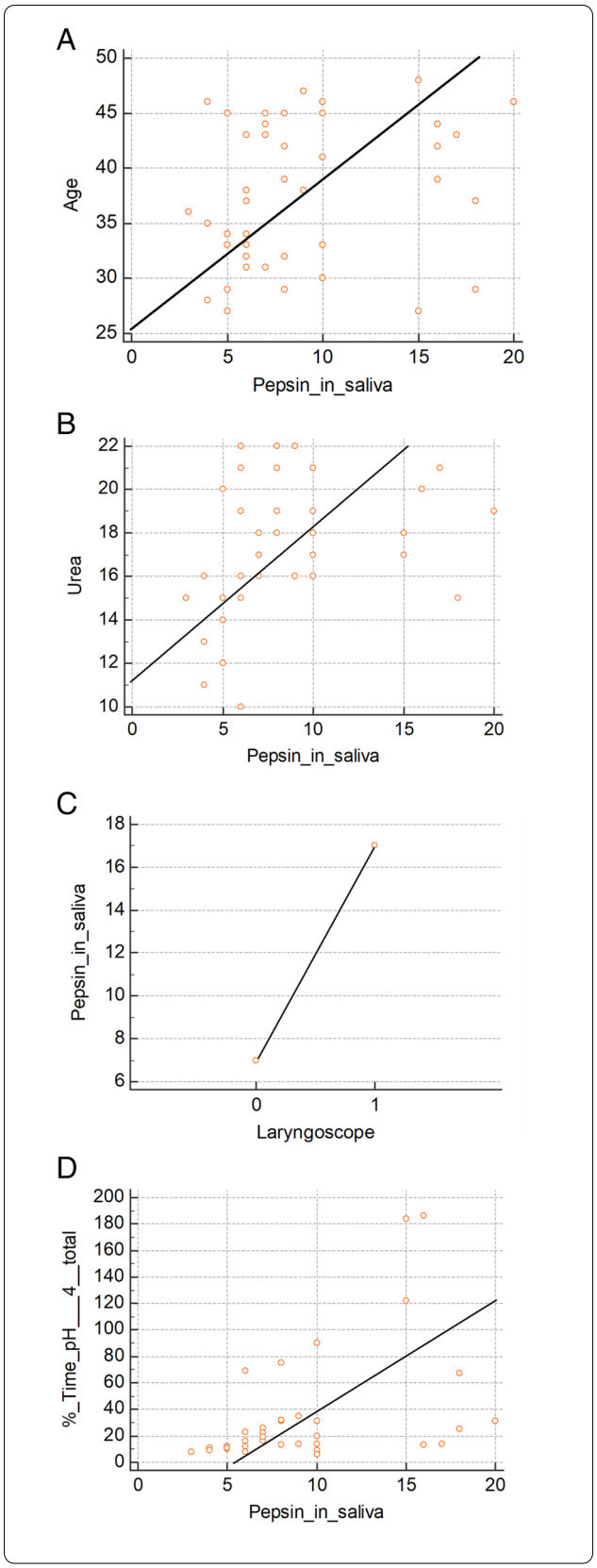

Fig. 2 **A** Correlation between salivary pepsin and age. **B** Correlation between salivary pepsin and urea. **C** Correlation between salivary pepsin and laryngoscope abnormality. **D** Correlation between salivary Pepsin and pH-metry (% Time pH < 4)

Discussion

Larygeopharyngeal mucosa is affected directly and indirectly by gastroduodenal refluxate, which results in morphological changes in the upper aerodigestive tract known as LPR [8].

Patients with LPR complain mostly of hoarseness, sore throat, odynophagia, cough, throat clearing, globus sensation, and excessive phlegm [9]. Throat clearing was the most common symptom for LPR in the study by Noordzij et al. [10] while Kamel et al. [11] found that throat burning and cough were more intense than throat clearing in their patients. In our study population, the most prevalent symptoms were chronic cough and halitosis (76%), 56% had sensation of postnasal drip, and 40% of patients complained of throat pain.

In 2005, Knight et al. [5] evaluated the detection of pepsin in throat sputum in comparison to 24-h double-probe pH monitoring for diagnosis of LPR and suggested that the finding of pepsin in the airway (using a sputum sample) is as diagnostic of LPR as is abnormal pH-metry. When the pepsin assay results were compared with the pharyngeal pH data for detecting reflux (events with pH < 4), the pepsin immunoassay was 100% sensitive and 89% specific for LPR.

The level of salivary pepsin collected upon awakening was found to be higher than its level at any other time [6]. They suggested that fasting salivary pepsin level might be useful in the diagnosis of LPR. This was also confirmed by Na et al. [12], where fasting salivary pepsin was found to be the highest throughout the day and showed a significant increase in LPR patients which led them to the conclusion that it can be a useful non-invasive marker for diagnosis of LRP.

In this study, symptomatic patients and asymptomatic controls underwent esophageal pH-metry which showed a significant increase in the percentage of time with pH < 4 in LPR patients compared to control group ($p < 0.0001$). Asymptomatic controls had a median of 12% of time with pH less than 4 as opposed to 26% in LPR patients. The percentage of time with pH less than 4 in healthy individuals might be considered slightly elevated than that in some of the previously mentioned studies [5, 12] and this can be explained by the difference in dietary habits, ethnicities and environmental factors. All participants in control group were completely asymptomatic prior to and during the study. Considering that healthy individuals have around 40 reflux episodes over a 24-h period as

Table 4 Logistic regression model for the factors affecting LPR occurrence using Forward method

Predictor factor	Coefficient	OR	*P* value
(Constant)	− 4.86864		
pH-metry (% Time pH < 4)	0.31250	1.3668	0.005**

Other factors excluded from the model as (p value > 0.1)

OR Odds ratio

** statistically significant

measured by impedance-pH [13], it is expected to find some decline in oesophageal pH through-out the day.

Salivary pepsin level upon awakening showed a significant increase in LPR patients compared to healthy individuals ($p < 0.001$) (Table 1, Fig. 1). Salivary pepsin at a cut-off point > 5 ng/ml diagnosed patients with LPR, with fair (77.9%) accuracy, sensitivity of 100% and specificity of 56% (Fig. 3).

Accordingly, salivary pepsin at a cut-off point > 5 ng/ml was highly sensitive in diagnosis of patients with LPR with fair accuracy and specificity (Fig. 3). A study by Zhang et al. [14] compared salivary pepsin to 24-h dual pH/impedance probe in diagnosis of LPR. They concluded that salivary pepsin detection is an inexpensive and non-invasive first-line alternative in diagnosing LPR at a cut-off value of 16 ng/ml. Knight et al. [5] found that pepsin at a cut-off value of 6.5 ng/ml was diagnostic of LPR. Barona-Lleo et al. [15] recruited 221 subjects and compared their Reflux Symptom Index scale with salivary pepsin and concluded that salivary pepsin can be used as an alternative diagnostic tool for LPR. Salivary pepsin was also a reliable biomarker for diagnosis of LPR in patients referred to ENT voice clinics [1]. Weitzendorfer et al. [16] also found out that salivary pepsin measurement could assist office-based diagnosis of LPR.

In 2017, after examining 12 studies evaluating pepsin as a diagnostic tool of LPR through a systematic review, Calvo-Henríquez et al. [17] concluded that pepsin can be used as a reliable marker for LPR. Only two of the 12 of the studies did not find a relation between pepsin and LPR.

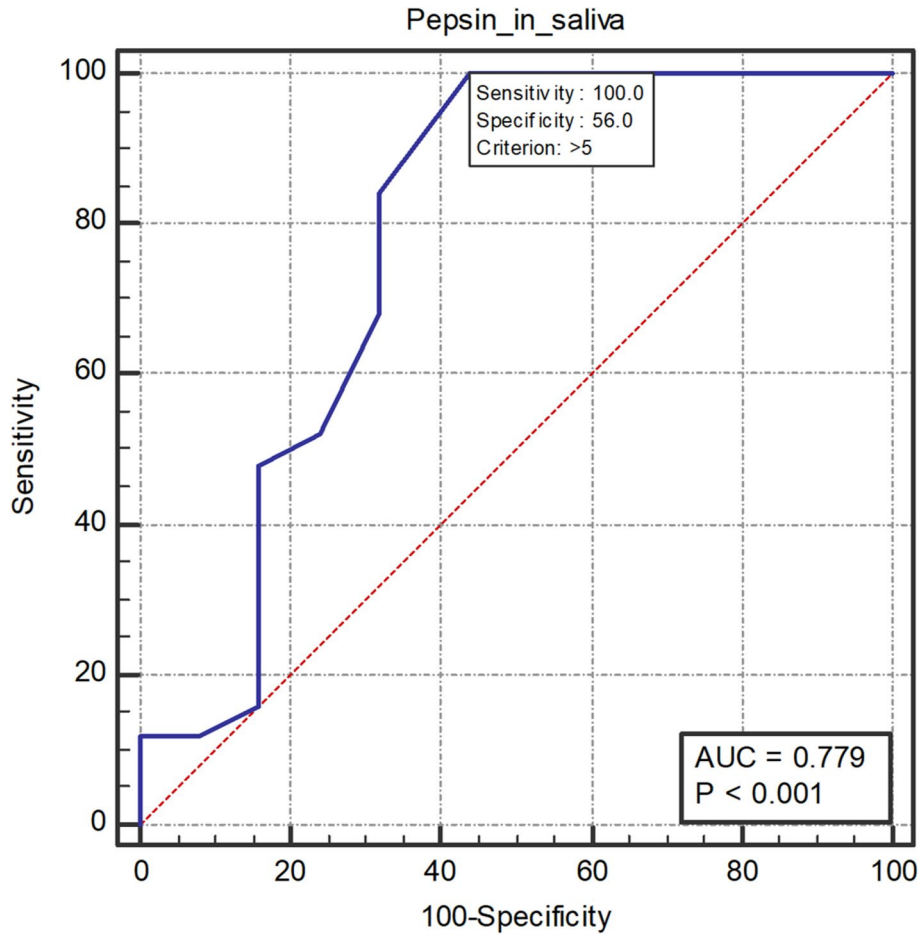

Fig. 3 ROC curve for salivary pepsin (LPR)

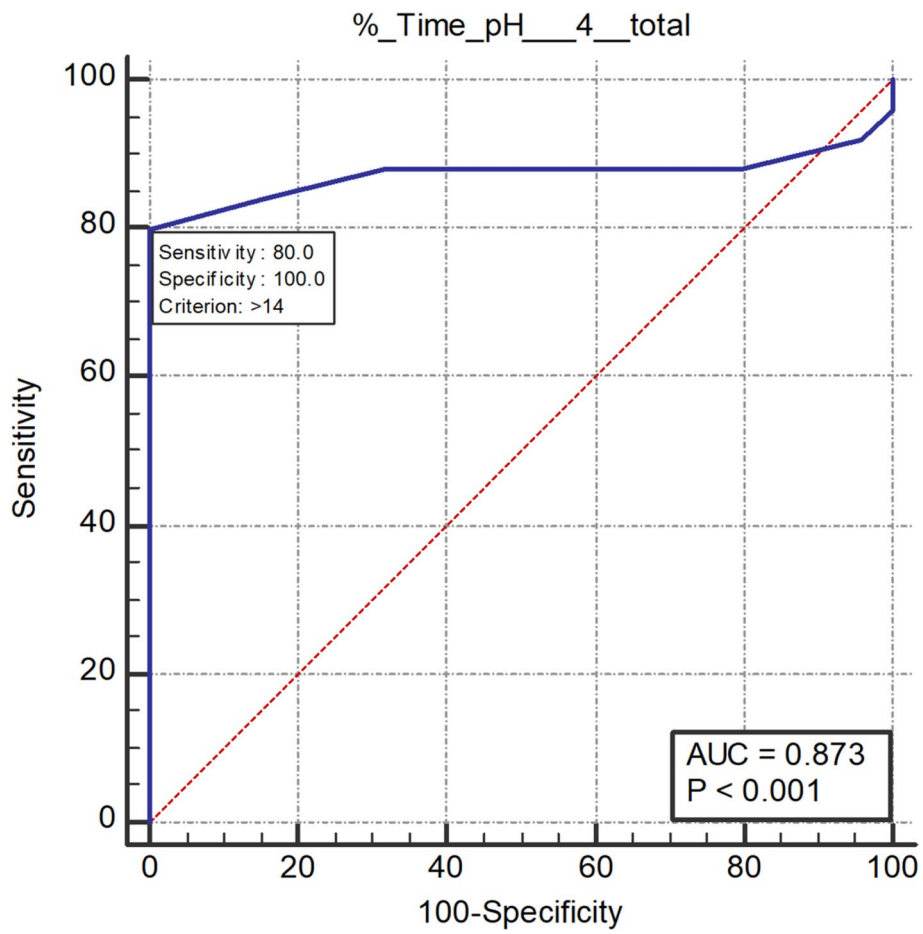

Fig. 4 ROC curve of pH-metry (% Time pH < 4) (LPR)

In our study, the percentage of time with pH less than 4 on pH-metry testing at a cut-off point > 14% diagnosed patients with LPR, with higher accuracy (87%), specificity (100%), and good sensitivity (80%). This indicates that although pH-metry could be considered a superior diagnostic tool for LPR according to our results, salivary pepsin can be used as a non-invasive primary method to detect LPR. Consequently, pH-metry as an invasive method can be used in patients where diagnosis is suspected and measurement of salivary pepsin is inconclusive.

Multiple regression analysis showed that the increase in age, urea, presence of laryngoscope abnormality, and increased percent of time with pH less than 4 in pH-metry were independent factors for salivary pepsin increment with significant statistical difference (Table 3, Fig. 2).

The presence of laryngoscope abnormality as well as lower pH on pH-metry testing is an expected finding in patients with LPR; therefore, it is understandable that they have a significant correlation with increased salivary pepsin.

In fact, it was interesting to find a significant correlation between increase in age and elevated levels of salivary pepsin. The eldest patient in the LPR group was 48 years meaning that no elderly patients were included in our study. A study of the prevalence of laryngopharyngeal reflux in the English population [18] found that LPR incidence was higher in age groups of 41–50 and 51–60 years and that the age group between 41 and 60 years had a higher overall incidence of LPR symptoms. This shows a similarity to the age of the patients in our study aged 27–48 years as with progression of age, awareness of the symptoms increases in addition to the cumulative effect of irritant foods, smoking, and alcohol throughout the years.

Although higher levels of serum urea were considered statistically significant predictors for increased salivary pepsin levels, this cannot be considered useful in practical clinical application since serum levels of urea in the LPR group and controls were all within the normal ranges despite being a little higher in the LPR group. Further studies with wider range of changes in serum urea levels in relation to salivary pepsin are needed in order to evaluate the possible correlation.

Limitations of the study are mainly due to the small study population which might not be conclusive to detect accurate cut-off value of salivary pepsin. This study was mainly concerned with Egyptian patients, further studies with larger cohort and different ethnicities would be needed to apply these results on different populations.

In conclusion, we believe that fasting salivary pepsin level at a cut-off value of > 5 ng/ml is a reliable, non-invasive method for detection of LPR. It can be used as an easily accessible and rapid means of diagnosis to guide proper treatment especially in resource-limited settings. Middle age and serum urea levels should be evaluated as possible predictors for elevated levels of salivary pepsin in symptomatic patients.

Abbreviations

MII: Multichannel intraluminal impedance; PPI: Proton-pump inhibitor; LPR: Laryngopharyngeal reflux; CBC: Complete blood count; RBS: Random blood sugar; ENT: Oto-rhinolaryngology.

Acknowledgements

Not applicable.

Authors' contributions

AMM and TGM carried out the experiment. TGM wrote the manuscript with support from MSM. TMY and MSM helped supervise the project. TGM and AMM conceived the original. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Available upon request.

Declarations

Ethics approval and consent to participate

Every patient signed an informed consent after full explanation of the study procedures and was given the choice to join the study population and/or decide to withdraw from the study at any time point. Before starting the study, the Faculty of Medicine, Ain Shams University Ethical Committee, approved the study protocol. The study had been performed in accordance with the ethical guidelines of the 2013 Declaration of Helsinki [7]. Ethical approval number: FMASU MS 17585/2018. Date 15 May 2018.

Consent for publication

I, the undersigned, give my consent for the publication of the manuscript details, which can include figures, tables and/or details within the text to be published in The Egyptian Journal of Internal Medicine. Consent for publication was taken from all subjects in the study.

Competing interests

The authors declare that they have no competing interests.

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