

Salivary Pepsin as an Intrinsic Marker for Diagnosis of Sub-Types of GERD

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ABSTRACT

Background: A stomach-based protease called pepsin has been linked to the etiology of GERD and may function as a prospective biomarker for disease evaluation.

Aim: Our study's objective is to evaluate salivary pepsin's diagnostic accuracy as an intrinsic marker for GERD diagnosis, and its utility to assess disease severity.

Methods: 90 Egyptian patients were enrolled in this study (45 patients with GERD and 45 healthy volunteers). Pepsin level was estimated in saliva samples using ELISA kit. The disease severity was assessed according to the Los Angeles classification and compared to the pepsin levels.

Results: Increased salivary Pepsin was shown to be highly statistically significant ($p\text{-value} < 0.001$) in Cases group (108.5 ± 46.6 ng/ml) when compared with Control group (24.4 ± 9.9 ng/ml). The present study revealed significant correlation between salivary pepsin and Los Angeles classification of GERD severity. We observed statistically significant ($p\text{-value} < 0.001$) increased salivary Pepsin in grade C cases (191.9 ± 27.4 ng/ml) when compared with grade B cases (98.2 ± 22.9 ng/ml) and grade A cases (84.5 ± 25.5 ng/ml). It was demonstrated that salivary pepsin may be utilized to distinguish between the patients and control groups using a roc curve at a cutoff level of > 46.5 , with 95.6% sensitivity, 97.8% specificity, 97.8% PPV and 95.7% NPV.

Conclusion: Salivary pepsin can be used as cost-effective test to assess the severity of GERD and follow up of GERD patients after therapy.

Key Words: Gastroesophageal reflux, intrinsic marker, PPI, salivary pepsin.

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INTRODUCTION

GERD, which is "A disorder that arises when the reflux of stomach contents into the oesophagus occurs causing troublesome symptoms and complications," has become more common in recent years and has a major negative influence on people's quality of life. The two most prevalent signs of GERD are regurgitation and heartburn, which affect 40–60% of patients overall. In contrast, 70–90% of extraesophageal symptoms manifest as pharyngitis, cough, hoarseness, and asthma; these symptoms are sometimes misinterpreted as respiratory or throat disorders and treated poorly or slowly^[1].

According to the American Gastroenterological Association (AGA) Gastroesophageal reflux disease

(GERD) treatment depends mainly on proton pump inhibitors (PPI) for patients who complain of heartburn or regurgitation. The trial therapy duration is about 8-weeks in case of absent risk factors or complications^[2].

Moreover, hypersensitive esophagus (HE) is defined as the presence of heartburn symptoms in the absence of acid reflux as detected by endoscopy or pH monitoring, in case of PPI-responsive patients^[3].

The "PPI test," endoscopy, ambulatory oesophageal reflux monitoring, and GERD questionnaires are among the current techniques used to diagnose GERD. Nonetheless, others have questioned the sensitivity and specificity of these techniques^[4].

Salivary pepsin has shown variable diagnostic accuracy for GERD. Salivary pepsin in a small study cohort shown low levels in the asymptomatic individuals while there was higher levels in HE and GERD symptomatic patients^[5].

AIM OF THE STUDY

Our study's objective was to evaluate salivary pepsin's diagnostic accuracy as an intrinsic marker for GERD diagnosis and subtypes stratification.

MATERIALS AND METHODS

Ninety Egyptian patients presenting at the Outpatient Clinic were included in the study after meeting our eligibility criteria from August 2023 to January 2024. All included patients signed an informed consent. For GERD sub-types stratification, Los Angeles classification was applied depending on patients' endoscopic findings^[6].

Eligibility criteria: Patients aged >18 years old who were diagnosed to have GERD by both clinical and endoscopic findings. Patients with heartburn and/or regurgitation, two or more episodes per week, and chronic or recurring symptoms for more than three months are considered to have typical GERD symptoms.

All patients didn't take PPI for the last two weeks before salivary pepsin sampling, nor multivitamins containing biotin for the last 12 hours prior to testing.

Healthy controls were selected from the clinic with absent symptoms or history of GERD.

Exclusion criteria include the following: use of H. pylori eradication medications, proton pump inhibitors, and histamine type 2 receptor antagonists during the previous month; individuals with a history of gastric surgery; patients with anatomic facial abnormalities and oral or dental problems; patients with esophageal malignancy or gastric carcinoma; patients on non-steroidal anti-inflammatory drugs; patients with alkaline or biliary reflux; and pregnant females.

ETHICS COMMITTEE

The study was approved by the Ethics Committee, Faculty of Medicine, Ain Shams University. Ethical approval number FMASU-MS-550-2023. Every participant gave written informed consent after being fully informed about the purpose and procedures of the present investigation.

The following measures were applied to all patients and healthy controls:

I. History taking and Clinical Examination:

1. Complete history: which include demographic data, history of present illness (heartburn, regurgitation, chest pain, dyspepsia, anorexia, etc), and history of additional coexisting illnesses, including diabetes mellitus, heart disease, and renal failure.
2. Thorough Clinical Examination: An assessment of the patient's vital signs was done and, as part of a comprehensive clinical examination. chest, abdomen, and heart examination results were evaluated with an emphasis on gastrointestinal symptoms.
3. Anthropometric measurements: After taking measurements of height and weight, the body mass index (BMI) was calculated by dividing the weight in kilograms. By square the height in meters.

II. Laboratory Investigations: Following blood samples collection, the following investigations were done:

- Full blood picture: results were obtained showing leucocytic count, haemoglobin concentration and platelet count.
- Alanine aminotransferase (ALT), aspartate aminotransferase (AST).
- Morning fasting plasma glucose.
- Serum creatinine and urea.

III. Salivary Pepsin Collection and Detection:

- Sampling time: In the event that the classic GERD symptoms appeared, at least 1 millilitre of saliva from the throat was collected into a clean collection tube. The sample was obtained within 15 minutes after the onset of symptoms; if this was not possible or the patient was unable to assess for themselves, the sample was taken one hour after supper.
- Pre-sample measures: smoking, carbonated drinks, and caffeine were avoided one hour before sampling. Antacids, alginate antacids, and alkaline drinks or beverages were not consumed 48 hours before to sample collection, nor was sampling immediately following strenuous activity.
- Estimation of pepsin level in saliva: Pepsin level was estimated in saliva samples using ELISA kits (Bioassay Technology laboratory Shanghai Korain Co., Ltd, Zhejiang, China). The test principle was based on Biotin antibody sandwich technology in which pepsin was added to the wells which

are coated with monoclonal antibodies labeled with biotin. This assay employed the competitive inhibition enzyme immunoassay technique.

IV. Imaging: Patients in the study were screened with an abdominal ultrasonography to evaluate:

- liver size in both midline and mid-clavicular line, liver surface and echogenicity. The same radiologist conducted all of the ultrasonographic exams while being blind to the patients' clinical and laboratory information.
- Splenic size: either average size or enlarged.
- Portal vein patency and whether there is ascites or not.

V. Upper gastrointestinal endoscopy:

After the patient was well prepared, this was performed through a sterile upper gastrointestinal video scope. An overnight fast was conducted followed by an endoscopic examination. Olympus GIFQ-40 was used for all endoscopies, and the throat was sprayed locally with xylocain. Every patient's esophageal and stomach examination results were documented.

Results of upper gastrointestinal endoscopy were recorded and included comment on the following: competence of cardia, inflammation, ulceration, masses, stricture of esophagus, and presence of hiatus hernia. Endoscopic classification of severity of GERD (erosive esophagitis) was based on Los Angeles classification of GERD severity

Analysis of statistics:

The data was analyzed using version 24 of the Statistical Program for Social Science (SPSS). The mean \pm SD was used to express quantitative data. Frequencies and percentages were used to express the qualitative data. The following tests were used in analysis:

- When comparing two means, the independent sample T test (T) is employed. (for data that is normally distributed). As for comparing abnormally distributed data, the Mann Whitney U test (MW) was used.
- Chi-square test: used to qualitative data comparisons.
- To correlate data, Pearson's correlation coefficient (r) was employed.
- Using the Receiver Operating Characteristic Curve (ROC curve), cutoff value, sensitivity, specificity,

positive predictive value (PPV), and negative predictive value (NPV) were all determined.

- The likelihood that a test will come back positive while the illness is present is known as sensitivity.
- The likelihood that a test will come back negative in the absence of the condition is known as specificity.
- When a test is positive, the likelihood that the illness exists is known as the positive predictive value.
- When the test is negative, the likelihood that the illness is absent is known as the negative predictive value.
- *P values* >0.05 were statistically non-significant, *P values* <0.05 were considered statistically significant and *P values* <0.01 were considered highly significant.

RESULTS

This case-control study was conducted on 90 individuals: 45 patients with GERD, and 45 healthy volunteers as control group. The mean age of cases group was 43.2 ± 4.8 years while the mean age in control group was 42.7 ± 6.01 years. There is no statistically significant difference (*p-value* = 0.658) between cases and control as regard age. The study included 26 males (57.8%) and 19 females (42.2%) in case group while there were 32 males (71.1%) and 13 females (28.9%) in Control group. There is no statistically significant difference (*p-value* = 0.186) between studied groups (cases and control) as regard sex.

The results of current study showed statistically significant (*p-value* <0.001) increased BMI in Cases group (32.2 ± 3.9 kg/m²) when compared with Control group (27.8 ± 2.4 kg/m²). We found statistically significant (*p-value* = 0.046) increased percentage of smokers among Cases group (20 patients, 44.5%) when compared with Control group (11 patients, 24.4%).

The results of current study showed highly significant statistical (*p-value* < 0.001) increase in salivary Pepsin in Cases group (108.5 ± 46.6 ng/ml) when compared with Control group (24.4 ± 9.9 ng/ml). The present study revealed significant correlation between salivary pepsin and Los Angeles classification of GERD severity. We noticed statistically significant (*p-value* < 0.001) increase in salivary Pepsin in grade C cases (191.9 ± 27.4 ng/ml) when compared with grade B cases (98.2 ± 22.9 ng/ml) and grade A cases (84.5 ± 25.5 ng/ml).

Table 1: Comparability of the analysed groups with respect to demographic information.

Variable		Cases (N = 45)		Control (N = 45)		Stat. test	P-value
Age (years)	Mean	43.2		42.7		T = 0.44	0.658 NS
	±SD	4.8		6.01			
Sex	Male	26	57.8%	32	71.1%	X ² = 1.74	0.186 NS
	Female	19	42.2%	13	28.9%		
BMI (kg/m ²)	Mean	32.2		27.8		T = 6.4	< 0.001 HS
	±SD	3.9		2.4			
Chronic diseases	Non	32	71.1%	39	86.7%	X ² = 10.4	0.016 S
	DM	9	20%	1	2.2%		
	HTN	2	4.4%	5	11.1%		
	BA	2	4.4%	0	0%		
Smoking	No	25	55.6%	34	75.6%	X ² = 3.98	0.046 S
	Yes	20	44.5%	11	24.4%		

S: *p*-value < 0.05 is considered non-significant; T: independent sample T test. HS: *p*-value < 0.001 is considered highly significant.
X²: Chi-square test. NS: *p*-value > 0.05 is considered non-significant.

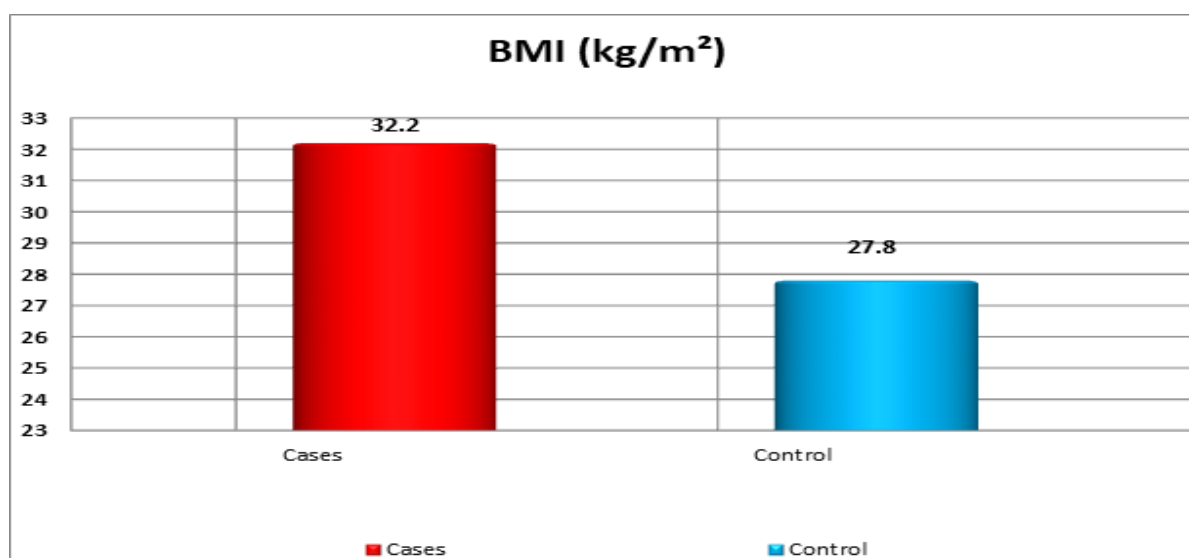
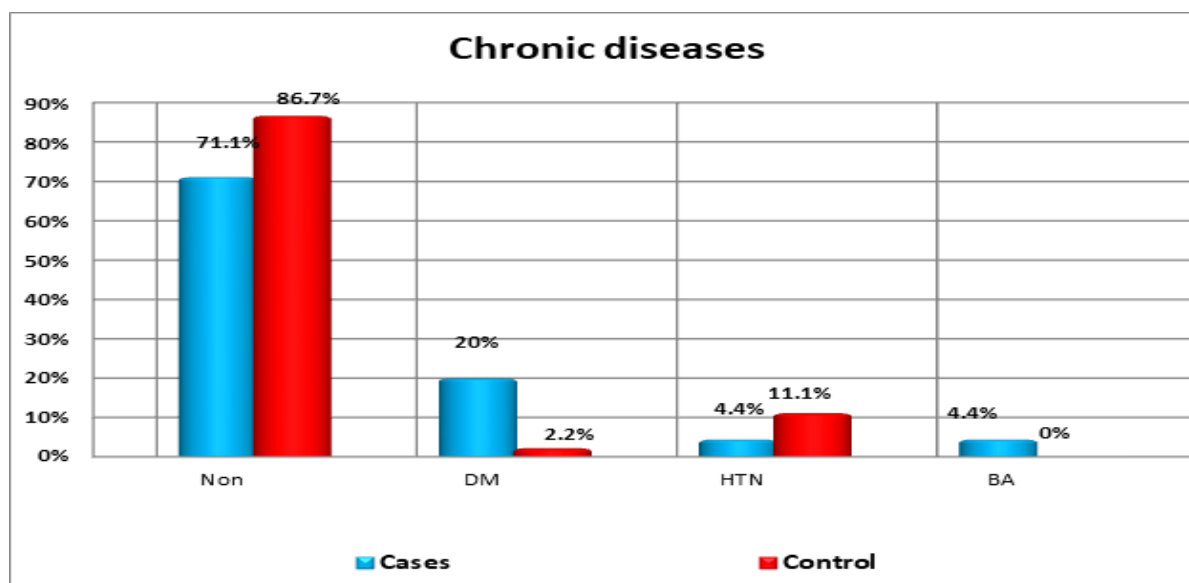
**Fig. 1:** Comparison of the BMIs of the studied groups.**Fig. 2:** Comparison of chronic diseases prevalence among studied groups.

Table 2: Comparison of laboratory data between different studied groups.

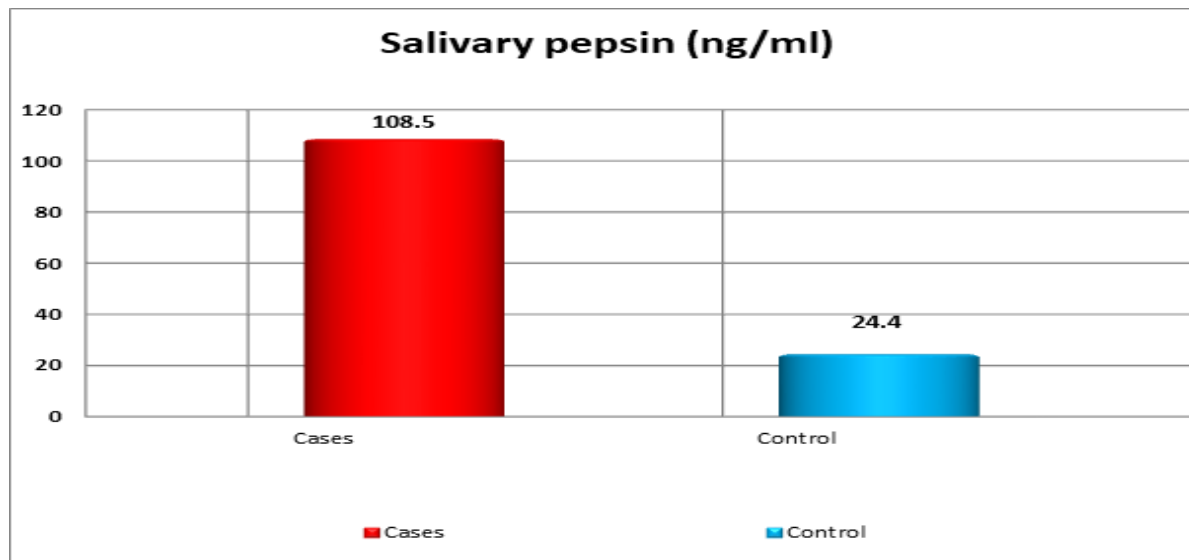
Variable		Cases (N = 45)	Control (N = 45)	MW	P-value
Hb (g/dl)	Mean	12.4	12.9	821.5	0.123 NS
	±SD	1.6	1.5		
WBCs (x10 ³ /ul)	Mean	6.0	5.8	971.5	0.741 NS
	±SD	1.8	1.7		
PLTs (x10 ³ /ul)	Mean	216.6	225.0	892	0.331 NS
	±SD	56.5	58.7		
FBS (mg/dl)	Mean	117.4	107.1	922	0.465 NS
	±SD	45.9	33.8		
ALT (U/L)	Mean	48.8	41.3	808	0.099 NS
	±SD	20.2	22.1		
AST (U/L)	Mean	28.6	29.3	989	0.849 NS
	±SD	17.2	16.8		
ALB (g/dl)	Mean	4.2	4.1	879.5	0.281 NS
	±SD	0.5	0.4		
T. Bil (mg/dl)	Mean	0.73	0.70	934.5	0.524 NS
	±SD	0.19	0.21		
Creat (mg/dl)	Mean	1.18	1.15	927.5	0.488 NS
	±SD	0.19	0.19		

MW: Mann Whitney U test.

NS: *p-value* > 0.05 is considered non-significant.**Table 3:** Comparison between studied groups showing salivary pepsin levels.

		Cases (N = 45)	Control (N = 45)	MW	P-value
S. pepsin (ng/ml)	Mean	108.5	24.4	10	< 0.001 HS
	±SD	46.6	9.9		

MW: Mann Whitney U test.

NS: *p-value* > 0.05 is considered non-significant.**Fig. 3:** Comparison between studied groups as regard salivary pepsin.**Table 4:** Diagnostic performance of salivary Pepsin in discrimination between studied groups:

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
S. Pepsin	> 46.5	0.99	95.6%	97.8%	97.8%	95.7%	< 0.001

PPV: positive predictive value.

NPV: negative predictive value.

AUC: Area under curve.

The diagnostic performance for salivary Pepsin using ROC curve showed that it can be used to discriminate between cases group and Control group at a cutoff level of > 46.5 , with 95.6% sensitivity, 97.8% specificity, 97.8% PPV and 95.7% NPV (AUC = 0.99 & p -value < 0.001).

Salivary Pepsin (Cases vs Control)

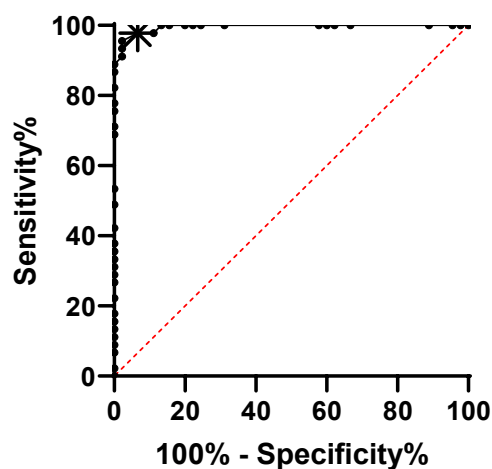


Fig. 4: Diagnostic performance of salivary pepsin using ROC curve.

Table 5: Description of Endoscopic findings in Cases group.

		Cases group (N = 45)	
Endoscopic findings	Erosive esophagitis	45	100%
	Relaxed cardia	16	35.6%
	BE	2	4.4%
	Hiatus hernia	3	6.7%

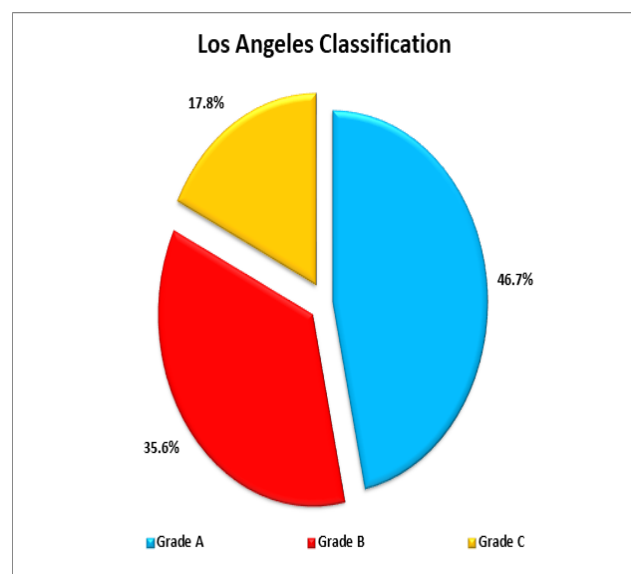


Fig. 5: Description of Los Angeles Classification in Cases group.

Table 6: Correlation between salivary Pepsin and Los Angeles Classification in cases group.

		Salivary Pepsin (ng/ml)	KW	P -value
Los Angeles Classification	Grade A (n = 21)	84.5 \pm 25.5	20.4	<0.001 HS
	Grade B (n = 16)	98.2 \pm 22.9		
	Grade C (n = 8)	191.9 \pm 27.4		

KW: Kruskal Willis test.

HS: p -value < 0.001 is considered highly significant.

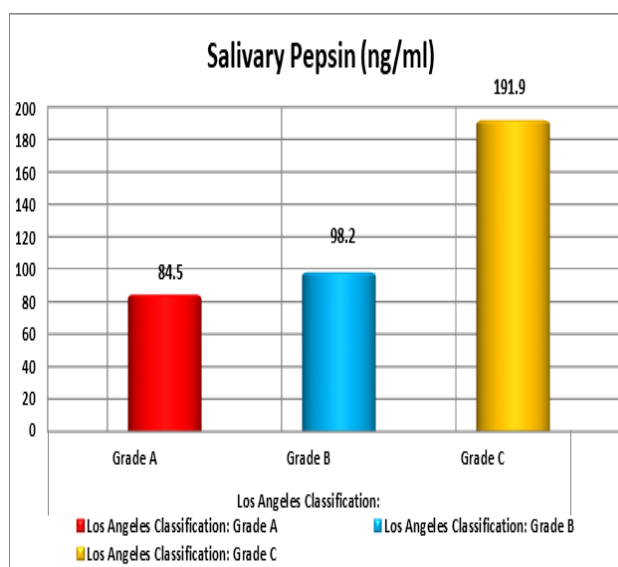


Fig. 6: Relation between salivary Pepsin and Los Angeles Classification in cases group.

Table 7: Correlation between salivary Pepsin and other studied parameters in all studied groups.

Salivary Pepsin	Cases group		Control group	
	r	p -value	r	p -value
Age	-0.03	0.852	-0.1	0.473
BMI	0.08	0.626	-0.1	0.636
Hb	0.10	0.499	0.1	0.641
WBCS	0.24	0.11	-0.1	0.511
PLTs	0.16	0.281	-0.1	0.463
FBS	0.12	0.441	0.1	0.606
ALT	0.18	0.226	0.0	0.958
AST	-0.18	0.241	-0.2	0.309
ALB	0.15	0.321	-0.2	0.223
T	0.15	0.335	-0.1	0.586
Bilirubin				
Creat	0.10	0.51	0.0	0.785

(r): Pearson correlation coefficient.

NS: p -value > 0.05 is considered non-significant.

DISCUSSION

This study was conducted on 90 individuals. The cases included 45 GERD symptomatic patients and were compared to 45 healthy controls.

A metanalysis conducted on 2018 by *Guo et al.*, it showed that in a total of five studies pepsin showed a sensitivity of 60%, specificity of 71%, and area under the curve of 70% in diagnosing GERD. Thus salivary pepsin has only a moderate diagnostic prediction of GERD^[7].

In a cross sectional study on Egyptian medical students, it was found that the prevalence of GERD symptoms reaches 17%, where smoking and family history of GERD are important risk factors^[8]. In our study it was found that higher BMI is found in the cases of GERD as compared to the controls.

The threshold for diagnosing GERD by pepsin using “Peptest”, in a cross-sectional study on Vietnamese patients with extraesophageal symptoms, was 31.2 ng/mL with 86.7% sensitivity, and 27.5% specificity^[9].

Another case control study using Peptest versus ELISA measuring of pepsin on GERD patients. They found that Peptest was reflecting the diagnosis of GERD than salivary pepsin. Peptest was not affected by measuring diurnally, or 60 minutes after the occurrence of symptoms^[10]. This could be explained by; that the optimal collection of pepsin samples for the Peptest is at the postprandial time or at night time (PM), as the levels of the pepsin are higher than the morning time (AM)^[11].

Different subtypes of GERD had a significant increase in salivary pepsin as compared to healthy control including non-acid reflux disease (NERD), erosive esophagitis, Barrett’s esophagus, and typical GERD. In addition, salivary pepsin can be used as a complementary test to 24 hour- esophageal pH monitoring in GERD diagnosis^[12].

A small group of patients with laryngeal cancer had greater levels of salivary pepsin and salivary bile acids than healthy controls, suggesting that both may have contributed to the disease's development^[13].

Moreover, salivary pepsin was found to independently predict treatment response of laryngeal reflux disease^[14].

In a small cohort salivary pepsin was shown to exhibit a positive predictive value (PPV) of 81% and a negative predictive value (NPV) of 78% in diagnosing GERD as compared to endoscopy and 48 hours pH esophageal monitoring^[15].

Finally, in our study we found that the higher the grade of GERD (according to the Los Angeles classification) the higher the level of salivary pepsin level. Thus salivary

pepsin can be used to predict the severity of GERD and for the follow up of the GERD patients after therapy.

DISCLOSURE OF INTEREST

There are no conflicting interests to disclose, according to the authors.

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AUTHORS CONTRIBUTION

Prof. Sarah El Nakeep designed and approved the whole research protocol and amended the final paper version to be published. Professor Essam Bayoumy contributed to the protocol design and revised the manuscript draft version to be published. Mostafa Abbas supervised sample collection according to inclusion criteria, revised clinical data, diagnosis, and patient classification. Dr. Mariam El Sayed monitored data collection process and carried out the laboratory work. Asst. prof. Hager ELessawy collected the samples and patient’s clinical data, carried out statistical analysis of the results, and wrote the manuscript draft. The manuscript was revised and approved by all authors.

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الببسين اللعابي كعلامة داخلية لتشخيص الأنواع الفرعية من مرض الارتجاع المعدي المريئي (GERD)

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الخلفية: يعد مرض ارتجاع المريء من الامراض الشائعة التي تؤثر بشكل كبير على جودة الحياة. وقد تم ربط إنزيم الببسين، وهو بروتياز موجود في المعدة، كمسبب للمرض، وقد يعمل كمؤشر حيوي محتمل لتقييم الحالة.

الهدف: يهدف بحثنا إلى تقييم دقة إنزيم الببسين اللعابي كمؤشر داخلي لتشخيص مرض الارتجاع المعدي المريئي.

الطرق: شملت هذه الدراسة على 90 مريضاً مصرياً يعاني 45 منهم من مرض ارتجاع المريء و 45 من المتطوعين الأصحاء. تمت مقابلتهم في العيادات الخارجية بعد استيفائهم لمعايير الإدراج، وذلك في الفترة من أغسطس 2023 حتى يناير 2024. تم تقدير مستوى الببسين في عينات اللعاب وتم تقييم شدة المرض وفقاً لتصنيف لوس أنجلوس ومقارنتها بمستويات الببسين.

النتائج: أظهرت الدراسة الحالية زيادة ذات دلالة إحصائية عالية (قيمة $p < 0.001$) في مستوى الببسين اللعابي في مجموعة المرضى (46.6 ± 108.5 نانوغرام/مل) مقارنة بمجموعة الشواهد (24.4 ± 9.9 نانوغرام/مل). كما كشفت الدراسة عن وجود علاقة ذات دلالة إحصائية بين مستوى الببسين في اللعاب وتصنيف لوس أنجلوس لشدة المرض. وقد لوحظ ارتفاع ذو دلالة إحصائية.

(قيمة $p < 0.001$) الثالثة في مستوى الببسين اللعابي في الحالات من الدرجة (27.4 ± 191.9 نانوغرام/مل) مقارنة بالحالات من الدرجة الاولى الدرجة الثانية (22.9 ± 98.2 نانوغرام/مل) و (25.5 ± 84.5 نانوغرام/مل).

ومن خلال منحنى تبين أن الببسين اللعابي يمكن استخدامه للتمييز بين مجموعة المرضى ومجموعة الشواهد عند مستوى قطع < 46.5 ، بحساسية 95.6%، ونوعية 97.8%، وقيمة تنبؤية إيجابية 97.8%، وقيمة تنبؤية سلبية 95.7% ($AUC = 0.99$)، وقيمة $p < 0.001$.

الاستنتاج: يمكن استخدام الببسين اللعابي كاختبار تشخيصي فعال من حيث التكلفة لتقييم شدة المرض ولمتابعة مرضى بعد العلاج.