Expression of metalloproteinase-9 in esophageal tissue related to degree of gastroesophageal reflux disease

Hanan Ahmed Abdel Sattar
M.B,B.Ch, Menoufiya University, hanan.ahmed5055@gmail.com

Hassan Elsayed Zaghla
Professor of Hepatology, Hepatology Department, National Liver Institute, Menoufiya University

Medhat Assam Mahrous
Professor of Hepatology, Hepatology Department, National Liver Institute, Menoufiya University

Ahmed Mohamed EL-Refaie
Assistant professor of Pathology, Pathology Department, National Liver Institute, Menoufiya University

Amr Ragab Shalaby
Lecturer in Hepatology, Hepatology Department, National Liver Institute, Menoufiya University

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Expression of Metalloproteinase-9 in Esophageal Tissue Related to the Degree of Gastroesophageal Reflux Disease

Hanan Ahmed Abdel Sattar [a,*], Hassan Elsayed Zaghla [b], Medhat Assam Mahrous [b], Ahmed Mohamed EL-Refaie [c], Amr Ragab Shalaby [b]

[a] Menoufiya University, Egypt
[b] Hepatology Department, National Liver Institute, Menoufiya University, Egypt
[c] Pathology Department, National Liver Institute, Menoufiya University, Egypt

Abstract

Background: The condition known as gastroesophageal reflux disease arises when the contents of the stomach result in bothersome symptoms and issues. Matrix metalloproteinases are endopeptidases that may break down extracellular matrix elements. They are crucial for tumor invasion and metastasis.

Objective: To correlate the expression of metalloproteinase-9 (MMP-9) in patients with mild and severe gastroesophageal reflux disease in their esophageal samples.

Method: Cross-sectional investigation: Patients with mild and severe GERD had their esophagus tissue biopsied to assess the expression of MMP-9. Age, sex, diagnosis, smoking, body mass index (BMI), and MMP-9 expression were the included variables. Descriptive statistics were used, including the X2 and Kappa tests for diagnosis concordance.

Results: There were 50 patients with 27 (54 %) men and 23 (46 %) women, with an average age of 55.6 14.75. Nineteen people (38 %) were smokers. The average BMI ranged from 20 to 31; 12 (24 %) people had obesity. For histological diagnosis, the interobserver concordance was 1.0, and for the severity of esophagitis, it was 0.84. There were 16 patients (32 %) with Barrett's esophagus, 30 (60 %) with mild to moderate esophagitis, and 4 (8 %) with esophageal cancer. Patients with mild esophagitis (3.4 $\pm$ 7.78), moderate esophagitis (12.2 $\pm$ 5.23), Barrett's esophagus (25.7 $\pm$ 2.8), and esophageal cancer (38.6 $\pm$ 5.26) all had MMP-9 expression.

Smoking and histological diagnosis showed statistically significant correlations with MMP-9 expression.

Conclusions: Expression MMP-9 showed a significant positive correlation with severity of GERD.

Keywords: Biopsy, Esophageal, Gastroesophageal, Metalloproteinase-9, Reflux

1. Introduction

The condition known as gastroesophageal reflux disease (GERD) develops when the stomach contents are refluxed and cause unpleasant symptoms or issues. Heartburn is a condition that 35−44 % of people experience at least once a month.[1]

Extra esophageal instances are further separated into those with postulated and those with established links. Separated from extra esophageal cases are those with esophageal injury, such as those with reflux esophagitis, reflux stricture, Barrett's esophagus, and esophageal cancer, as well as those with normal reflux syndromes and reflux chest pain syndrome.[2]

Recurrent otitis media, reflux tooth erosion syndrome, reflux cough syndrome, reflux laryngitis syndrome, reflux asthma syndrome, and reflux cough syndrome have all been linked in studies. Pharyngitis, sinusitis, idiopathic pulmonary fibrosis, and pharyngitis are among more conditions that have been linked.[3]

The symptoms were categorized into four groups in the same consensus. It is important to rule out a

* Corresponding author.
E-mail address: hanan.ahmed5055@gmail.com (H.A.A. Sattar).

cardiac origin for chest discomfort because it can resemble ischemic heart pain. Serious sleep disorders: GERD patients frequently experience nighttime awakenings or difficulty falling asleep as a result of their symptoms, which are exacerbated when lying down and markedly alleviated by proton-pump inhibitors. Asthma, laryngitis, and cough are all respiratory issues. These signs are connected to the traditional heartburn and regurgitation signs. To focus on specific therapeutic modalities that would benefit each individual group of patients, paying attention to the lesion of the esophageal mucosa and esophageal symptoms, proposed the categorization of GERD in only three patient groups in 2002: nonerosive reflux disease, erosive reflux esophagitis, and Barrett's esophagus.[4]

Thus, erosive and nonerosive esophagitis were the moderate kinds, whereas Barrett's esophagus and esophageal cancer were the severe ones.[5]

The MMP family of zinc-dependent endopeptidases includes matrix metalloproteinases. They can all break down the extracellular matrix's individual components when acting as a group. There is a lot of proof that MMPs are crucial for tumor invasion and metastasis because they break down the extracellular matrix and connective tissue around tumor cells and the basement membrane.[6]

Yet, there are no Egyptian studies concerning the relationship between MMPs and severity of GERD. Therefore, the objective of this study is to present the association of MMP-9 expression in patients with GERD in its mild and severe forms in a tertiary center, National Liver Institute (NLI).

2. Patients and methods

Between December 2020 and May 2022 a cross-sectional comparative study was carried out in which the expression of MMP-9 was compared in 50 patients with esophagitis in its mild and severe forms. The material endoscopic biopsy blocks and patient data were retrieved from the archives of the Department of Cellular Pathology, National Liver Institute, Menoufia University. Biopsies were reported. Ethical approval for the study was obtained from the Local Research Ethics Committee.

Patients with GERD in mild and severe forms, of either sex, between 18 and 60 years, who voluntarily agreed to participate in the study and filled out the Carlsson Dent questionnaire for gastroesophageal reflux and patients with GERD diagnosed by the Carlsson Dent questionnaire, whose score had been ≥4, who met the inclusion criteria. We used this questionnaire because with this cutoff level of 4 points, it had a sensitivity of 70 % and a specificity of 46 % for suffering from esophagitis, and it did not require complementary studies, which were included in our study. Patients with digestive tract cancer at other levels, clinical data of gastrointestinal obstruction, insufficient sample due to defect or errors in taking it, and gastroesophageal reflux, but with normal histopathological results, were excluded from our study.

The biopsies comprised the following (15/50 were mild GERD biopsies, 15/50 were moderate GERD biopsies, 16/50 were severe experiments or without dysplasia, 4/50 (three were adenocarcinoma biopsies and one squamous cell carcinoma).

These biopsies were selected on the basis of having paraffin blocks available for recut and histopathological reexamination. Four μ M thick paraffin sections were prepared from each paraffin block of the selected liver biopsies. Two of these sections were stained with Hematoxylin and Eosin (H&E) and examined to confirm the histopathological diagnosis before immunohistochemical procedures were started.

The MMP-9 used in this study was a commercial rabbit monoclonal (BioGenex, The Netherlands) antibody. Detection of the MMP-9 was done using the antigen–antibody complex and performed using a one-step supersensitive HRP stitch on kit HDAP (Bioforex, The Netherlands).

The MMP-9 steroid biopsies were first dewaxed using three 5-min cycles of fresh xylene. Following this, parts were hydrated using ethyl alcohol in descending concentrations (i.e., 100 % ethyl alcohol twice for 5 min each, followed by 95 % ethyl alcohol twice for 5 min each, and then 70 % ethyl alcohol once for 5 min). The liver tissue sections were treated with 3 % hydrogen peroxide in methanol for 10 min at room temperature to inhibit peroxidase activity. By incubating the MMP-9-stained liver sections with 5 % normal rabbit serum in Tris-buffered saline (TBS) for 10 min, nonspecific protein binding was also prevented. The primary antibody (monoclonal, rabbit antihuman, MMP-9; diluted 1:50) was then applied to sections for 30 min at room—MMP-9 temperature. This was followed by the detection of the MMP-9-positive cells using rabbit anti-monoclonal rabbit peroxidase anti-peroxidase serum (applied for 30 min; diluted 1:20). Finally, the color development in MMP-9-stained sections was achieved using $3'$, $3'$ diaminobenzidine (DAB; Sigma, U.K.), which renders the immunoreactive cells brown. This was followed by counterstaining the cosplayed biopsy AAL stained in biopsy sections with Mayer's Hematoxylin, dehydration through ascending ethyl alcohol concentrations, clearing using xylene, and mounting on DBX solution.

Table 1. Sociodemographic data of studied patients.

Sociodemographic	Studied patients (n = 50) Number (%)
Age	
Mean ± SD	55.6 ± 61.9
Range	23–82
Sex	
Male	27 (54.0)
Female	23 (46.0)
Smoker	19 (38.0)
Obesity	12 (24.0)

Table 2. Histopathological results of studied patients.

Histopathological	Studied patients (n = 50) Number (%)
Mild to moderate esophagitis	30 (60.0)
Severe esophagitis	16 (32.0)
Cancer	4 (8.0)

Table 3. Type of cancer of studied patients.

Histopathological	Studied patients (n = 4) Number (%)
Adenocarcinoma	3 (75.0)
Squamous cell carcinoma	1 (25.0)

Two controls were incorporated in each run; a positive control bone marrow biopsy with strong MMP-9 immunoreactivity, and a negative control the same control tissue section on which pre-dilated monoclonal universal rabbit negative control was applied instead of the MMP-9 primary antibody.

In Hematoxylin and Eosin-stained section, the cocophyed histopathological analysis of the biopsies was undertaken by two observers. The intra-observer agreement for the esophageal biopsy diagnosis was (1) Kappa.

MMP-9-stained sections were visualized by light microscopy. The positive staining of MMP-9 cells in tissues was indicated by the presence of brown color in the cytoplasm and the cells membrane esophageal mucosal. Each slide was evaluated with respect to the corresponding negative and positive control slides.

For every stained esophageal biopsy, the number of MMP-9-positive cells per 10 high positive field ($\times$ 40). Twice. Then, the two numbers were compared, and in the event of discrepancy an agreed number was deserved after further inspection of the sections.

Finally, for each stained esophageal biopsy, the summed MMP-9 pattern cell number per 10 high positive field ($\times$ 40) was calculated, and was recorded as the MMP-9 cell density'

MMP-9 cell density = Number of MMP-9 pattern cells/10 high-positive field ($\times$ 40).

2.1. Statistical analysis

Statistical Package for the Social Sciences, version 26 (SPSS Inc. Released 2018) was used on an IBM-compatible personal computer to gather, tabulate, and statistically analyze the data. Armonk, NY: IBM Corp., IBM SPSS Statistics for Windows, version 26.0. The test used included standard error (SEM) of the mean (X), using a Student (t) test, compare two means, when comparing more than two means, one-

Table 4. Relationship between histopathological results and MMP-9 expression in studied patients with and without heartburn, nausea and vomiting, epigastric pain, use of PPI, insomnia, and regurgitation.

	Mild esophagitis Mean ± SD	Moderate esophagitis Mean ± SD	Severe esophagitis Mean ± SD	Cancer Mean ± SD	Test of sig.	P value
Heartburn						
Positive	3.17 ± 2.44	8.113 ± 1.22	7.11 ± 1.23	9.65 ± 2.31	0.58	>0.05
Negative	1.022 ± 1.21	4.04 ± 3.22	18.23 ± 3.22	28.45 ± 3.4		
Nausea and vomiting						
Positive	2.04 ± 1.32	7.33 ± 2.44	3.18 ± 4.21	38.5 ± 5.25	0.73	>0.05
Negative	1.36 ± 7.24	4.32 ± 1.35	22.23 ± 1.4	0 ± 0		
Epigastric pain						
Positive	1.81 ± 4.75	8.95 ± 2.31	4.76 ± 2.45	9.6 ± 2.3	0.74	>0.05
Negative	1.54 ± 3.23	3.25 ± 5.25	20.64 ± 1.4	25 ± 3.44		
Use of PPI						
Positive	2.95 ± 3.12	7.32 ± 1.44	6.7 ± 2.1	28.35 ± 3.4	0.56	>0.05
Negative	10.45 ± 2.32	4.88 ± 5.25	18.7 ± 1.44	9.65 ± 2.3		
Insomnia						
Positive	0.45 ± 2.32	11 ± 3.25	22.13 ± 1.48	19.3 ± 2.21	0.82	>0.05
Negative	2.94 ± 3.12	11.38 ± 2.25	3.18 ± 1.44	19.3 ± 2.4		
Regurgitation						
Positive	1.13 ± 3.45	9.76 ± 2.3	6.2 ± 2.11	28.35 ± 3.4	0.68	>0.05
Negative	0.45 ± 32.32	4.88 ± 1.35	18.7 ± 4.4	9.65 ± 2.31		

Table 5. Relationship between histopathological results and demographic data of studied patients.

Demographic data	Mild to Moderate esophagitis (n = 30) Number (%)	Severe esophagitis (n = 16) Number (%)	Cancer (n = 4) No. (%)	Test of sig.	P value
Age					
Mean ± SD	58.35 ± 77.8	51.4 ± 20.59	50.6 ± 15.37	KW = 2.055	0.9978
Range	23–69	37–82	52–73		
Sex					
Male	12 (40.0)	12 (75.0)	3 (75.0)	χ^2 = 2.61	0.0519
Female	18 (60.0)	4 (25.0)	1 (25.0)		
Smoking					
Yes	7 (23.3)	9 (56.3)	3 (75.0)	χ^2 = 7.325	**0.0257***
No	23 (76.7)	7 (43.7)	1 (25.0)		
Obesity					
Yes	9 (30.0)	3 (18.8)	0 (0.0)	χ^2 = 2.097	0.3505
No	21 (70.0)	13 (81.2)	4 (100.0)		

Table 6. Relationship between MMP-9 expression and Histopathological diagnosis of studied patients.

Demographic data	MMP-9 expression Mean ± SD	Test of sig.	P value
Histopathological diagnosis			
Mild esophagitis	3.4 ± 7.78		
Moderate esophagitis	12.2 ± 5.23	**KW = 5.74**	<0.001**
Severe esophagitis	25.74 ± 2.82		
Cancer	38.6 ± 5.26		

Fig. 3. Esophageal biopsy with severe gastroesophageal reflux disease showing elongation of the lamina propria papillae and many inflammatory cells, H&E X100.
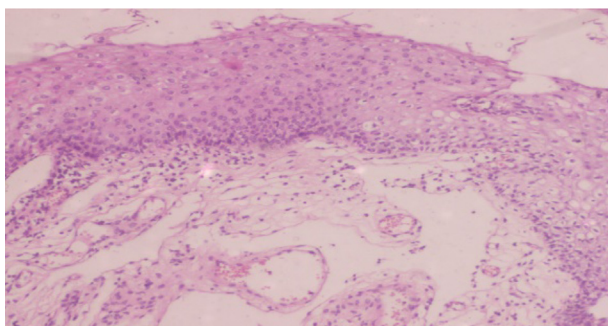

Fig. 1. Esophageal biopsy with mild gastroesophageal reflux disease showing basal cell hyperplasia, H&E X100.

Fig. 4. Esophageal biopsy with gastroesophageal reflux disease showing Barrett's esophagus, H&E X100.
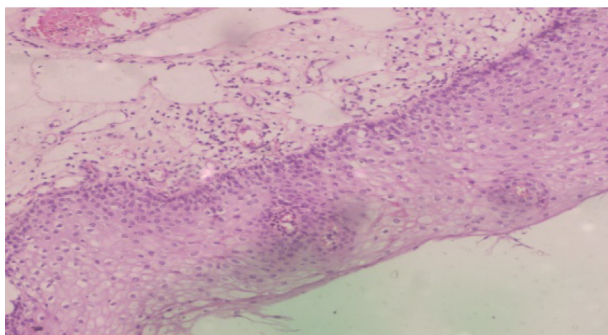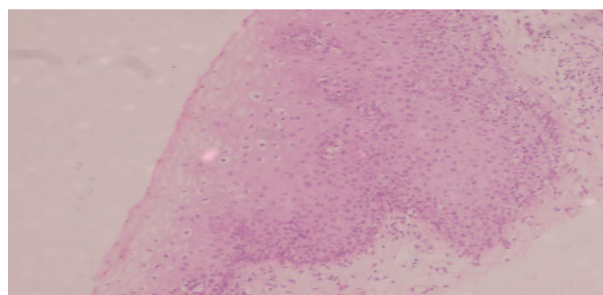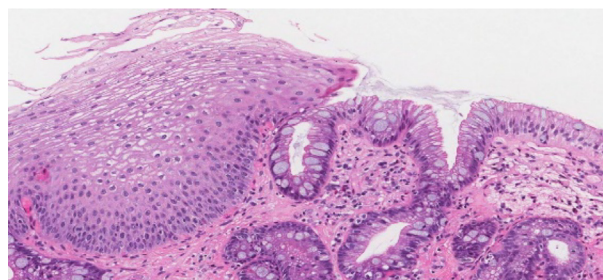

Fig. 2. Esophageal biopsy with moderate gastroesophageal reflux disease showing elongation of the lamina propria papillae, H&E X100.
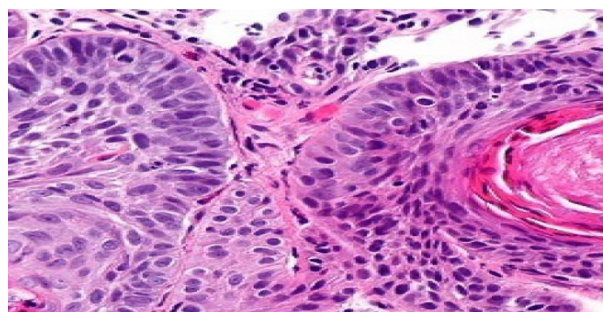

Fig. 5. Esophageal biopsy with gastroesophageal reflux disease and squamous cell carcinoma, H&E X400.
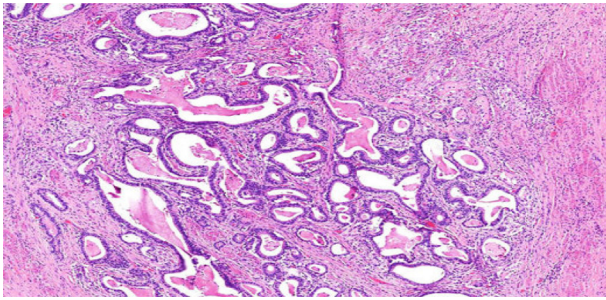
Fig. 6. Esophageal biopsy with gastroesophageal reflux disease and adenocarcinoma, H&E X200.
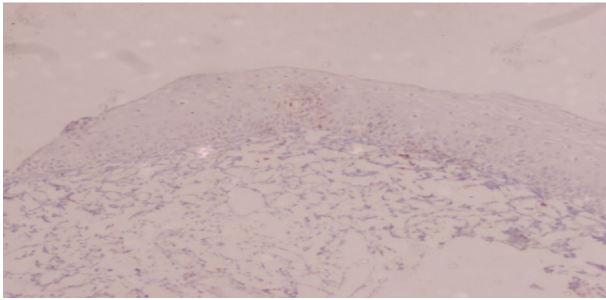

Fig. 7. MMP-9 immunostaining in esophageal biopsy with mild gastroesophageal reflux disease showing very few brown MMP-9-positive cells, X100.

way analysis of variance is equal to an ANOVA (F) test, where DF stands for the estimated degree of freedom. The values of P at each degree of freedom were then determined by comparing the computed (t) in the Student's (t) test or (F) in the ANOVA test with the tabulated (t) or (F) in (t) or (F) probability tables, where: $P > 0.05$ nonsignificant, P $0.05 =$ significant, and $P\,0.001 =$ extremely significant. The statistical software programs GraphPad Prism and Minitab were used to examine all the data.

3. Results

The mean $\pm$ SD of age in studied patients was 55.6 ± 61.9, ranged from 23 to 82; 54 % were male,
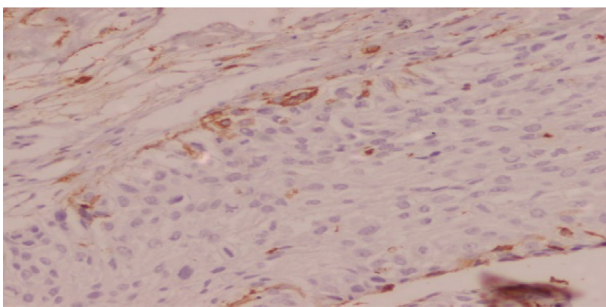

Fig. 8. MMP-9 immunostaining in esophageal biopsy with moderate gastroesophageal reflux disease showing some brown MMP-9-positive cells, X200.
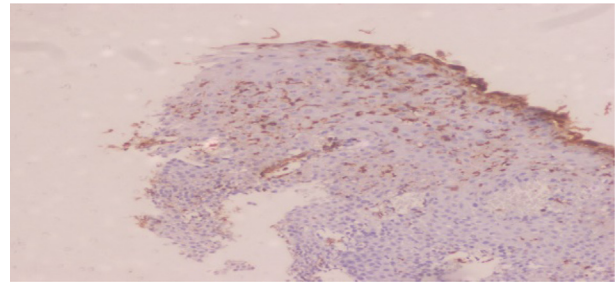

Fig. 9. MMP-9 immunostaining in esophageal biopsy with severe gastroesophageal reflux disease showing many brown MMP-9-positive cells, X100.

38 % smokers, and 24 % were obese (BMI ranged from 20 to 33 kg/m2) (Table 1).

According histopathological results of studied patients, 60 % were mild to moderate esophagitis,

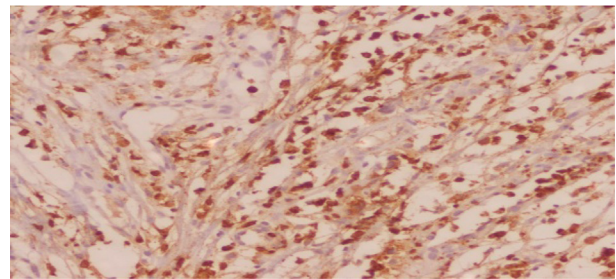
Fig. 10. MMP-9 immunostaining in esophageal biopsy with squamous cell carcinoma showing extensive brown MMP-9-positive cells, X200.
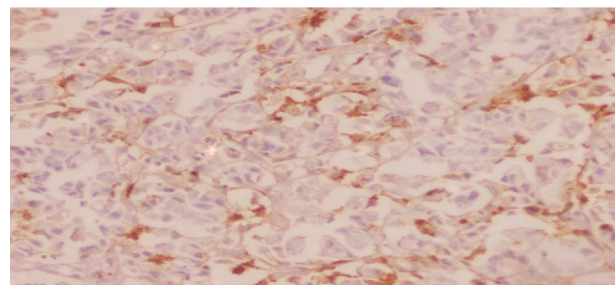

Fig. 11. MMP-9 immunostaining in esophageal biopsy with signet ring adenocarcinoma showing extensive brown MMP-9-positive cells, X200.
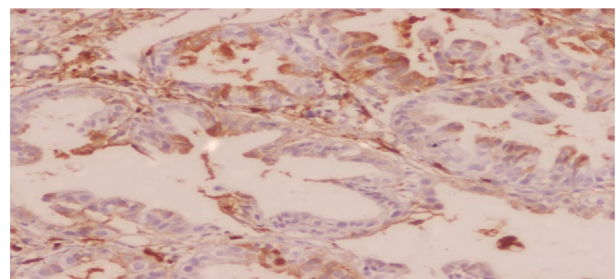

Fig. 12. MMP-9 immunostaining in esophageal biopsy with adeno-carcinoma showing extensive brown MMP-9-positive cells, X200.

32 % severe esophagitis, and 8 % were cancer (Table 2).

From the cancer cases, 75 % were adenocarcinoma and 25 % were squamous cell carcinoma (Table 3).

There were no association between MMP-9 expression in different degrees of histopathological results and heartburn, nausea, vomiting epigastric pain, use of PPI, insomnia, and regurgitation (Tables 4–6), Figs. 1–12.

4. Discussion

About 20 % of adults in high-income nations have GERD, which is characterized by persistent and bothersome heartburn, regurgitation, or GERD-specific problems.[7] Erosive esophagitis, esophageal strictures, Barrett's esophagus, esophageal malignancy, hiatus hernia, delayed stomach emptying, and visceral hypersensitivity are further side effects of chronic acid reflux.[8]

A few GERD risk factors that are more closely associated with the patient's way of life include the use of nonsteroidal anti-inflammatory drugs (NSAIDs), certain food and drink categories, smoking, family history, BMI, physical activity, salt and pickle use with meals, and fast food. Moreover, studies have linked GERD to factors such as age, gender, pregnancy, and geographic variation. It has been hypothesized that spine misalignment or vertebral fractures may influence the prevalence of GERD.[9] Reflux esophagitis (RE), bleeding, stricture, Barrett's esophagus (BE), and cancer are esophageal consequences of GERD. BE is a significant, potentially precancerous consequence of GERD, which is unmistakably linked to esophageal adenocarcinoma. BE is defined by a transition from the typical squamous esophageal epithelium to columnar epithelium, which happens as a side effect of persistent GERD. Recognizing BE is the main justification for evaluating patients with long-standing GERD. It is unclear if GERD-induced RE played a role in the development of BE; however, it has been proposed that cellular injury and subsequent repair with columnar epithelium may have occurred. Heartburn frequency and intensity are not helpful in predicting the presence of RE or BE.[10]

Close associations between disease, irritation, and MMPs are factors that are apparently gathered inside Barrett's pathogenesis. Further, the enzymatic action of the MMPs gives them the properties required for expected continuous imaging by applying activatable fluorescently marked substrates. Along these lines, we chose to assess in the event that MMP movement could be valuable for risk separation and illness determination in BE.

As proteolytic proteins, MMPs assume significant parts in the debasement of the extracellular lattice, prompting the proteolysis of microvessel storm cellar films and attack of the endothelium, which contribute exceptionally to the initial step of cancer improvement and metastasis.[11]

MMP-9 (gelatinase B), one of the MMPs, is particularly important in degrading collagen because it absorbs denatured collagen (gelatin), which is created when collagenases cleave the fibrillar collagen triple helix (MMP-1, MMP-8, MMP-13, and film type-1 MMP [MMP-14]). In addition, local type IV and type V collagens, elastin, aggrecan, and fibronectin are dynamic targets of MMP-9. In terms of transcriptional regulation and actuation procedure, MMP-9 varies typically. A few proteases, such as plasmin and MMP-3, can activate MMP-9 similar to other MMPs.[12]

The aim of the present study was to evaluate the association of MMP-9 expression in patients with GERD in its mild and severe forms in a tertiary center, National Liver Institute (NLI). The expression of MMP-9 was evaluated in 50 individuals with esophagitis in both moderate and severe forms in the current cross-sectional comparative investigation. According to the current study, 54 % of the patients were men, 38 % smoked, and 24 % were obese. The mean age of the patients was 55.6 61.9, ranging from 23 to 82. According to histological findings of research subjects, 32 % had severe esophagitis, 60 % had mild to moderate esophagitis, and 8 % had cancer. Gado et al. evaluated 433 patients with gastroesophageal RS who were having an endoscopy. The majority of the patients had mild esophagitis, reflecting grades 1 (93 %) and 2 (5 %), when RE was detected in 24 % of patients.[10]

Our discoveries were equivalent to those of the results by Montiel-Jarqun et al., which found that 32 patients (64 %) were men and 18 (36 %) were ladies, with a mean time of 52.13 14.75. Seven (14 %) had liquor abuse and 12 (24 %) were smokers.

The typical BMI ranged from 26.71 ± 4.07 kg/m^2 (15.3–33) with 40 (80 %) of them being obese. For histological conclusion, the interobserver concordance was 1.0, and for the seriousness of esophagitis, it was 0.84. Seven (14 %) people had esophageal disease, 16 (32 %) had Barrett's throat, and 27 (54 %) had esophagitis.[6] Gisbert et al.[13] uncovered that the middle patient age was 57 years of age and that 50–60 % of them were female. As per the study by Bruley des Varannes et al.,[14] the review populace's mean age was 54 years, 40 % of the patients were men, and the typical weight list was 26.6 kg/m2. GERD might make columnar and gastrointestinal metaplasia with potential

movement adenocarcinoma.[15] The current review showed that among cases with disease cases 75 % were adenocarcinoma and 25 % squamous cell carcinoma. Event of malignant growth in patients with GERD might be made sense of by esophageal aggravation and subsequent oxidative pressure prompting DNA harm. Both corrosive and bile are dynamic on oncogenic pathways. Corrosive prompts DNA harm, diminishes expansion, and increments apoptosis. Bile salts prompt DNA harm, influence expansion in a pH-subordinate way, and cause protection from apoptosis.[16]

The current investigation found no statistically significant correlation between age, sex, obesity, and either MMP-9 expression or histological findings. Similar findings were made by Li et al.,[12] who examined 58 surgically removed specimens of ESCCs and discovered that MMP-2 expression was unrelated to age or sex. According to Tomaszewski et al., individuals with esophagogastric cancer under the age of 60 years experienced more pain and discomfort than patients beyond the age of 60 years. The results of the current investigation revealed a statistically significant relationship between MMP-9 expression and smoking and the histopathological diagnoses of the patients who were the subject of the study. A significant positive correlation between MMP-9 and GERD severity was found. MMP-9 was expressed in four patients with esophagitis, five patients with Barrett's esophagus, and five patients with esophageal cancer, according to the Montiel-Jarqun et al. study. Smoking, MMP-9 expression, and histology diagnosis were found to be statistically associated with each other.[6]

MMP-9 has been linked to the pathophysiology of Barrett's esophagus, ESCC, and gastric cancer in previous research.[12,17,19] It has been demonstrated in the past that MMP-13 expression aids in the development of malignant cells in gastric cancer and ESCC specimens. It has also been proposed that its coordinated overexpression in conjunction with MMP-1 and/or MMP-2 may have a synergistic impact on the growth of tumors.[20,21]

According to Zeng et al.,[11] overexpression of MMP-9 was linked to poor cell differentiation, a poor TNM stage, lymph node metastasis, vascular invasion, and a poor prognosis for ESCC, indicating that MMP-9 may have been involved in the development of ESCC and may be a prognostic factor for the disease.

According to the research by Herszenyi et al.,[22] MMP-9 expression rises in the progressively aberrant tissues of Barrett's pathogenic sequence. The EAC had the highest MMP-9 expression, which is consistent with our findings. Moreover, they noticed

a much increased expression of the MMP-9 protein even in the early stages of esophagitis when compared with the control squamous tissues. This trend suggests that because this particular MMP is either elevated or chosen throughout Barrett's pathogenesis, it may have application as a predictive biomarker in BE. In addition to depth of invasion, lymphatic permeation, and nodal metastasis, Tanioka et al.[22] demonstrated that MMP-9 expression for the entire tumor was associated with the esophageal tumor differentiation grade as well.

Moreover, we found no correlation between histological findings and clinical history determining patients' quality of life. The research by Zuberi et al.[23] on patients who reported having heartburn and/or acid regurgitation at least twice per week for at least 3 months is consistent with our findings. The clinical intensity of epigastric discomfort and reflux did not significantly correlate with histology results. However, Paice et al.[24] contend that pain was a major symptom that had a significant impact on the HRQoL of cancer patients; 25%–75 % of the patients reported varying levels of pain, depending on the disease stage. Patients with cancer may struggle with anxiety, depression, and suicidal thoughts.[25]

In contrast to the general population, gastric cancer patients reported more preoperative symptoms such sleeplessness, decreased sexual activity, and appetite loss, according to the Svedlund et al.[24] research. According to Kidane et al.,[26] patients with clinical T1 esophageal cancer tend to have considerably lower patient-reported HRQOL scores than those with higher clinical T stages. After treatment, Liu et al.[27] found that EC drastically reduced the daily HRQoL of Chinese patients. The disparity between our study and others may be the result of varying sickness severity and posttreatment recovery times. According to the current study, there is no connection between MMP-9 expression at different histological levels and symptoms such heartburn, nausea, vomiting, epigastric discomfort, PPI use, regurgitation, or insomnia.

However, MMP-9 expression was strongly associated with unfavorable clinicopathological characteristics, including lymph node metastasis, vascular invasion, and tumor differentiation according to Tullavardhana et al.[28] We discovered strong proof that MMP-9 may be utilized as a marker to gauge how aggressive an esophageal carcinoma will be. Genes linked to the key EC pathogenic factor expression were found by Klimczak-Bitner et al.[29] In this population-based investigation of EC, the three most predictive genes were MMP-9, SERPINE1, and miR-134. Patients with AEGs may have a worse prognosis if their tumors express MMP-9 more

strongly according to the Lu et al. research.[30] Due to the various tissue specimen sources used for MMP-9 analysis, the various types of antibodies, and the various cutoff levels used to determine the positive MMP-9 expression, our results might be different from those of prior studies.[31]

Our study was limited by the small sample size and symptoms we had asked in our questionnaire; they were subjective, can happen in GERD, and can happen in many other diseases. We excluded as much as we can but could not exclude all.

4.1. Conclusion

MMP-9 expression showed significant positive correlation with severity of GERD in Egyptian patients.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article.

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Conflicts of interest

The authors declared that there were no conflicts of interest.

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