

Eosinophilic Esophagitis in Patients with Refractory Gastroesophageal Reflux Disease (GERD).

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Background and study aim : Eosinophilic esophagitis (EE) is defined as the presence of an abundant eosinophil infiltrate of the esophageal mucosa observed at biopsy. For some time, the existence of a pathophysiological relationship between GERD and EE has been speculated. The aim of this work is to estimate the frequency of eosinophilic esophagitis among patients with refractory reflux esophagitis.

Patients and Methods: We selected fifty patients diagnosed previously as refractory GERD (group I). Another fifty patients corresponding in age and sex with group I with esophageal and extra-esophageal clinical symptoms suggestive of GERD were enrolled in this study as control (group II). All patients were subjected to upper GIT endoscopy (multiple biopsies were taken), 24 hrs esophageal PH monitoring, esophageal motility study, histopathology and immune-histochemistry of esophageal biopsies.

Results: Two patients with EE were found among patients with refractory GERD. Dysphagia, heart burn, and food impaction were the common presenting symptoms of patients with EE. Rings and furrow were the most common and significant endoscopic pictures in cases of EE. Eosinophils, microabscess, basal zone hyperplasia and increased lamina propria papillae were the significant specific finding for EE in esophageal biopsies. Esophageal dysmotility and occurrence of reflux were common and significant in patients with EE. Immuno-histochemistry had high sensitivity in detection of eosinophils and its degradation product in esophageal biopsies.

Conclusion: eosinophilic esophagitis is one of the causative factors of refractory GERD and its frequency is about 4%. It should be put in differential diagnosis of cases of refractory GERD. Endoscopic picture is suggestive but biopsy for EE is confirmatory.

INTRODUCTION

Eosinophilic esophagitis (EE) is defined as the presence of an abundant eosinophil infiltrate of the esophageal mucosa observed at biopsy [1]. While it is true that there is still controversy about the number of eosinophils that must be observed per high power field (HPF) for the diagnostic standard of EE, most authors have used a number equal or greater than 15 cells [2].

This disease was described in children and in adults [3]. An increased prevalence has been observed which may be

partially explained by the more diligent search for the disease or etiology-related changing mechanisms [4].

The prevalence of EE is highly variable and appears to depend on the characteristics of the study population. In a prospective studies indicated a low prevalence (0.05–0.4%) in general population [5]. However, it may be up to 15% in patients with dysphagia [6] or as high as 48% in patients with food bolus impaction [7].

The clinical features of EE are not completely known due to the fact that few prospective studies have been conducted. However, EE is frequently associated with dysphagia, esophageal food impaction, allergic processes (aeroallergens, food, asthma) and some endoscopic abnormalities (concentric rings, longitudinal furrows, mucosal white plaques, strictures and narrowing of the esophageal lumen [8]. EE may also be associated with clinical symptoms suggestive of gastro-esophageal reflux disease (GERD) such as heartburn and regurgitation in about 30% of cases [9]. Also cases with non-specific symptoms and normal endoscopy have been reported from 8% to 28% of cases [10].

For some time, the existence of a pathophysiological relationship between GERD and EE has been speculated, as eosinophilic infiltrates have been observed in the esophageal mucosa of patients with GERD. It has also been suggested that EE may cause secondary GERD [11]. Some authors have reported more than 20 eosinophils \times HPF in patients with GERD, which disappeared after treatment with gastric acid inhibitors [12]. In other results it was observed that patients with EE are hypersensitive to acid perfused in the oesophagus, thus acid may play a role in the symptoms [11].

Although there may be overlapping of the two diseases, it is currently accepted that the EE is a pathological entity different from GERD, whose etiology seems to be related to allergic and genetic factors [13]. Consequently, EE symptoms respond well to topical steroid administration, while the response to gastric acid inhibitors is very limited.

We hypothesized that some patients with GERD symptoms who do not respond to conventional treatment may have EE. Currently this disease is not routinely considered in the differential diagnosis of refractory GERD. For this reason, we proposed as main objective to prospectively evaluate the frequency of EE in a consecutive population of patients with GERD who were refractory to conventional therapy, and as secondary objective to describe the clinical features and predictors of EE in this subset of patients.

The aim of this work is to estimate the frequency of eosinophilic esophagitis among patients with refractory reflux esophagitis, to study its effect on the course of the disease and to find in what way it can be differentiated from non refractory GERD.

PATIENTS AND METHODS

Patients and study design :

This study was done in Tropical Medicine and Pathology departments Faculty of Medicine Zagazig University, from July 2008 to December 2012. We prospectively studied patients who were treated at the outpatient clinic of the Gastroenterology Unit. We selected fifty patients diagnosed previously as refractory GERD as they had persistent esophageal or extra esophageal symptoms suggestive of GERD and are refractory to full dose PPI (40 mg) treatment for 8 weeks (group I). Failure of response to 8 weeks treatment is taken as a base of refractory GERD according to Locke et al. [14]. Another fifty patients corresponding in age and sex with group I with esophageal and extra-esophageal clinical symptoms suggestive of non refractory GERD according to the GERD Symptoms Checklist were enrolled in this study as control (group II).

Exclusion Criteria :

We excluded patients diagnosed with :

- 1- Crohn's disease.
- 2- Scleroderma.
- 3- Fungal infections.
- 4- Collagen disease.
- 5- Esophageal carcinoma.
- 6- Drug induced esophagitis.

This study was performed on these patients to study the frequency and the role of eosinophilic infiltration on the course of GERD. All patients underwent a complete history taking included demographic data, history of environmental, food, and drug allergies, clinical symptoms suggestive of GERD, length of symptoms, type and duration of GERD treatment, adherence to treatment, and associated co-morbid states and diagnostic testing for GERD.

All patients are exposed to the following investigations :

- 1- CBC especially eosinophilic count.
- 2- Chemical laboratory tests including LFTs, KFTs and fasting blood sugar to exclude hepatic, renal and diabetes which affect esophageal motility.
- 3- Esophagogastroduodenoscopy: Using Pentax (EG-2940) endoscope to assess the presence of esophagitis, multiple biopsies were taken from the upper, middle and lower third of the esophagus. Esophageal biopsy specimens were obtained by direct endoscopic vision using fenestrated, ellipsoid, spiked 7mm open

span biopsy forceps. Four quadrant biopsies were taken from each subject essentially

including areas suffer from sings of esophagitis.

Grading were done according to Los Angeles classification of esophagitis [15] :

Grade A	One (or more) mucosal break no longer than 5 mm that does not extend between the tops of two mucosal folds
Grade B	One (or more) mucosal break more than 5 mm long that does not extend between the tops of two mucosal folds
Grade C	One (or more) mucosal break that is continuous between the tops of two or more mucosal folds but which involve less than 75% of the circumference
Grade D	One (or more) mucosal break which involves at least 75% of the esophageal circumference

4- Oesophageal Manometry Study : We studied our cases using Sandhill Smart Lab. Computer Polygraph Manometry system.

2- Oesophageal body motility : Measures are made for the peristaltic parameters: amplitude, and duration. Usually 10 wet swallows are assessed and parameters are based on the mean.

Interpretation of the results :

1- Resting lower esophageal sphincter (LOS)

Pressure : Two popular ways to measure the LOS pressure are from gastric baseline to the either mid-expiration or end-expiration pressure at the highest point. We used mid-expiration pressure because it provides a resting LOS pressure measurement that most reliably distinguishes patients with normal of gastro-oesophageal reflux from those with abnormal amounts. Thus the pressure component contributed by the diaphragm during respiration may be an important component of the antireflux mechanism of the LOS and should be included in the assessment of overall resting pressure. We considered normal value of mid-expiration LOS pressure 24.4 ± 10.1 mmHg according to Ott et al. [16].

The values given below are considered to be standard for wet swallow [16]:

Normal oesophageal body pressure data :

Wet swallows	Duration (sec)
Amplitude (mmHg)	
62 ± 29	2.8 ± 0.8
70 ± 32	3.5 ± 0.7
99 ± 40	3.9 ± 0.9

5- Ambulatory Oesophageal Ph Monitoring

Interpretation : Acid reflux was defined where the PH in the oesophagus dropped to 4.0 or less. In the analysis of oesophageal PH recording, different parameters were estimated including :

The variables advocated by Johnson and Demeester [17] :

a- Parameter : Percent of time pH <4	Normal value
% time reflux upright	< 6.3
% time reflux supine	< 1.2
% time reflux total	< 4.2
b- Number of episodes:	
Total episodes	< 50
Episodes longer 5 min	< 3
Longest episode (min)	< 9.2
c- Composite score	< 22

6- Histological Examination : Diagnosis of EE is based on criteria described in diagnosis and treatment guidelines published in 2007. According to these guidelines, diagnosis is confirmed in patients with :

- Symptoms suggesting esophageal dysfunction,
- ≥ 15 eosinophils per HPF ($\times 400$) in at least one esophageal biopsy samples, and
- Exclusion of other causes of esophageal eosinophilia [18].

Biopsy specimens were fixed in 4% formalin, embedded in paraffin, serially, sectioned and then stained with hematoxylin and eosin. Biopsy. Preparation and step serial sections of biopsy specimens were performed to enhance detection of eosinophils [19].

7- Immunohistochemistry : To detect eosinophilic count in oesophageal biopsies in both groups which refers to the process of detecting antigens of eosinophiles in tissue sections by exploiting the principle of antibodies binding specifically to antigens in biological tissues [20].

RESULTS

The mean , SD ($X \pm SD$) and range of patients ages with refractory GERD (group I) were 37 ± 14 and 20-70 years respectively. In addition 23(46%) were males and 27(54%) were females. While the demographic data in patients with GERD (group II) show the mean , SD and range of ages were 32.9 ± 13 and 18-68 respectively in addition 24 (48%) were males and 26 were females (52%). There is no statistical significant difference in demographic data between the two groups.

The clinical picture of the studied patients showed that in group I heart burn, epigastric pain, vomiting, dysphagia, haematemesis and cough were 60%, 40%, 32%, 30%, 8%, 18% respectively while in group II they were 82%, 50%, 24%, 14%, 22% and 20% respectively. There is statistical highly significant difference between the two groups only as regard heart burn.

The associated allergic diseases in the studied patients such as bronchial asthma, allergic rhinitis, allergic conjunctivitis and autoimmune diseases in group I were 10%, 10%, 4% and 8% while in group II were 12%, 4%, 4% and 2% respectively without statistical significant differences between both groups.

The endoscopic finding in the studied groups showed pathological changes as esophageal rings and furrows in 2 patients of group I (eosinophilic esophagitis patients). Los Angeles classification showed that grade A, B, C and D were 50%, 26%, 16% and 8% in group I while in group II were 82%, 2%, 2% and 2% respectively. There was highly statistically significant difference between the two groups as regard endoscopic grading (table 1).

The motility study in group I showed hypo peristalsis in 94%, hyper peristalsis in 6% and hypotensive lower oesophageal sphincter (LES) in 92% while in group II were 70% hypo peristalsis, 30% hyper peristalsis and 80% hypotensive LES. There was highly significant difference between the two groups as regard the peristalsis but no significant statistical difference in the lower esophageal sphincter tone. 24- pH monitoring of group I showed that reflux state was present in 18% and absent in 82% of patients but in group II present in 86% and absent in 14%. Reflux frequency were few in 10% and many in 90% in group I patients while was few in 68% and many in 32% in group II. There is highly statistical difference between both groups as regard the state of reflux (frequency and number of attacks).

There was no statistical significant difference between both groups as regard the count of blood eosinophils and esophageal biopsies eosinophils by histopathology . The eosinophilic count in the esophageal biopsies by immunohistochemistry revealed mean \pm SD of eosinophilic count in esophageal biopsies of group I was 2.72 ± 4.6 (range 0-27) while in group II were 0.76 ± 1.5 (range 0-5). There was highly significant difference between both groups (table 2). There was strong correlation between the eosinophilic count in esophageal biopsies by histopathology and immunohistochemistry ($r \geq .97$, $p < 0.001$).

The histopathological features in patients of EE patients (2 patients) showed the characters of EE; eosinophils ≥ 15 /HPF, increased lamina propria papillae in 100%, basal zone hyperplasia in 50% and microabcess in 50% while in other refractory GERD patients (48 patients) showed oedema in 58%, neutrophils in 16%, fibrosis in 4.8% and eosinophils ≤ 6 cells /HPF in 6.3% with highly significant statistical difference between the two groups in microabcess, lamina propria papillae, oedema, basal zone hyperplasia, fibrosis and eosinophilic count and significant difference in neutrophils count (table 3).

Table (4) shows the histopathological findings of studied groups that reveal eosinophilic count (mean \pm SD) in group I: 2.6 ± 4.5 (rang 0-27), lymphocyte not detected in 78% and detected in 22%, microabcess in 4%, fibrosis of lamina propria detected in 2% while leucocytes detected in 2% but group II showed eosinophilic count: 8 ± 1.5 (range 0-5), lymphocyte, fibrosis, microabcess and leucocyte were not detected. There was highly significant difference between both groups as

regard eosinophilic count and fibrocytes detected in the esophageal biopsies.

Table (1) : Upper GIT Endoscopy of studied patients (Los Angeles classification)

	Group I		Group II		X ²	P
	No	%	No	%		
Pathological finding						
Rings	2	4	0	0	2.04	0.15 (NS)
Furrows	2	4	0	0	2.04	0.15 (NS)
Stricture	2	4	0	0	2.04	0.15 (NS)
Hiatus hernia	10	20	41	82	2.99	0.08 (NS)
Grade of GERD						
A	25	50.0	41	82.0	9.2	0.001* (HS)
B	13	26.0	1	2.0	8.9	0.004* (HS)
C	8	16.0	1	2.0	7.8	0.002* (HS)
C	4	8.0	1	2.0	12.9	0.004* (HS)

Table (2) : Immunohistochemistry of esophageal biopsies of the studied patients

	Group I	Group II	X ²	P
Eosinophils				
X ± SD	2.72 ± 4.6	0.76 ± 1.5	10.09	0.0014** (HS)
Range	0 - 27	0 - 5		

Table (3) : Histopathological features in patients with EE and gastro-esophageal reflux

	EE patients (N = 2) Mean (range)		Refractory GERD (N = 48) Mean (range)		X ²	P
	No	%	No	%		
Eosinophil/HPF	20 (12-25)		3 (0-6)			
Micoabcess	1	50	0	0	9.4	0.001 (HS)
Increase lamina propria papillae	2	100	3	6.3	12.1	0.003(HS)
Basal zone hyperplasia	1	50	4	8.3	8.1	0.004 (HS)
Oedema	0	0	28	58.3	7.6	0.004 (HS)
Neutophils	0	0	8	16.6	5.4	0.03 (SD)
Eosinophils	2	100	3	6.3	10.4	0.001(HS)
Fibrosis	1	50	2	4.8	6.7	0.003(HS)

Table (4) : Histopathology of studied groups

	Group I		Group II		X^2	P
	No	%	No	%		
Eosinophil count					8.6	0.003* (HS)
X \pm SD	2.6 \pm 4.5		0.8 \pm 1.5			
Range	0 - 27		0 - 5			
Lymphocytes					12.36	0.001* (HS)
Not detected	39	78.0	50	100.0		
Detected	11	22.0	0	0.0		
Leucocytes					2.04	0.15 (NS)
Not detected	48	96.0	50	100.0		
Detected	2	4.0	0	0.0		
Microabscess					2.04	0.15 (NS)
Not detected	48	96.0	50	100.0		
Detected	2	4.0	0	0.0		
Fibrosis lamina pro.					2.04	0.15 (NS)
Not detected	48	96.0	50	100.0		
Detected	2	4.0	0	0.0		

DISCUSSION

Although we had known that eosinophilic esophagitis (EE) prevalence is low among the population, this study was conducted to through the light towards this disease.

One hundred patients were enrolled in this study, 50 patients with refractory GERD (group I) and the other with non refractory GERD (group II). All patients were studied clinically, endoscopically, manometrically and histopathologically to evaluate the presence of eosinophilic infiltration and its role in refractory esophageal pathology.

The demographic data of this study showed no statistical significant difference among the patients sex of two groups, with GERD either with or without refractory entity. This finding is in agreement with Vindigni et al. [21] but in contrary with Forountan et al. [22] who mentioned that the disease show female predominance. This difference can be explained by age and parity difference. The same findings of non-statistical significant difference were founded as regarding the age. This is consistent with Dellon et al. [23], but in contrary with Liacouras et al. [24] who mentioned that refractory GERD was more predominant in old ages. This can be explained by the effect of aging on the LES tone [25].

The clinical picture showed no statistical significant difference between both groups as regard dysphagia, vomiting, haematemesis, itching, cough, pallor, wheezes and urticaria, but there is a statistical significant difference as regard heart burn. These results were in agreement with Molina-Infante et

al. [26], and not in agreement with Aceves et al. [27] who mentioned that the dysphagia is the most common presenting symptoms in patients with refractory GERD. This difference can be explained by variability in the duration of disease. Where Richter [28] reported that the more advanced pathological findings were encountered in patients with long history of disease, as they selected their patients with long history of GERD.

The associated disease as allergic rhinitis, allergic conjunctivitis, bronchial asthma and auto immune diseases showed no statistical significant difference between the studied groups and these results were in agreement with Martinez et al. [29], but in contrary with Liacouras et al. [24] who stated that 28% of their patients suffer from associated allergic disease and this can be explained by relative low rate of allergic diseases in both groups of patients in this study.

The endoscopic finding in the studied groups showed pathological changes as esophageal rings and furrow in eosinophilic esophagitis patients (2 patients) and this was in agreement with Venge et al. [30] but in contrary with Remedios et al. [9] who stated that, plaques were the most common endoscopic findings in their patients but none was diagnosed with a ringed esophagus which is one of the most typical findings in the previous studies, together with linear furrows. This difference can be explained by seasonal variation and multiple variety in the endoscopic picture of EE [31]. There was a statistically significant difference between the two groups as regard endoscopic grading of esophagitis. These result

was in agreement with Dellon et al. [23], but in contrary with Shah et al. [32] who stated that no endoscopic difference among their studied groups. This variation can be explained by difference in operator experience, prevalence of EE and treatment used before endoscopy. The more pathological changes may be attributed to the events which reported by Hirano [33] as prolonged exposure of esophageal mucosa to acid, partial response of EE to PPI therapy and toxic effect of eosinophilic degranulation into the tissue.

The motility study of both groups showed highly statistical significant difference between the two groups as regard the peristalsis but no statistical difference in the lower esophageal sphincter tone. These result were in agreement with Pandolfino et al. [34] but in contrary with Tian et al. [35] and this difference can be explained by finding of Mueller et al. [36] as variations in factors controlling esophageal peristalsis and lower esophageal sphincter such as diet, hormonal, nervous, drugs and eosinophilic granule constituents which are toxic to a variety of tissues, including esophageal epithelium. Study of 24-ph monitoring of patients group shows a significant statistical difference between both groups as regard the state of reflux (frequency and number of attacks). These result in agreement with Weusten et al. [37], but differ with Mattioli et al. [38] who stated that 25% of GERD with esophagitis have 24 h pH monitoring within normal range. This variation can be explained by difference in defensive factories such as LES and luminal clearance of acids [39] or variation of injurious factors such as prolonged transient esophageal sphincter relaxation, hiatus hernia and blood supply [40].

As regard the eosinophilic count in peripheral blood which showed no statistical significant difference between patients of both groups and this finding is in agreement with Gonsalves et al. [41]. It is known that EE is a chronic immunological disorder characterized by clinical symptoms related to esophageal dysfunction and eosinophilic infiltration in the esophagus regardless the peripheral blood eosinophilic count [42]. The relation between peripheral blood eosinophils and esophageal biopsies eosinophilis showed no statistical significant difference. This result was in agreement with Attwood et al. [25], however, the result of this study was differing with Brown et al. [43]. This variation can be explained by more advances in diagnostic tools and nowadays EE is considered a local allergy of esophagus to foods (asthma of esophagus) [44]. Regarding to

the eosinophilic count in the esophageal biopsies by immuno-histochemistry revealed a statistical significant difference between both groups. These result was in agreement with Leader et al. [45]. Study of esophageal eosinophilia by H&E and immune histochemistry showed highly statistical significant difference between eosinophilic count by H&E stain and immuno-histochemistry stain. This result was in agreement with Mueller et al. [36] who stated that the immunohistochemistry detected up to two times more eosinophils than routine haematoxylin and eosin staining and can perhaps be used to discover minimal change EE. We did not find any study differ with this result as eosinophils morphologically are easily to see in conventional histology. Moreover, it is almost impossible to identify degranulated eosinophils with haematoxylin and eosin staining [46].

Correlation between eosinophilic count in esophageal biopsies by histopathology and immune histochemistry showed highly statistical significant correlation. This results were in agreement with Onbasi et al, [47] who reported that immune histochemistry using monoclonal anti body is the most sensitive method for eosinohils detection.

The histopathological finding in group I that showed a highly statistical significant difference between the 2 EE patients and other 47 refractory GERD patients as regard eosinophilic count, fibrocytes, oedema, basal cell hyperplasia and fibrosis detected in the esophageal biopsies these finding were in agreement with Spergel et al. [48] who founded that eosinoplic infiltration of esophageal mucosa ≥ 15 cells/hpf, oedema, basal cell hyperplasia and fibrosis are characterstic of EE.

The histopathological finding in both groups showed highly statistical significant differences in tissue eosinophilic count and lymphocyte but no statistical differences in leucocyte, microabcess and fibrosis lamina propria. These were in agreement with Straumann et al. [49]. The cause of eosinophilic infiltration is poorly understood, but allergy has been implicated. The majority of patients have evidence of food and aeroallergen hypersensitivity, as defined by skin prick test responses, however, only a minority have a history of food anaphylaxis [50], this indicating distinct mechanisms compared with classical IgE-mediated mast cell/basophil activation. Substantial evidence is accumulating that EE is associated with TH2-type immune responses. In

particular, increased levels of eosinophil-active TH2 cytokines (eg, IL-4, IL-5, and IL-13), as well as mast cells, are present in the esophagi of patients with EE [51].

CONCLUSION

Finally it can be concluded that the eosinophilic esophagitis is one of the causative factors of refractory GERD and its frequency is about 4%. It should be put in differential diagnosis of cases of refractory GERD. Endoscopic picture is suggestive but biopsy for EE is confirmatory. H&E stain appearance is cost-effective approach for diagnosis.

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Ethical approval: The study was approved by the Ethical Committee of Zagazig Faculty of Medicine and a written informed consent was taken from each participant that follows principles in the Declaration of Helsinki.

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