Serum eosinophilic cationic protein is correlated with food impaction and endoscopic severity in eosinophilic esophagitis

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ABSTRACT

Background/Aims: *The aim of the present study was to analyze the diagnostic accuracy of serum eosinophilic cationic protein (ECP) for eosinophilic esophagitis (EoE) and the correlation of ECP with clinical, histopathological, laboratory, and endoscopic features of EoE.*
Materials and Methods: *Fifteen patients with EoE and 14 healthy controls were included in the study. Demographic parameters were recorded. EoE Endoscopic Reference Score (EREFS) was calculated according to endoscopic features, and esophageal biopsies were obtained by a single experienced endoscopist in a patient group. Serum ECP levels (μg/mL), absolute eosinophil count (U/mm³), and maximum peak of eosinophils/high-power field in esophageal biopsies were analyzed.*
Results: *The median age of all participants was 33.0 (min-max: 18-46) years. There were 27 (93.1%) male patients. Serum ECP level was significantly higher in patients with EoE than in healthy volunteers (20.4 vs. 8.8, p<0.0001). According to the receiver operating characteristic (ROC) curve analysis, ECP had 80% sensitivity and 92.8% specificity to diagnose EoE with a cut-off value of 13.9 μg/mL (area under the ROC curve 0.895; p<0.0001; 95% CI: 0.725-0.978). EREFS (p<0.0001) and the presence of food impaction (p=0.04) were significantly correlated with ECP.*
Conclusion: *Serum ECP is an accurate non-invasive biomarker for EoE with high specificity and sensitivity. In addition, ECP is strongly correlated with EREFS and the symptom of food impaction.*
Keywords: *Eosinophilic esophagitis, eosinophilic cationic protein, diagnosis*

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic esophageal inflammatory disease that is mediated by immunogenic/antigenic (primarily food allergen) mechanisms. It is characterized by esophageal symptoms (dysphagia, heartburn, and chest pain) caused by esophageal dysfunction and eosinophilic esophageal inflammation (>15 eosinophilic granulocytes/high-power field (hpf)) (1,2). The prevalence of EoE in children and adults has been increasing steadily over the past decade (3-5). However, it is not clear if this is a true increase in prevalence or an artificial result due to improved understanding of the disease by physicians, enabling a higher rate of diagnosis (6).

Eosinophilic esophagitis Endoscopic Reference Score (EREFS) has been recently described to guide physicians for recognizing and reporting the main endoscopic findings of EoE (Table 1) (8). It has been documented that EREFS has a good inter- or intra-observer agreement. It is a simple scoring system with an accurate diagnostic utility that shows the severity of the disease and probability of response to treatment (8,9).

In addition to endoscopic/histopathological findings, some non-invasive serological markers have been studied for the diagnosis of EoE. Increased serum total IgE levels have been shown in active EoE disease (10). The diagnostic role of peripheral blood eosinophil count and eosinophil-derived proteins (eosinophil-derived neurotoxin and major basic protein) has been documented in previous studies. Another eosinophil-derived protein, eosinophilic cationic protein (ECP), has been studied and shown to be accurate in the clinical monitoring of asthma and allergic rhinoconjunctivitis (11). However, data regarding the diagnostic accuracy of ECP for EoE are extremely rare.

In the present study, we aimed to analyze serum ECP levels in patients with EoE and controls to predict the diagnostic accuracy of ECP for EoE and to determine the correlation of ECP with clinical symptoms, eosinophilia (histological/peripheral blood), and endoscopic reference score of EoE in a patient group.

MATERIAL AND METHODS

Fifteen adult patients with EoE were included consecutively according to the current diagnostic criteria (12) as

≥15 eosinophils/hpf (eo/hpf) in esophagus biopsies before and after treatment with proton pump inhibitors for 8 weeks. The control group included 14 healthy participants to provide blood sample only for ECP.

Endoscopic procedures were performed by an experienced gastroenterologist (CC) using a flexible 9.9 mm gastroscope with a 2.8 mm work channel (GIF-H180J; Olympus. Hamburg, Germany). Biopsies from the proximal esophagus were obtained using conventional forceps when there is an endoscopic stigmata of EoE. No complications were observed during the endoscopic procedure.

Biopsy specimens were sent to the pathology department of the TOBB University of Economics and Technology Hospital. A diagnosis of "histologically compatible with EoE" was made with the presence of ≥15 eo/hpf clustered in microabscesses.

Venous blood was collected from the participants. Absolute eosinophil count (AEC) was analyzed via an automated hematology analyzer at the central laboratory of the TOBB Economy and Technology University Hospital. ECP assay was performed in an affiliated commercial laboratory specialized for this type of tests.

ECP assay procedure
1. All reagents, samples, and standards were prepared according to the manufacturer's instructions.
2. One hundred μL of sample was added into each well and incubated for 2 h at 37°C.
3. The liquid of each well was removed, and 100 μL of biotin antibody was added into each well and incubated for 1 h at 37°C.
4. Each well was aspirated and washed three times.
5. One hundred μL of horseradish peroxidase-avidin was added into each well and incubated for 1 h at 37°C.
6. Each well was aspirated and washed five times.
7. Ninety μL of 3,3',5,5'-tetramethylbenzidine substrate was added into each well and incubated for 15-30 min at 37°C.
8. Fifty μL of stop solution was added into each well, and the assay was read at 450 nm within 5 min.

Statistical analysis
The Statistical Package for the Social Sciences (SPSS) version 16 software (SPSS Inc.; Chicago, IL, USA) was used for statistical analysis. Kolmogorov-Smirnov test was used to determine the normality of the distribution of data. Comparisons between the groups were performed by the Mann-Whitney U test for non-parametric variables. The group characteristics were compared using the Fisher's exact test and the chi-square test. A receiver operating characteristic (ROC) curve was used to determine the diagnostic ability of ECP for EoE. Pearson's test was used for correlation analyses. A p-value of <0.05 was considered statistically significant.

RESULTS

Study population
Our study included 29 participants (15 consecutive patients with EoE and 14 age-sex-matched control group). The median age of all participants was 33.0 (min-max: 18-46) years. Twenty-seven (93.1%) participants were male. Table 2 shows the demographic characteristics of the participants.

Table 1. Eosinophilic Esophagitis Endoscopic Reference Score (EREFS)

| | Classification and grading | | | |
	0	1	2	3
Rings	None	Mild (subtle circumferential ridges)	Moderate*	Severe**
Exudates	None	Mild¶	Severe¶¶	
Furrows	Absent	Present		
Edemas	Absent	Loss of clarity, absence of vascular markings		
Strictures	Absent	Present		

*Distinct rings that do not impair passage of a standard diagnostic adult endoscope of outer diameter 8-9.5 mm
**Distinct rings that do not permit passage of a diagnostic endoscope
¶Lesions involving <10% of the esophageal surface
¶¶Lesions involving >10% of the esophageal surface

Table 2. Demographic, clinical characteristics, and laboratory findings of the study groups

|	EoE (+), n=15	EoE (-), n=14	p
Age (median, min-max)	33.0 (18-44)	33.0 (18-46)	0.7
Male gender (n, %)	14 (93.3)	13 (92.9)	1.0
Atopic disease			
Asthma (n, %)	4 (26.7)	-	
Allergic rhinitis (n, %)	3 (20.0)		
Other AD (n, %)	1 (6.7)		
Symptoms			
Dysphagia (n, %)	9 (60.0)		
Heartburn (n, %)	6 (40.0)		
Food impaction	2 (13.3)		
Laboratory/histopathological finding			
Peripheral blood eosinophilia (n, %)	4 (26.7)		
EC/hpf (esophageal biopsy) (median, min-max)	40 (15-100)		
AEC (median, min-max)	285 (205-450)		
EREFS (median, min-max)	3.0 (1.0-6.0)		

EoE: eosinophilic esophagitis; AD: allergic disease; EC: eosinophil count; hpf: high-power field; AEC: absolute eosinophil count; EREFS: Eosinophilic Esophagitis Endoscopic Reference Score

### Clinical characteristics, laboratory findings, and EREFS of patients with EoE

Eleven (73.3%) patients with EoE had at least one allergic disease. Eight out of the 11 patients had food allergy, three had allergic rhinitis, and four (26.7%) had bronchial asthma. The most common symptom was dysphagia (9/15, 60.0%) among patients with EoE. The median eo/hpf count in esophageal biopsy was 40 (min-max: 15-100) while peripheral blood eosinophilia was observed in 4 (26.7%) patients with EoE. The median EREFS was found to be 3.0 (min-max: 1.0-6.0) in patients with EoE. Table 2 shows the clinical characteristics and laboratory findings of patients with EoE.

Predictive value of ECP for EoE

Eosinophilic cationic protein level was significantly higher in patients with EoE than in healthy volunteers (20.4 vs. 8.8, $p<0.0001$) (Figure 1). An ROC curve was used to
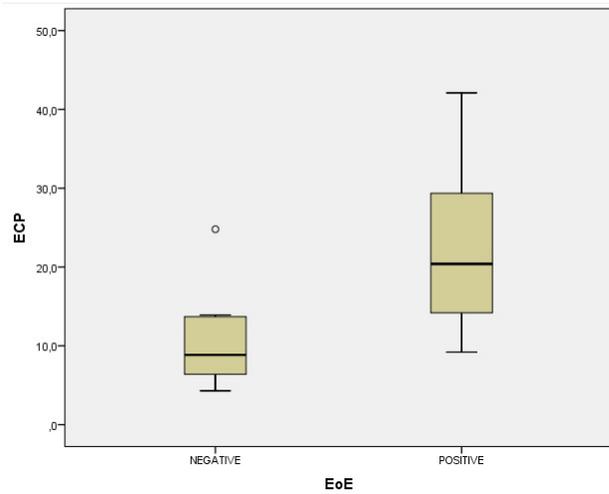
Figure 1. Comparison of the median ECP between patients with EoE and control group
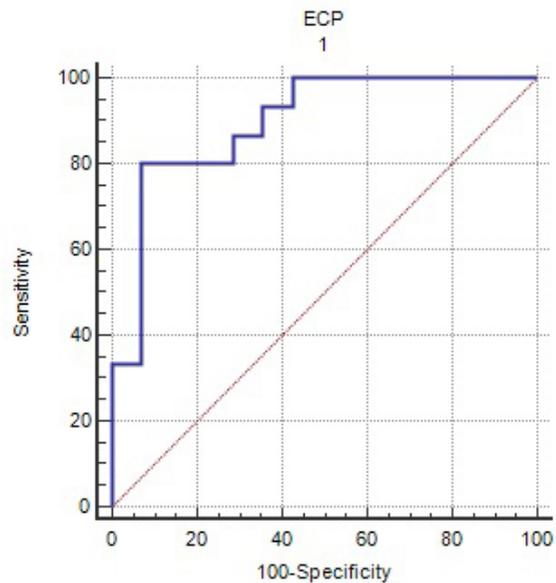
Figure 2. A receiver operating characteristic curve analysis regarding the diagnostic ability of ECP for EoE

determine the predictive value of ECP for EoE. According to this ROC curve analysis, the cut-off value of ECP was found to be 13.9 μg/mL (sensitivity: 80% (95% CI: 51.9-95.7), specificity: 92.8% (95% CI: 66.1-99.8), positive predictive value: 92.3 (95% CI: 64.0-99.8), and negative predictive value: 81.2 (95% CI: 54.4-96.0), with an area under the ROC curve (AUC) 0.895; $p<0.0001$; 95% CI: 0.725-0.978) (Fig. 2).

Table 3. Correlation of ECP with symptoms, laboratory findings, and EREFS

	Laboratory		Symptoms		Endoscopy	
	AEC	HPEC	Heartburn	Dysphagia	FI	EREFS
r	-0.077	0.072	-0.201	0.398	0.525	0.900
p	0.7	0.7	0.4	0.1	0.04	<0.0001

ECP: eosinophilic cationic protein; EREFS: Eosinophilic Esophagitis Endoscopic Reference Score; AEC: absolute eosinophil count; HPEC: histopathological eosinophil count; FI: food impaction

Correlation of ECP with symptoms, laboratory findings, and EREFS

When correlation of ECP with AEC, esophageal tissue eosinophil count, EREFS, and EoE symptoms was analyzed, only EREFS (p<0.0001) and the presence of food impaction (p=0.04) were significantly correlated with ECP (Table 3).

DISCUSSION

Eosinophilic esophagitis is predominantly seen in male patients between 30 and 40 years old. Although it is assumed that the prevalence of EoE is increasing due to improvement in the understanding of the disease, it is still apparent that EoE is an underdiagnosed entity with a delay of diagnosis (6).

Histological examination of the esophageal mucosa is required for the exact diagnosis of EoE. Non-invasive diagnostic markers have been analyzed in previous studies to assist in the diagnosis and monitoring of EoE. With the establishment of these non-invasive markers, it is aimed to prevent multiple endoscopies that are costly, invasive, and associated with risks (13). Several prospective studies have assessed the potential role of various biomarkers, including peripheral eosinophil count, cytokines, and chemokines involved in the pathogenesis of EoE (14-17). Contradictory results have been documented in studies analyzing the diagnostic role of several non-invasive biomarkers for EoE. In the study by Dellon et al., 14 different serum biomarkers showed no significant correlation or difference between subjects with EoE and controls (18).

Eosinophils have cytoplasmic granules that degranulate on stimulation and release toxic mediators, causing tissue damage and inflammation (19). Several studies have reported that in addition to the aforementioned non-invasive markers, the presence of these degranulation products in the esophageal tissue can help to diagnose EoE (13,17). In a recent study, Min et al. showed that serum ECP is significantly higher in patients with EoE than in controls (13). Since elevation of ECP may also be due to bronchial inflammation, it is suggested that ECP will be beneficial only in patients without asthma (6). However in the study by Min et al., higher serum ECP level was an independent predictor of EoE when age, sex, and atopic disease were evaluated in multivariate analysis. The authors concluded that although ECP can be elevated in other atopic diseases, such as asthma or allergic rhinitis, it was noteworthy to report since it has remained significantly associated with EoE by the multivariate analysis (13). In our study, we determined that ECP levels were significantly higher in subjects with EoE than in controls. In addition, we documented that ECP has very accurate sensitivity and specificity values (AUC 0.895; p<0.0001) to diagnose EoE.

There are contradictory results in previous studies analyzing the correlation of ECP with AEC and esophageal tissue eosinophil count. In the study by Chehade et al., ECP has been found to be significantly higher in patients with EoE, and a positive correlation was obtained between ECP and peripheral eosinophilia, whereas this correlation could not be observed between ECP and eosinophil count in esophagus biopsy (20). The findings of the study by Rodriguez-Sanchez were similar with the aforementioned study (21). However, Min et al. showed a significant correlation between ECP and esophageal tissue eosinophil count (13). In our study, differently from previous studies, ECP was correlated neither with AEC nor with esophageal tissue eosinophil count.

Endoscopic reference score is a recent classification system that is used to define the endoscopic features (edemas, rings, exudates, furrows, and strictures) of EoE. This grading system has an accurate intra- and inter-observer agreement that has been independently validated by several investigators from North America and Europe (7,22). Shoepfer et al. (23) showed that EREFS is associated with symptom severity due to a validated patient-reported outcome instrument for EoE.

Although correlation of non-invasive biomarkers of EoE and laboratory/histopathological parameters has been studied in several studies, to the best of our knowledge,

there is no study analyzing the correlation of non-invasive biomarkers with endoscopic features. In the current study, we analyzed the correlation of ECP (a non-invasive biomarker) with endoscopic grade of EoE (EREFS), AEC, esophageal tissue eosinophil count, and EoE symptoms. Importantly, we found that EREFS and the presence of food impaction symptom were strongly correlated with ECP.

In conclusion, we have shown that a non-invasive biomarker ECP is an accurate diagnostic tool with high specificity and sensitivity. The most striking finding of the present study is the significant correlation between ECP and EREFS that has not been studied in the literature to date. However, a small sample size is a limitation of the present study, and further prospective studies with larger sample size are needed to confirm the role of biomarkers in assessing the diagnosis and symptomatic/endoscopic severity of EoE.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of TOBB University of Economics and Technology (Decision No: MED/2171/2011; Decision Date: August 8, 2011).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The author has no conflict of interest to declare.

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