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THE UTILITY OF FAECAL CALPROTECTIN, ECP, IL-5 AND IL-13 IN THE DIAGNOSIS OF EOSINOPHILIC ESOPHAGITIS

EOZİNOFİLİK ÖZOFAJİT TANISINDA FEKAL KALPROTEKTİN, ECP, IL-5 VE IL-13'ÜN KULLANIMI

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ABSTRACT

Objective: Eosinophilic esophagitis is a Th2, antigen driven disease in which chronic, eosinophilic inflammation results with symptoms of esophageal dysfunction. There are many diseases in the differential diagnosis, the most important one is gastroesophageal reflux diseases. We aimed to investigate the use of fecal calprotectin, ECP, IL-5 and IL-13 in distinguishing these two diseases and in the diagnosis of eosinophilic esophagitis.

Method: Forty children who had gastroscopic examination and who had macroscopic and/or microscopic pathologic findings compatible with eosinophilic esophagitis or gastroesophageal reflux disease were enrolled. The FC level, complete blood count, C reactive protein (CRP), eosinophilic cationic protein (ECP), total immunoglobulin E (IgE), specific IgE, IL-5, IL-13 were studied and compared with healthy controls.

Results: The eosinophil number, CRP, total IgE, serum IL-5, IL-13 and FC levels were significantly higher in the eosinophilic esophagitis group compared to the healthy controls (P<0.05). The leukocyte count, AEC, CRP, total IgE, ECP and serum IL-5 levels were significantly higher in the eosinophilic esophagitis group (P<0.05). The FC level was higher in patients with eosinophilic esophagitis than ones with gastroesophageal reflux disease, but there was no statistically significant difference (P=0.055). Total IgE value (r=0.489) and ECP (r=0.810) were correlated with the tissue eosinophil count in eosinophilic esophagitis (P=0.001; <0.001).

Conclusion: It is difficult to differentiate eosinophilic esophagitis from gastroesophageal reflux disease clinically and the gold standard test for differentiation is still biopsy. In this study a remarkable result was that ECP may be helpful for the diagnosis of eosinophilic esophagitis.

Keywords: Children, eosinophilic, calprotectin, interleukin, esophagitis

ÖZ

Amaç: Eozinofilik özofajit, kronik, eozinofilik inflamasyonun özofagus fonksiyon bozukluğu semptomlarıyla sonuçlandığı, Th2, antijen kaynaklı bir hastalıktır. Ayırıcı tanıda pek çok hastalık vardır, en önemlisi gastroözofageal reflü hastalıdır. Bu iki hastalığın ayrımını yapmada ve eozinofilik özofajit tanısında fekal kalprotektin, ECP, IL-5 ve IL-13'ün kullanımını araştırmayı amaçladık.

Yöntem: Gastroskopi uygulanan, makroskobik ve/veya mikroskobik patolojik bulguları eozinofilik özofajit veya gastroözofageal reflü hastalığı ile uyumlu olan 40 çocuk çalışmaya alındı. Kalproteiktin düzeyi, eozinofilik katyonik protein (ECP), IL-5, IL-13 incelendi ve sağlıklı kontrollerle karşılaştırıldı.

Bulgular: Eozinofil sayısı, CRP, toplam IgE, serum IL-5, IL-13 ve kalprotektin düzeyleri, kontrol grubuna kıyasla eozinofilik özofajit grubunda anlamlı derecede yüksekti (P<0.05). Lökosit sayısı, AEC, CRP, toplam IgE, ECP ve serum IL-5 düzeyleri eozinofilik özofajit grubunda anlamlı derecede yüksekti (P<0.05). Kalprotektin düzeyi eozinofilik özofajitli hastalarda gastroözofageal reflü hastalığı olan hastalara göre daha yüksekti ancak istatistiksel olarak anlamlı bir fark yoktu (P=0.055). Total IgE değeri (r=0,489) ve ECP (r=0,810) eozinofilik özofajitdeki doku eozinofil sayısı ile koreleydi (P=0,001; <0,001)

Sonuç: Eozinofilik özofajiti gastroözofageal reflü hastalığından klinik olarak ayırmak zordur ve ayrım için altın standart test hala biyopsidir. Bu çalışmada ECP'nin eozinofilik özofajit tanısında yardımcı olabileceği dikkat çekici bir sonuçtur.

Anahtar Kelimeler: Çocuklar, eozinofilik, interlökin, kalprotektin, özofajit

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Introduction

Eosinophilic esophagitis (EoE) is a chronic, immune-mediated disease affecting both children and adults.[1] It results in esophageal dysfunction. Since children with EoE have many symptoms such as abdominal pain, vomiting, failure to thrive, food refusal, and heartburn, those symptoms may be confused with many diseases including gastroesophageal reflux disease (GERD), food allergies, inflammatory bowel diseases, and functional disorders.[1-3] Among those gastroesophageal reflux disease is mostly confusing that symptoms such as heartburn, chest pain, food refusal, or vomiting overlap with EoE. For pediatricians, it is a challenge to differentiate EoE from GERD. Gastroscopy and biopsy are more definite diagnostic methods.[4-9] Exploration of the upper gastrointestinal system by endoscopy is an invasive procedure which should be absolutely performed in individuals with intractable gastrointestinal complaints.[4] There are limited data about validated non-invasive laboratory tests which will help to differentiate EoE from GERD and to evaluate EoE disease activity.[4,6,9]
Faecal calprotectin is a simple, inexpensive, sensitive, and non-invasive method that can be used in the diagnosis and follow-up of intestinal inflammation in children,[10] and can be used as erythrocyte sedimentation rate (ESR) or C reactive protein (CRP) of the intestines.[11] Calprotectin is a calcium-binding, neutrophilic, and cytosolic protein with immunomodulatory, antimicrobial and antiproliferative properties. It is mostly found in the cytoplasm of neutrophils (constitutes 60% of cytoplasmic protein) and with a lower rate in monocytes and reactive macrophages.
Pathological conditions which change the permeability of the intestinal mucosa led to an increase in neutrophil and granulocyte migration and FC levels. An increased calprotectin level may also be found in patients with upper gastrointestinal damage.[12] Therefore, we hypothesize that measurement of FC may be performed as a noninvasive method in specifying the etiology of esophagitis. We aimed to assess the utility of eosinophilic cationic protein (ECP), interleukin-5 (IL-5), interleukin-13 (IL-13), and FC in differentiation of EoE from GERD.

Methods

Study Design and Participants

We enrolled patients aged between 6 months and 18 years who presented to Istanbul University, Cerrahpasa Medical Faculty, Pediatric Gastroenterology Outpatient Clinic between April 2012 and June 2014 with treatment-resistant upper gastrointestinal system complaint and who were found to have EoE or GERD on gastroscopy prospectively. The diagnosis is based on the 2011 updated diagnostic criteria of the 2007 consensus.[5] Pediatric patients whose upper gastrointestinal system complaints did not clinically respond to three-week or longer anti-reflux treatment were included. Patients with other causes of esophageal eosinophilia were excluded.
The patients were interrogated in terms of presentation complaints, accompanying conditions, complaints related to atopy, family history and compliance with previous treatment. A detailed physical examination was performed. Complete blood count, CRP, and immunoglobulins (IgG, IgA, IgM, IgE) were studied. Specific IgE f1 (egg white specific IgE), f2 (cow milk specific IgE), and food mixed panel; egg white, milk, codfish, wheat flour, peanut) were studied in patients below two years of age. Skin prick test was performed on the patients above four years of age.
Gastroscopy was performed in the Pediatric Gastroenterology Endoscopy Unit. A total of ≥4 biopsy samples were obtained from the upper, middle, and lower parts of the esophagus and these samples were evaluated in Pathology Laboratory. Samples from the stomach and the duodenum were also taken to exclude concomitant eosinophilic gastroenteritis.
On biopsy assessment, the patients in whom at least ≥15 eosinophils were observed on each high-power field (HPF) (x400) in the esophageal epithelium and no eosinophil was found in the stomach and duodenum were diagnosed as EoE. GERD was diagnosed in patients who had less than 15 eosinophils in the biopsy sample and whose histopathological diagnosis was compatible with reflux esophagitis. Serum IL-5, IL-13, ECP, and FC levels were studied in both EoE and GERD groups and compared with healthy controls (HCs).
As HCs, we included 24 children who had no allergic or gastrointestinal findings and had presented to outpatient clinics for routine follow-up or vaccination. They had no underlying diseases. Care was taken such that no infectious diseases was present at the time of serum and faecal sampling. Their physical examinations were normal and there were no signs of infection. Complete blood cell counts were normal and CRP levels were negative.
Written informed consent was obtained from patients or their legal caregivers. The study was approved by Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethics Committee (2012/10051).

Laboratory

Specific IgE (f1, f2 ve f5) was studied using Phadia 100 Unicap device (Phadia Austria GmbH Donau-City-Str. 1, AT-1220 VIENNA, Austria). Total IgE was studied using Siemens BN2 nephelometer device (Siemens, Munich, Germany) nephelometrically in Cerrahpasa Medical Faculty, Pediatric Immunology Laboratory. Age-dependent values were considered in the assessment of serum total IgE levels, whereas specific IgE levels above 0.35 kU//L were considered positive.
Serum IL-5 and IL-13 were studied with Platinum Elisa kit using Biotech Instruments, Inc. ELX 800 (USA) ELISA device in Cerrahpasa Medical Faculty Central Biochemistry Laboratory.
After the faecal samples were obtained, they were processed on at least the second day. A 15 mg was weighed by highly sensitive scale, 0.75 ml buffer solution was added and mixed by vortex for 20 minutes and

centrifuged at 10 000 rpm for 5 minutes. After the supernatant of the solution was taken, it was placed in a small storage tube and kept at -80°C. Faecal calprotectin was studied using Biotech Instruments, Inc. ELX 800 (USA) ELISA device and Faecal Calprotectin MRP8/14 Elisa kit in Biochemistry Laboratory.

Statistics

Statistical analysis of the data was performed using SPSS 22.0 package program (SPSS, IBM Ltd, UK). The categorical variables were expressed as digits and percentages. The continuous variables were expressed as mean and standard deviation. In-group comparisons were performed by student's t-test. Non-parametric Kruskal-Wallis analysis and Man Whitney U test were used in comparison between the groups. Spearman analysis was used in correlation analysis. Binary logistic regression analysis was performed for dependent variables. A *P* value of <0.05 was considered statistically significant.

Results

During the study period, a total of 218 patients had gastroscopy and 40 of them were enrolled (27 patients with GERD and 13 patients with EoE). The study flow diagram is given in Figure 1. In the GERD group more than half of the patients were female (n=17, 63%). The median (IQR) age of the patients with GERD was 12 (9) years. Nine (69.2%) of 13 patients with EoE were male. The median (IQR) age of the patients with EoE was 4 (9.8) years. Patients with EoE were younger than patients with GERD (*P*=0.005). Two patients with EoE were siblings and one was the cousin of those siblings. Demographics, complaints, and physical examination findings are summarized in Table 1.

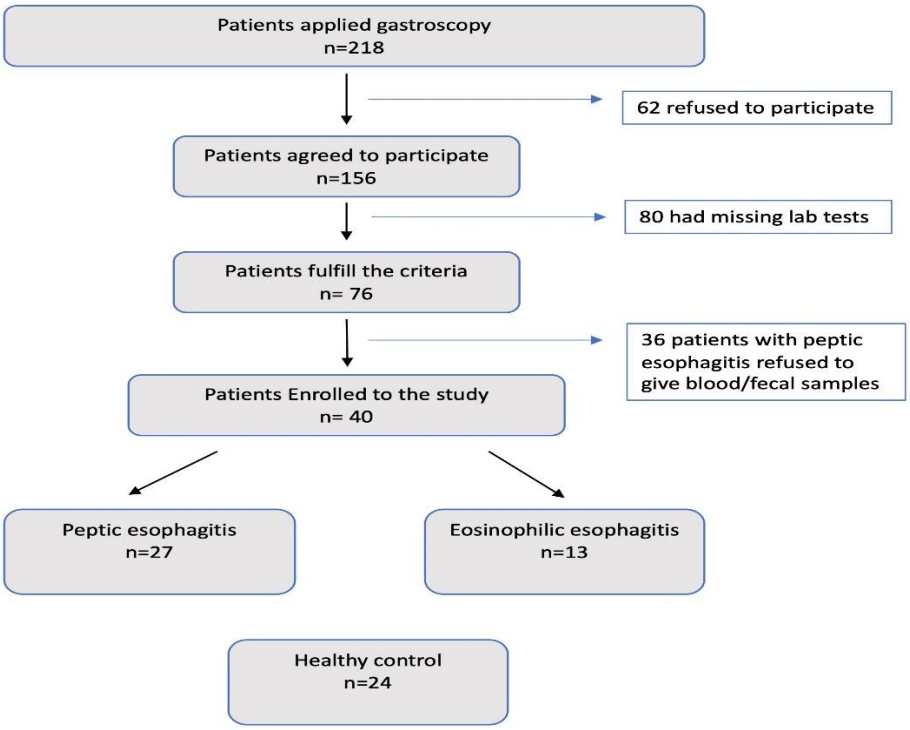

Figure 1. Study flow diagram

In the EoE group, family history of atopy, refusal of feeding, abdominal distension, inability to gain weight, feeling of abdominal discomfort, and history of reactive airway disease, history of atopic dermatitis was higher compared to the GERD group (*P* <0.05). At admission, abdominal pain was the most common complaint in both groups.

No significant difference was present between the GERD and HCs in terms of FC (*P*= 0.571) (Figure 2).

The skin prick test was positive in nine (62.9%) patients with EoE. A positive response against house dust mite was present in six of them and a positive response against food panel was also present. Serum-specific IgE levels were increased in two of the patients with EoE. One had increased f1 and f2 (>0.35); the other one had increased f1 and f5.

On gastroscopic examination, nodular appearance was present in nine patients (62.9%) with EoE, hyperemia was present in five (38.46%) and striation was in four (30.7%) (Table 2). On microscopic examination of biopsy samples, the mean eosinophil count on HPF was 80.9 (Figure 3). There was an eosinophilic nodule on the biopsy sample of a patient shown in Figure 3.

Absolute eosinophil count (AEC), CRP, total IgE, IL-5, IL-13, and FC levels were significantly higher in the EoE group compared to the HCs (*P*= 0.013; <0.001; <0.001; 0.010; 0.003; 0.033, respectively) (Figure 2). The FC levels were higher in the EoE group than in GERD, but there was no statistically significant difference (*P*=0.055; 0.292) (Figure 2). The white blood cell (WBC) count, AEC, CRP, total IgE, ECP, and IL-5 levels were significantly higher in

the EoE group compared to GERD (*P*= 0.003; 0.001; 0.016; 0.005; <0.001; 0.005, respectively) (Figure 2).

Correlation analysis was performed to evaluate if there was a significant correlation between the gastroscopic findings (nodular appearance, striation, hyperemia and stenosis) and laboratory findings in the patients with EoE. There was a statistically significant correlation between nodular appearance, and WBC count, AEC, and ECP level (Table 3). ECP level was an independent predictor of nodular appearance with regression analysis (including WBC, AEC, and ECP level) (β=0.049; *P*=0.016).

When correlation analysis was performed for striation in the esophagus and laboratory data, there was a significant correlation between striation in the esophagus and AEC, total IgE and IL-5 levels (Table 3). No independent predictor was found in the regression analysis.

Table 1. Demographic and clinical characteristics of patients with esophagitis

Characteristics	Eosinophilic esophagitis	Peptic esophagitis	*P*
No of Patients, n (%)	13 (32.5)	27 (67.5)	NA
Sex F/M (n, %)	4 (30.8) / 9 (69.2)	17 (63) /10 (37)	NA
Age, years, median (IQR)	4 (9.8)	12 (9)	NA
Height SDS ±SD	-0.60±2.25	0.06±0.91	NA
Weight SDS ±SD	-1.09±1.70	-0.09±0.85	NA
Complaints and Findings (n, %)			
Family history of the same complaint	7 (53.8)	13 (48.1)	0.50
Atopy in a family member	10 (76.9)	2 (7.4)	**<0.001**
Refusal of feeding	9 (69.2)	1 (3.7)	**<0.001**
Vomiting	7(53.8)	9 (33.3)	0.180
Regurgitation	4 (30.8)	8 (29.6)	0.609
Abdominal distension	7 (53.8)	4 (14.8)	**0.015**
Abdominal pain	11 (84.6)	24 (88.9)	0.531
Epigastric pain	11 (84.6)	20 (74.1)	0.376
Dysphagia	4 (30.8)	6 (22.2)	0.414
Weight gain failure	8 (61.5)	2 (7.4)	**<0.001**
Feeling floating	3 (23.1)	0	**0.029**
History of reactive airway disease	9 (69.2)	1 (3.7)	**<0.001**
Atopic dermatitis	11 (84.6)	4 (14.8)	**<0.001**
Abdominal tenderness	3 (23.1)	6 (22.2)	0.624

F/M: Female/Male, n: number, SD: Standard deviation, SDS: Standard deviation score

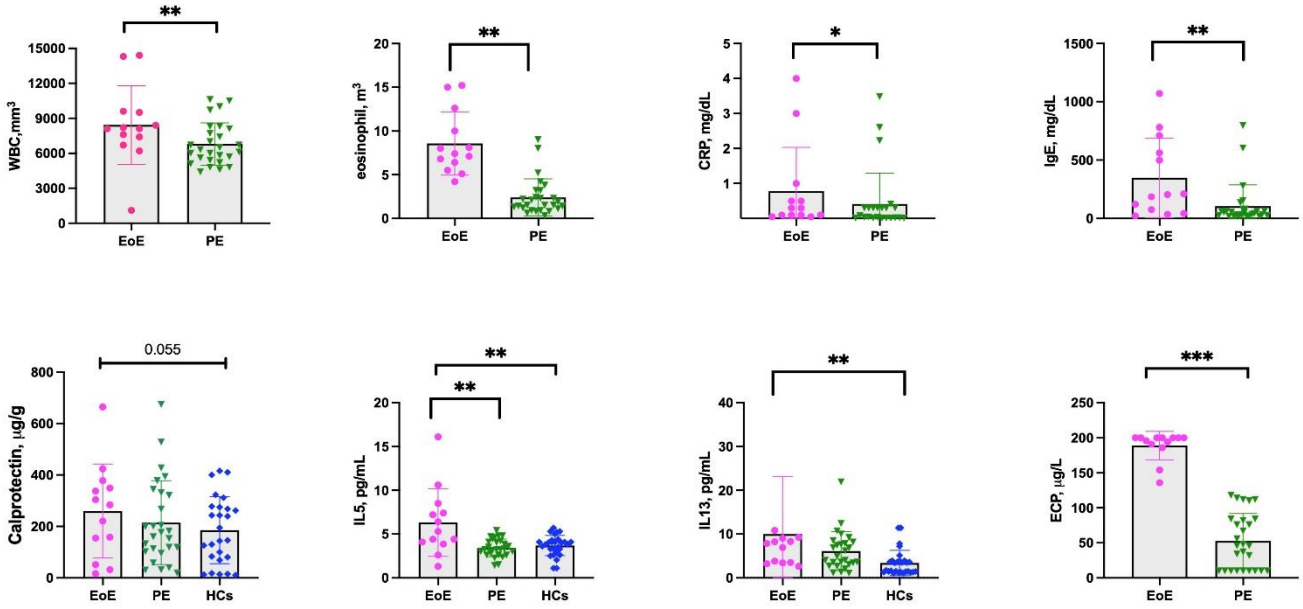

Figure 2. Laboratory results
*P<0.05; ** P<0.01; *** P<0.001

Table 2. Gastroscopic and histopathological findings of the patients with eosinophilic esophagitis

	Gastroscopic findings				Histopathological findings				
Patient number	**Nodular appearance**	**Striation**	**Hyperemia**	**Lump**	**Peak eosinophil count/HPF***	**Microabscess with eosinophilia**	**Superficial eosinophilia**	**Squamous epithelial infiltration**	**Papillary extension**
1	+	-	-	-	too much	-	+	-	-
2	+	-	+	-	16	-	+		-
3	+	+	-	-	very dense >55	+	+	+	+
4	+	-	-	-	18	-	+	-	-
5	-	+	+	-	25	-	+	-	+
6	+	-	+	-	20	-	+	-	-
7	+	-	+	-	17	-	-	-	+
8	+	-	-	-	16	-	+	-	-
9	-	-	+	-	>55	-	+	+	-
10	-	+	-	-	24	-	+	+	-
11	+	+	-	-	17	-	+	+	-
12	-	+	-	-	230	-	+	+	+
13	+	-	-	-	16	-	+	-	-

*HPF: High power field

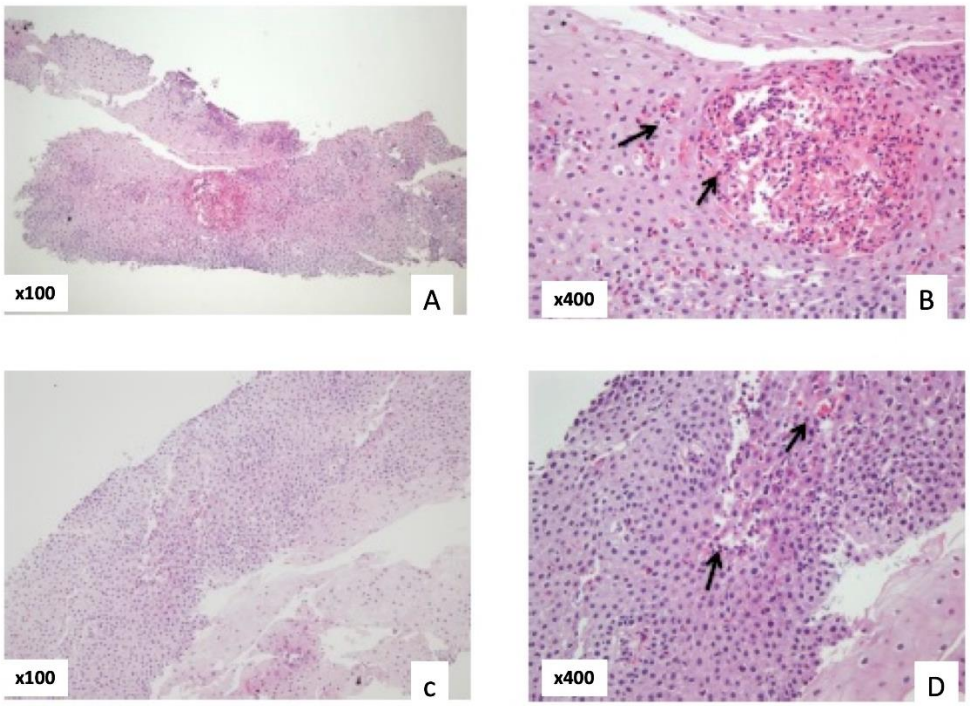

Figure 3. Microscopic examinations of biopsy samples of patients with eosinophilic esophagitis (A, B, C, D). Mean eosinophil count was 80.9/HPF. Arrows show eosinophils. **B.** Patient 3 had eosinophilic micro-abscess formation

Table 3. Correlation analysis of nodular appearance and striation in the esophagus of EoE patients

	Nodular appearance		Striation in the esophagus	
	r*	***P***	**r***	***P***
White cell count, mm³	**0.335**	**0.035**	0.119	0.464
Eosinophil count, mm³	**0.387**	**0.014**	**0.310**	**0.051**
CRP, mg/dL	0.239	0.138	**0.447**	**0.004**
Total IgE, mg/dL	**0.667**	**<0.001**	0.379	0.160
ECP, µg/L	0.273	0.089	**0.314**	**0.048**
IL-5, pg/mL	0.018	0.911	0.195	0.228
IL-13, pg/mL	0.27	0.083	0.116	0.478

CRP: C reactive protein, ECP: Eosinophilic cationic protein, IL-5: interleukin-5, IL-13: interleukin-13
*r: Correlation coefficient

Discussion

In this prospective study which includes children with EoE and GERD, we identified some laboratory tests that have the potential as noninvasive diagnostic tests that may be helpful in the diagnosis of EoE. In addition, unlike previous studies, we evaluated whether FC has a place in the diagnostic evaluation.[13-19] We showed that EoE patients had higher FC levels compared to GERD patients and HCs, but further studies are needed to support this conclusion. Inflammatory markers such as white blood cell count, AEC, CRP, serum total IgE, ECP and IL-5 values were significantly higher in the EoE group compared to GERD patients. Another remarkable finding was that ECP was an independent predictor of nodular appearance in EoE patients.

The cause of EoE is not fully understood, but presence of food or aeroallergen hypersensitivity has been reported in most patients.[1,2,20] Increased AEC, increased total IgE level and abnormal allergy tests are helpful in the diagnosis.[5,6] Many studies have evaluated AEC as biomarker for EoE.[13-19] Weschler JB et al.[21] concluded that AEC correlated with tissue eosinophil density. Similarly, we found that AEC was increased in patients with EoE and there was a statistically significant correlation between the blood AEC and esophageal tissue eosinophil count. This relationship may be a helpful marker for predicting tissue eosinophilia in future studies. Moreover, there was a statistically significant correlation between serum total IgE level and tissue eosinophil count. In the regression analysis, the total IgE level was found to be an independent predictor of tissue eosinophil count. This may be related to the fact that most of patients (62.9%) had a positive skin prick test and thus with IgE-mediated allergic status. On the other hand, the presence of non-IgE-mediated allergic responses may explain why the role of IgE in EoE disease activity is limited.[15-22] Rodríguez-Sánchez et al.[22] reported there is no correlation between serum total IgE level and tissue eosinophil count.

Whilst ECP is widely used in the assessment of atopic conditions, there are a limited number of studies demonstrating its use in the assessment of EoE.[14-16] Chehade et al.[23] and Rodríguez-Sánchez et al.[22] had reported that the ECP levels were higher in EoE group than in the HCs. In our study the mean ECP level was higher in patients with EoE than in GERD patients. These results lead us to think that ECP may be a useful marker for EoE as in other allergic diseases.

There are few ancillary laboratory tests that are significantly associated with the endoscopic appearance of EoE and tissue eosinophil count.[6,13-23] In view of our findings, considering the statistically significant association of ECP with both nodular appearance and tissue eosinophil count, ECP may be a useful surrogate marker in the evaluation of EoE.

In some studies, FC level was evaluated in patients with food allergy and GERD. Beşer et al.[24] reported higher level of FC in patients with cow's milk allergy (CMA) compared to HCs. Canani et al.[25] found the FC levels of 17 patients with GERD to be significantly higher compared to HCs. However, no significant difference was found between FC levels of GERD patients and HCs in this study.

One of the limitations in our study is the relatively small cohort of patients with EoE. Our results suggest that FC and ECP can be used in the differential diagnosis of EoE. However, further studies involving larger numbers of patients are needed before FC can be used as a diagnostic marker in clinical practice. Another limitation is the fact that we could not analyse the longitudinal levels of the tests we measured. Lastly, concentrations were measured only at admission and serial measurements at specific time intervals might better elucidate their role on disease activity.

In conclusion, it is difficult to clinically differentiate EoE from GERD, but allergic findings such as atopic dermatitis, reactive airway disease and family history of atopy may be prominent in patients with EoE. The gold standard for the diagnosis of EoE is biopsy findings showing increased intraepithelial oesophageal eosinophils without concomitant eosinophilic infiltration in the stomach or duodenum. A remarkable result of this study was that ECP was an independent predictor of nodular appearance, the most common endoscopic finding in patients with EoE.

Compliance with Ethical Standards

The study was approved by Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethics Committee (2012/10051).

Conflict of Interest

The authors have indicated they have no potential conflicts of interest to disclose.

Author Contribution

TE, OFB, FÇÇ and TK: Conceptualized and designed the study and reviewed and revised the manuscript; SLG: Conceptualized the study, collected data, carried out the analyses and drafted the initial manuscript; DK: Performed laboratory analysis; SE and NK: Performed pathologic examinations. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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