

## **Role of Filaggrin in Histopathological Expression in cases of childhood Eosinophilic Esophagitis**

**Salma Ahmed Mohammed Watfa<sup>1</sup>, Mohammed Sanad Naguib<sup>1</sup>, Hosam Fathy Elsaadany<sup>1</sup>, Raafat Awad Mostafa Hegazy<sup>2</sup>**

**<sup>1</sup> Pediatrics, <sup>2</sup> Pathology Departments, Faculty of Medicine, Zagazig University, Egypt.  
Corresponding author: Salma A. M. Watfa, Email: [salmawatfa@yahoo.com](mailto:salmawatfa@yahoo.com)**

### **Abstract**

**Purpose:** Previous data have suggested that Filaggrin (FLG) gene may be dysregulated in eosinophilic esophagitis (EoE). We aimed to further evaluate the expression patterns of FLG protein in esophageal tissue samples of pediatric patients with EoE, as compared to those of normal controls.

**Patients and methods:** During the period from September 2018 to February 2021, a total of 970 prospectively collected pediatric cases aging from 1 to 13 years including 474 females and 496 males that presented to our tertiary care center for upper gastrointestinal symptoms that necessitated upper gastrointestinal endoscopy and biopsy were included in our study. The immunohistochemical expression of Filaggrin was evaluated in esophageal biopsies obtained from patients and controls, and the results were correlated with EoE-related clinicopathological parameters.

**Results:** There were 970 patients in this research. Primary eosinophilic esophagitis represented 1.9 percent of the subjects included in this study and had significantly lower Filaggrin expression. All endoscopic results improved considerably following therapy, as did the symptoms and the expression of Filaggrin was dramatically increased.

**Conclusion:** When compared to normal controls, the immunohistochemistry expression of Filaggrin was down-regulated in the esophageal mucosa of children with EoE in our research. Following therapy, the aforesaid changes were reversed in a statistically significant percentage of instances.

**Keywords:** eosinophilic esophagitis; immunohistochemistry; Filaggrin; esophageal eosinophilia.

### **Introduction**

Eosinophilic esophagitis (EoE) is a clinicopathological esophageal illness characterized by significant eosinophilic infiltration of the esophageal tissue and clinical signs of upper GIT dysfunction (1). EoE has become more common since it was initially reported in the 1990s. It appears to cause substantial morbidity when compared to other chronic pediatric disorders (2). The pathophysiology of EoE has been linked to environmental and genetic variables that cause polysensitization to diverse meals (3). As a result, atopic predisposition and EoE appear to be linked, as both produce particular IgE in response to diverse allergens (4). The "atopic march" occurs when children with allergic disorders such as atopic dermatitis acquire food allergies, asthma, and other atopic problems. Filaggrin loss of function mutations are one of the risk factors for such disorders (5).

Filaggrin is a protein that plays a crucial role in the formation of subcutaneous tissue. It is a component of the protein-lipid cell envelope, which helps to prevent water loss as well as the entry of bacteria and allergens (6). Filaggrin and other tight junction proteins play a crucial role in the esophageal epithelium's barrier function. Although the specific mechanism is yet unknown, it appears to be involved in the pathophysiology of EoE. (7). A loss of function mutation in the Filaggrin gene has been associated to atopic disorders. As a consequence, Filaggrin expression may have a role in the pathogenesis of eosinophilic esophagitis and therefore be treated.

Previous research has revealed that epithelial barrier abnormalities play an important role in the pathophysiology of primary eosinophilic esophagitis. Furthermore, in situations of eosinophilic esophagitis, the Filaggrin gene may be dysregulated. We compared the histological expression of Filaggrin in instances with primary pediatric eosinophilic esophagitis to cases of secondary esophageal eosinophilia and those with no esophageal eosinophilia in this research. We also looked at how this marker changed after patients with primary eosinophilic esophagitis were treated.

## **Patients and Methods**

### **Study setting and population:**

Between September 2018 and February 2021, we studied 970 prospectively collected pediatric cases ranging in age from 1 to 13 years, including 474 females and 496 males who presented to our tertiary care center with upper gastrointestinal symptoms requiring upper gastrointestinal endoscopy and biopsy.

### **Diagnosis of eosinophilic esophagitis:**

Symptoms that were resistant to treatment included vomiting, stomach pain, reflux, dysphagia, and/or food impaction. After a complete endoscopic examination and biopsy, the patients were divided into three groups based on histological H&E examination and diagnostic criteria of eosinophilic esophagitis by Evan (8): There were eighteen cases that met the diagnostic criteria for primary eosinophilic esophagitis; 223 cases that had esophageal eosinophilia without meeting the diagnostic criteria and were labelled as secondary esophageal eosinophilia; and 729 cases that did not have esophageal eosinophilia and did not meet the diagnostic criteria (Table **Error!**

### **Reference source not found.**

### **Study procedures:**

The following tests were performed on all of the patients who took part in the study: (3) Laboratory testing: CBC, Liver function tests, Renal function tests, Bleeding profile; (4) Upper GIT endoscopy and biopsy; (5) biopsy samples from the patients' esophagus; and (6) histological evaluation.

### **The Histopathological examination was done as follows:**

- **Light microscope:** Tissue specimens were fixed for 12 hours in 10% neutral buffered formalin before being dehydrated in successive grades of alcohol and paraffin embedding, as directed by the manufacturer (9). The hematoxylin and eosin-stained slides were examined for evidence of eosinophil infiltration and eosinophilic esophagitis using the Binuclear Olympus microscope XL31.
- **Immunohistochemical processing:** (Kim, Roh, Park, & medicine, 2016) have made the following changes: The paraffin-embedded blocks were cut into 5m thick slices and put on slides coated with poly-L-lysine (Sigma) (10). After being deparaffinized, the slides were rehydrated in distilled water, then transferred to sodium citrate buffer (pH 6.0) and heated twice for 10 minutes in an 800 W microwave oven. Between microwave irradiations, the slides were cooled for 5 minutes. The slides were then washed twice in 10mM PBS, pH 7.4, and then incubated in the following order: 1 percent normal goat serum + 1 percent hydrogen peroxide in PBS for 30 minutes (at room temperature); 0.5 percent Triton X-100 in PBS for 30 minutes; Filaggrin, mouse monoclonal antibody, ready to use (DAKO, USA) for 12 hours at 4°C in PBS A biotinylated secondary antibody was used for 2 hours at room temperature on the slides, followed by conjugated streptavidin-peroxidase for 1 hour. The slices were washed twice with PBS in between incubations. Peroxidase activity was demonstrated using a 3, 3'-diaminobenzidine chromogen solution in the presence of hydrogen peroxide for 10 minutes. After washing, sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted with a cover slip (DPX). Negative Control sections were treated with pre-immune goat serum instead of the main antibody (1:2000). Skin biopsies from psoriasis patients were used to collect positive control sections.
- **Immunohistochemical analysis:** The slides were examined under a binocular microscope for Filaggrin cytoplasmic reaction (granular brown). A negative reaction occurs when only 0-10% of cells react to pale staining. Weak reaction is defined as 10% to 50% of the sample responding with a brownish hue. A moderate reaction is defined as 50-75 percent of the cells responding. A severe response was defined as more than 75% of cells displaying a strong cytoplasmic reaction with a dark brown color (9, 10).

### **Management:**

Those with a confirmed diagnosis of primary eosinophilic esophagitis were given the following treatment: (1) An elimination diet was used with or without corticosteroids (oral budesonide), and clinical symptoms were evaluated. (2) Eight weeks later, the patients were admitted to the hospital for a second upper gastrointestinal endoscopy and esophageal biopsy. (3) The histopathological expression of Filaggrin in the second biopsy samples was assessed using immunohistochemistry.

### **Ethical Consideration:**

The Institutional Review Board (IRB) and the ethical committee of Zagazig University approved this study, (IRB No.: ZU-IRB #4816/26-8-2018) and at least one parent or caregiver of the children participating in the study supplied informed written consent. The study was conducted in accordance with the Declaration of Helsinki's guidelines.

### **Statistical Analysis:**

Quantitative variables were given as mean values, whereas qualitative data were reported as absolute and relative frequencies (SD). To compare proportions between groups, Chi-square and Fisher's exact tests were performed. Student's t-tests and analysis of variance (ANOVA) were used to compare mean values. The Bonferroni correction was used in multiple comparisons to adjust for type I error. Throughout the follow-up period, the changes in EOS found among distinct therapy groups were examined using repeated measurements analysis of variance (ANOVA). All of the p values shown are two-tailed. p0.05 was used as the statistical significance criterion. The analyses were carried out using the SPSS statistical program (version 19.0).

**RESULTS:**

***Demographics, clinicopathological data and allergy testing results***

The demographics and clinicopathological features of the patients are summarized in Tables **Error! Reference source not found.** The participants ranged in age from one to thirteen years old, with an average of 5.6 years. They weighed 19.8 kg on average. Females made up 48.9% of the total number of participants. According to the study, 1.9 percent of the participants got primary eosinophilic esophagitis (Table **Error! Reference source not found.**).

There were no significant differences between the research groups in terms of all CBC items, liver function tests, BUN, and creatinine (Table **Error! Reference source not found.**). In terms of symptoms, those with primary eosinophilic esophagitis had more vomiting, and stomach pain, whereas those with secondary esophageal eosinophilia had more reflux (Table **Error! Reference source not found.**).

***Endoscopy and Immunohistochemistry***

Endoscopic signs such as furrowing, trachealization, exudate, and edema were more common in patients with primary esophageal eosinophilia (Table **Error! Reference source not found.**). Patients without esophagitis had considerably increased Filaggrin expression. Patients with primary eosinophilic esophagitis, on the other hand, had a lower rate (Table **Error! Reference source not found.**).

***Treatment***

Following therapy, all endoscopic results improved significantly, as did the symptoms (Table **Error! Reference source not found.**). Furthermore, Filaggrin expression rose considerably following therapy (Table **Error! Reference source not found.**).

**Table (1): Basic characteristics of the studied group:**

Variable	Studied group (n=970)	
<b>Age:</b>		
Mean ± SD	5.67 ± 2.44	
Range	0 - 13	
<b>Weight:</b>		
Mean ± SD	19.8 ± 5.9	
Range	8 - 40	
	No	%
<b>Sex:</b>		
Female	474	48.9
Male	496	51.1

**Table (2): Clinical types of lesions among the studied group:**

Variable	Studied group (n=970)	
	No	%

<b>Types:</b>		
Primary eosinophilic esophagitis:	18	1.9
Secondary eosinophilic esophagitis:	223	23
No esophageal eosinophilia:	729	75.1

**Table (3): Complete Blood Count Liver and Renal function tests among the studied groups:**

Variable	PrimaryEoE (n=18)	Secondary eosinophilia (n=223)	No eosinophilia (n=729)	Test	P-value
<b>Total Leukocyte Count:</b>					
Median	5.9	6.8	6.8	0.701	0.704
Range	4.2 - 12	3.4 – 15.8	3.5 – 14.3		(NS)
<b>Eosinophils:</b>					
Median	0.20	0.20	0.20	4.120	0.127
Range	0.2 – 0.5	0.1 – 0.9	0.1 – 0.6		(NS)
<b>Hemoglobin:</b>					
Mean ± SD	9.83 ± 1.1	10.3 ± 1.4	10.1 ± 1.2	2.690	0.068
Range	9 - 12	8 - 14	7.4 – 14.1		(NS)
<b>Platelets:</b>					
Median	284.5	314	344	3.396	0.183
Range	150 - 556	160 - 620	82 - 640		(NS)
<b>Total Serum Bilirubin:</b>					
Median	0.19	0.19	0.20	5.848 <sup>^</sup>	0.054
Range	0.1 – 0.2	0.1 – 2.2	0.1 – 3.5		(NS)
<b>Direct Serum Bilirubin:</b>					
Median	0.04	0.04	0.05	1.878 <sup>^</sup>	0.155
Range	0.01 – 0.1	0.01 – 0.5	0.01 – 2.2		(NS)
<b>Total protein:</b>					
Mean ± SD	5.92 ± 1.1	6.43 ± 1.2	6.52 ± 1.2	2.543*	0.079
Range	4.2 - 7	4 – 8.6	3.2 – 8.7		(NS)
<b>Albumin:</b>					
Mean ± SD	3.56 ± 0.68	3.43 ± 0.6	3.47 ± 0.59	0.638*	0.529
Range	2.7 – 4.6	2.1 – 4.5	2 – 4.8		(NS)
<b>ALT:</b>					
Mean ± SD	20.9 ± 2.9	22.4 ± 5.9	23.1 ± 6.1	2.170*	0.115
Range	10 - 25	10 - 44	10 - 45		(NS)
<b>AST:</b>					
Mean ± SD	29.9 ± 6.1	33.5 ± 7.7	32.6 ± 7.5	2.544*	0.079
Range	15 - 35	22 - 48	18 - 48		(NS)

<b>Blood Urea Nitrogen:</b>					
Median	10.2	8	8	4.816	0.090
Range	5 - 15	4 - 22	3 - 27		(NS)
<b>Creatinine:</b>					
Median	0.40	0.40	0.40	3.449	0.980
Range	0.2 – 0.5	0.2 – 0.9	0.1 – 0.9		(NS)

**Table (4): Clinical presentation and Endoscopic findings among the studied groups:**

Variable	PrimaryEoE (n=18)	Secondary eosinophilia (n=223)	No eosinophilia (n=729)	$\chi^2$	P-value
	N (%)	N (%)	N (%)		
<b>Clinical presentation</b>					
<b>1.Vomiting:</b>					
No:	3 (16.7)	104 (46.6)	537 (73.7)	<b>76.22</b>	<b>&lt;0.001 (HS)</b>
Yes:	15 (83.3)	119 (53.4)	192 (26.3)		
<b>2.Abdominal pain:</b>					
No:	9 (50)	198 (88.8)	696 (95.5)	<b>64.83</b>	<b>&lt;0.001 (HS)</b>
Yes:	9 (50)	25 (11.2)	33 (4.5)		
<b>3.Reflux:</b>					
No:	15 (83.3)	40 (17.9)	288 (39.5)	<b>53.22</b>	<b>&lt;0.001 (HS)</b>
Yes:	3 (16.7)	183 (82.1)	441 (60.5)		
<b>4.Dysphagia:</b>					
No:	12 (66.7)	202 (90.6)	642 (88.1)	<b>9.279</b>	<b>0.01 (S)</b>
Yes:	6 (33.3)	21 (9.4)	87 (11.9)		
<b>5.Food impaction:</b>					
No:	18 (100)	223 (100)	714 (97.9)	5.037	0.081 (NS)
Yes:	0 (0)	0 (0)	15 (2.1)		
<b>Endoscopic findings:</b>					
<b>1.Furrowing:</b>					
No:	3 (16.7)	223 (100)	729 (100)	<b>805</b>	<b>&lt;0.001 (HS)</b>
Yes:	15 (83.3)	0 (0)	0 (0)		
<b>2.Trachealization:</b>					
No:	3 (16.7)	223 (100)	729 (100)	<b>805</b>	<b>&lt;0.001 (HS)</b>
Yes:	15 (83.3)	0 (0)	0 (0)		
<b>3.Exudate:</b>					
No:	6 (33.3)	217 (97.3)	729 (100)	<b>429</b>	<b>&lt;0.001 (HS)</b>
Yes:	12 (66.7)	6 (2.7)	0 (0)		
<b>4.Edema:</b>					
No:	3 (16.7)	217 (97.3)	729 (100)	<b>576</b>	<b>&lt;0.001 (HS)</b>
Yes:	15 (83.3)	6 (2.7)	0 (0)		

**Table (5): Filaggrin among the studied groups:**

Variable	PrimaryEoE (n=18)	Secondary eosinophilia (n=223)	No eosinophilia (n=729)	$\chi^2$	P-value
	N (%)	N (%)	N (%)		
<b>Epithelial Filaggrin:</b>				<b>348</b>	<b>&lt;0.001 (HS)</b>
No:	15 (83.3)	87 (39)	6 (0.8)		
Yes:	3 (16.7)	136 (61)	723 (99.2)		
<b>Stromal Filaggrin:</b>				<b>348</b>	<b>&lt;0.001 (HS)</b>
No:	15 (83.3)	87 (39)	6 (0.8)		
Yes:	3 (16.7)	136 (61)	723 (99.2)		

**Table (6): Filaggrin before and after treatment:**

Variable	PrimaryEoE before treatment (n=18)	PrimaryEoE after treatment (n=18)	$\chi^2$	P-value
	N (%)	N (%)		
<b>Epithelial Filaggrin:</b>			<b>25.71</b>	<b>&lt;0.001 (HS)</b>
No:	15 (83.3)	0 (0)		
Yes:	3 (16.7)	18 (100)		
<b>Stromal Filaggrin:</b>			<b>25.71</b>	<b>&lt;0.001 (HS)</b>
No:	15 (83.3)	0 (0)		
Yes:	3 (16.7)	18 (100)		

**Table (7): Clinical presentation and Endoscopic findings before and after treatment:**

Variable	PrimaryEOE before treatment (n=18)	PrimaryEoE after treatment (n=18)	Test	P-value
	N (%)	N (%)		
<b>Clinical presentation</b>				
<b>1.Vomiting:</b>			<b>25.7</b>	<b>&lt;0.001 (HS)</b>
No:	3 (16.7)	18 (100)		
Yes:	15 (83.3)	0 (0)		
<b>2.Abdominal pain:</b>			<b>12.00</b>	<b>&lt;0.001 (HS)</b>
No:	9 (50)	18 (100)		
Yes:	9 (50)	0 (0)		
<b>3.Reflux:</b>			0.00	1.00 (NS)
No:	15 (83.3)	15 (83.3)		
Yes:	3 (16.7)	3 (16.7)		
<b>4.Dysphagia:</b>			<b>7.2</b>	<b>0.007 (S)</b>
No:	12 (66.7)	18 (100)		
Yes:	6 (33.3)	0 (0)		

<b>5.Food impaction:</b> No: Yes:	18 (100) 0 (0)	18 (100) 0 (0)	0.00	1.00 (NS)
<b>Endoscopic findings:</b> <b>1.Furrowing:</b> No: Yes:	3 (16.7) 15 (83.3)	18 (100) 0 (0)	25.7	<0.001 (HS)
<b>2.Trachealization:</b> No: Yes:	3 (16.7) 15 (83.3)	18 (100) 0 (0)	25.7	<0.001 (HS)
<b>3.Exudate:</b> No: Yes:	6 (33.3) 12 (66.7)	18 (100) 0 (0)	18.00	<0.001 (HS)
<b>4.Edema:</b> No: Yes:	3 (16.7) 15 (83.3)	15 (83.3) 3 (16.7)	16.00	<0.001 (HS)

## DISCUSSION

The age of the participants in the current study varied from 1 to 13 years old, with a mean of 5.6 years. They weighed an average of 19.8 kg. Females accounted for 48.9% of the participants. The majority of the patients (76.7 percent) had no eosinophilic esophagitis, whereas 1.9 percent had primary eosinophilic esophagitis and 21.5 percent had secondary esophageal eosinophilia. This was in line with Politi et al. (2017), who discovered that the average age of the patients investigated was 6.7 years. Females made up 22.5 percent of those who took part (11).

Some symptoms, such as vomiting and stomach discomfort, were shown to be more common in individuals with primary eosinophilic esophagitis, while reflux was found to be more common in those with secondary esophageal eosinophilia. Wong et al.(2020) observed that reflux was more common in those with secondary esophageal eosinophilia than in people with primary eosinophilic esophagitis(12). Furthermore, Liacouras et al. (2003) discovered that EoE presents a variety of symptoms, including difficulties feeding, failure to thrive, vomiting, epigastric or chest discomfort, and dysphagia (13). Carrasco et al. (2017) discovered that stomach discomfort (78.6%), vomiting (50%) regurgitation (50%) and dysphagia (50%) were all present in primary EoE patients (14).

The epithelial barrier gene Filaggrin (FLG) was discovered by Kubo et al. (2012) to be another genetic locus connected to a variety of allergy disorders, including EoE. (15). When the epithelium is damaged, it creates a protective barrier against environmental antigens, which can result in antigen hypersensitivity and amplified immunological responses. The creation of an unbroken stratum corneum, the epithelial layer that is primarily responsible for barrier formation, requires the accumulation of FLG monomers (5, 16).

Endoscopic abnormalities such as furrowing, trachealization, exudate, and edema were shown to be more common in individuals with primary eosinophilic esophagitis in the current investigation. This was in concordance with Shimura et al. (2014), who discovered that linear furrows were the most common endoscopic finding in patients with EoE, and that their diagnostic utility had just been described (17). Wong et al. (2020) discovered that individuals with primary eosinophilic esophagitis had more longitudinal furrows than those with secondary esophageal eosinophilia (12).

Filaggrin expression was shown to be considerably greater in patients without esophagitis in this investigation. Politi et al. (2017) found that FLG staining was positive in tissue sections from all normal subjects (14/14 cases, 100%) and the majority of cases in the GERD group (secondary eosinophilia) (12/21 cases, 57.1 percent), but negative in esophageal tissue sections (pretreatment biopsies) from all patients with EoE (39/39 cases, 100%) (11).

Symptoms and endoscopic findings in primary EoE were dramatically reduced following therapy in this research. This was in accordance with Lee et al. (2013), who showed that symptoms and eosinophil levels improved following therapy, although endoscopic findings of EoE were not changed. This disparity might be explained by persistent inflammation in their study(18). Munoz-Persy and Lucendo, (2018), and Rothenberg, (2009) both reported

symptomatic alleviation and eosinophil-mediated inflammation resolution as satisfactory treatment outcomes (19, 20). Furthermore, Abe et al. (2011) discovered that endoscopic results improved over time (21). Another research (Straumann et al., 2003) looked at the natural history of 30 EoE patients and found that endoscopic results did not alter substantially over time. All symptomatic patients had esophageal eosinophilic infiltration, although cell counts had reduced considerably. Chronic inflammation, according to the scientists, may cause irreversible anatomical changes in the esophagus, as well as a risk of reduced function (22).

Furthermore, Sherrill et al. (2011) reported that Filaggrin (FLG), a gene implicated in proper esophageal barrier function (Blanchard et al., 2010), was significantly decreased in Blanchard's dataset but not in ours (23, 24). Despite new evidence that thymic stromal lymphopoietin (TSLP) is important in the pathophysiology of EoE, previous gene expression studies have shown that EoE is characterized by dysregulated expression of various inflammatory mediators and epithelial differentiation complex genes, including FLG, presumably leading to impairment of the esophageal mucosa's epithelial barrier function and increased permeability to infectious and allergenic agents (24, 25).

Epithelial barrier abnormalities, as seen by lower expression of FLG and protease inhibitors such as the lymphoepithelial Kazal type-related inhibitor, may allow invading pathogens to penetrate the esophageal epithelium and initiate an inflammatory response, as Simon et al (2015) proposed (26). Blanchard et al (2010) found a 16-fold decrease in FLG mRNA levels in patients with active EoE when compared to normal controls in a cohort of 144 subjects; furthermore, FLG gene expression was not significantly different between successfully treated patients and controls, indicating that FLG expression changes in EoE may be restored to normal—or at least near normal—levels with adequate treatment (24). Matoso et al (2013) used gene expression microarray analysis (validated by reverse transcription-polymerase chain reaction) to find downregulation of the FLG gene in patients with EoE, as well as negative immunohistochemical expression of FLG protein in all EoE tissue samples, which was regained in the overwhelming majority (88 percent) of these cases after therapy (25).

Katzka et al (2014) found similar results, demonstrating that active EoE is related with increased spongiosis (dilation of intercellular gaps) and decreased FLG expression levels, and that steroid therapy can reduce spongiosis and restore FLG staining (27). Specifically, the authors concluded that, while it is unclear whether these findings are unique to EoE or are a nonspecific consequence of esophageal inflammation, it is possible that loss of FLG and histologic spongiosis are involved in the pathophysiology of EoE, and that the staining density of this protein may be altered by steroid therapy in patients with active disease (27).

## **Conclusion**

When compared to cases of secondary esophageal eosinophilia and normal controls, the immunohistochemistry expression of Filaggrin was down-regulated in the esophageal mucosa of children with primary eosinophilic esophagitis in our research. Following therapy, the aforesaid changes were reversed in a statistically significant percentage of instances.

## **Disclosure**

The author reports no conflicts of interest in this work

## **References**

1. Strasser DS, Seger S, Bussmann C, Pierlot GM, Groenen PMA, Stalder AK, et al. Eosinophilic oesophagitis: relevance of mast cell infiltration. *Histopathology*. 2018;73(3):454-63.
2. Rothenberg ME, Wen T, Greenberg A, Alpan O, Enav B, Hirano I, et al. Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis. *Journal of Allergy and Clinical Immunology*. 2015;135(2):500-7.
3. Rothenberg ME, Aceves S, Bonis PA, Collins MH, Gonsalves N, Gupta SK, et al. Working with the US Food and Drug Administration: Progress and timelines in understanding and treating patients with eosinophilic esophagitis. *Journal of Allergy and Clinical Immunology*. 2012;130(3):617-9.
4. Schoepfer AM, Simko A, Bussmann C, Safroneeva E, Zwahlen M, Greuter T, et al. Eosinophilic Esophagitis: Relationship of Subepithelial Eosinophilic Inflammation With Epithelial Histology, Endoscopy, Blood Eosinophils, and Symptoms. *Am J Gastroenterol*. 2018;113(3):348-57.
5. McAleer MA, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. *Journal of Allergy and Clinical Immunology*. 2013;131(2):280-91.
6. Brown SJ, Irwin McLean WH. One Remarkable Molecule: Filaggrin. *Journal of Investigative Dermatology*. 2012;132(3, Part 2):751-62.
7. Wu L, Oshima T, Li M, Tomita T, Fukui H, Watari J, et al. Filaggrin and tight junction proteins are crucial for IL-13-mediated esophageal barrier dysfunction. 2018;315(3):G341-G50.

8. Dellon ES, Liacouras CA, Molina-Infante J, Furuta GT, Spergel JM, Zevit N, et al. Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. *Gastroenterology*. 2018;155(4):1022-33.e10.
9. Hegazy RJAIMDR. Hegazy'simplified method of tissue processing (consuming time and chemicals). 2015;1(2):57-61.
10. Kim S-W, Roh J, Park C-SJop, medicine t. Immunohistochemistry for pathologists: protocols, pitfalls, and tips. 2016;50(6):411.
11. Politi E, Angelakopoulou A, Grapsa D, Zande M, Stefanaki K, Panagiotou I, et al. Filaggrin and Periostin Expression Is Altered in Eosinophilic Esophagitis and Normalized With Treatment. *J Pediatr Gastroenterol Nutr*. 2017;65(1).
12. Wong S, Smith G, Ruskiewicz A, Nguyen NQ. Distinguishing gastroesophageal reflux disease and eosinophilic esophagitis in adults: The role of esophageal mucosal immunoglobulin G4. 2020;4(5):851-5.
13. Liacouras CA, Spergel JM, Ruchelli E, Verma R, Mascarenhas M, Semeao E, et al. Eosinophilic Esophagitis: A 10-Year Experience in 381 Children. *Clinical Gastroenterology and Hepatology*. 2005;3(12):1198-206.
14. Carrasco AEAB, Machado RS, PATRÍCIO FRdS, Kawakami EJAdg. Histological features of eosinophilic esophagitis in children and adolescents. 2017;54:281-5.
15. Kubo A, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. *The Journal of Clinical Investigation*. 2012;122(2):440-7.
16. Simpson CL, Patel DM, Green KJ. Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. *Nature Reviews Molecular Cell Biology*. 2011;12(9):565-80.
17. Shimura S, Ishimura N, Tanimura T, Yuki T, Miyake T, Kushiyama Y, et al. Reliability of Symptoms and Endoscopic Findings for Diagnosis of Esophageal Eosinophilia in a Japanese Population. *Digestion*. 2014;90(1):49-57.
18. Lee JH, Kim MJ, Kim J-H, Youn YH, Kim N, Bak Y-T, et al. Clinical analysis of primary eosinophilic esophagitis. 2013;19(2):204.
19. Munoz-Persy M, Lucendo AJJEjop. Treatment of eosinophilic esophagitis in the pediatric patient: an evidence-based approach. 2018;177(5):649-63.
20. Rothenberg ME. Biology and Treatment of Eosinophilic Esophagitis. *Gastroenterology*. 2009;137(4):1238-49.
21. Abe Y, Iijima K, Ohara S, Koike T, Ara N, Uno K, et al. A Japanese case series of 12 patients with esophageal eosinophilia. 2011;46(1):25-30.
22. Straumann A, Spichtin H-p, Grize L, Bucher KA, Beglinger C, Simon H-u. Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. *Gastroenterology*. 2003;125(6):1660-9.
23. Sherrill JD, Rothenberg ME. Genetic dissection of eosinophilic esophagitis provides insight into disease pathogenesis and treatment strategies. *Journal of Allergy and Clinical Immunology*. 2011;128(1):23-32.
24. Blanchard C, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, et al. Coordinate Interaction between IL-13 and Epithelial Differentiation Cluster Genes in Eosinophilic Esophagitis. 2010;184(7):4033-41.
25. Matoso A, Mukkada VA, Lu S, Monahan R, Cleveland K, Noble L, et al. Expression microarray analysis identifies novel epithelial-derived protein markers in eosinophilic esophagitis. *Modern Pathology*. 2013;26(5):665-76.
26. Simon D, Radonjic-Hösli S, Straumann A, Yousefi S, Simon HU. Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation. *Allergy*. 2015;70(4):443-52.
27. Katzka DA, Tadi R, Smyrk TC, Katarya E, Sharma A, Geno DM, et al. Effects of Topical Steroids on Tight Junction Proteins and Spongiosis in Esophageal Epithelia of Patients With Eosinophilic Esophagitis. *Clinical Gastroenterology and Hepatology*. 2014;12(11):1824-9.e1.