

# Research Article:

## Serum Interleukin-17 Evaluation in Patients With Eosinophilic Gastrointestinal Disease



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## ABSTRACT

**Background:** Eosinophilic gastrointestinal disorders or diseases (EGIDs) may result from an abnormal immune-mediated response to food antigens. Activated eosinophils release various mediators with inflammatory properties which may result in tissue and subsequently organ damage. The cytokine milieu of these patients has revealed increased levels of pro-inflammatory cytokines, including interleukin (IL)-17. This study aims to evaluate the potential role of this cytokine in the pathogenesis of EGID.

**Materials and Methods:** This prospective study was conducted at the Allergy Outpatient Clinic, Mofid Children Hospital, Tehran, Iran from January 2016 to January 2017. In this study, Serum IL-17 was determined in all the patients referred to the Allergy Clinic with a pathological diagnosis of EGID.

**Results:** The median (minimum and maximum) serum IL-17 level in patients with EGID, gastroesophageal reflux disorder (GERD), and healthy controls were 4.32 (0.00-6.383), 2.82 (0.00-3.231), and 3.6 (0.00-7.165) pg/mL, respectively. The results of the non-parametric analysis revealed no significant difference between the three groups regarding IL-17 ( $P=0.16$ ). However, by classifying EGID in two separate groups with the involvement of upper (eosinophilic esophagitis and eosinophilic gastritis) and lower (eosinophilic enteritis, eosinophilic colitis) gastrointestinal (GI) tracts, a significant difference in serum IL-17 level became evident ( $P=0.004$ ).

**Conclusion:** We found a significant relationship between IL-17 and upper GI involvement in EGID.

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## Introduction

**E**osinophilic gastrointestinal disease (EGID) refers to a diverse group of gastrointestinal (GI) disorders with an increased number of eosinophils in one or more organs of the GI tract without an underlying systemic disorder or a secondary cause of eosinophilia [1]. Eosinophils are multifunctional circulating leukocytes, composing 1% to 5% of peripheral blood cells. They are involved in inflammatory and physiologic immune responses. They have been considered as destructive end-stage effector cells as well [2].

EGIDs seem to result from an abnormal immune-mediated response to food antigens [3]. Possible etiologies such as hyper-eosinophilic syndrome, drug reactions, and parasitic infestations have been suggested for EGID. Activated eosinophils release various mediators with inflammatory properties which may cause tissue and subsequently organ damage because of eosinophilic degranulation at the site of eosinophilic infiltrations [4]. By determining the serum cytokine milieu of these patients, increased levels of pro-inflammatory cytokines, including interleukin (IL)-17 has been detected [4].

IL-17 is mainly derived from activated T cells [5]. It is a ~ 20-kDa glycoprotein of 155 amino acids, with its secretion limited to T lymphocytes, predominantly in memory CD45RO cells [5]. In the GI tract, IL-17 is mainly expressed by CD4+ T cells [6]. Innate lymphoid cells may also produce IL-17 [7]. IL-17 and IL-17R signaling are essential factors in regulating mucosal host defense against invading pathogens [7]. The cellular type of inflammation in eosinophilic esophagitis is predominantly eosinophilic but also elevated numbers of T cells and mast cells have been noted [8]. Since IL-17 may act as a biomarker of disease progression in allergic disorders, we evaluated the potential role of this cytokine in the pathogenesis of EGID in this study.

## Materials and Methods

### Study participants

This prospective study was conducted on 82 children, at the Allergy Outpatient Clinic, Mofid Children Hospital, affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran from January 2016 to January 2017. All patients referred to the Allergy Clinic with a pathologic diagnosis of EGID were evaluated by an allergist expert in food allergic disorders. The diagnosis of

EGID was based on the microscopic evaluation of the endoscopic biopsy samples according to the quantity, location, and characteristics of the eosinophilic inflammation [9]. The patients were selected from different age groups; from infancy to adolescence.

### The inclusion criteria

Infants presenting with poor feeding and vomiting and adolescents presenting with dysphagia and food impaction with a history of atopy in the patient or his/her family with a pathologic report of the tissue sample obtained by endoscopy indicating tissue eosinophilia (at least 20 eosinophils per hpf) with increased thickness of basal layer and elongation of vascular papilloma were included in the study.

Infants with irritability and adolescents with heartburn and chest pain indicating GERD with a histologic diagnosis of esophagitis associated with the normal thickness basement membrane, normal length vascular papilloma, and sparse eosinophils were also considered in the study.

### The exclusion criteria

All patients with a history of consuming immunosuppressive agents, systemic steroids or nonsteroidal anti-inflammatory drugs, and autoimmune disorders were excluded from the study.

### Study design

Patients' enrolment in the study was done by convenience sampling method, in a continuous and non-random manner. A detailed medical history was taken from all patients (n=82) and a careful physical examination was performed on each one. A questionnaire was completed for each patient by an expert allergist in this field. Every patient underwent investigations, including skin prick test, determining serum IgA and IgE levels, peripheral blood absolute eosinophil counts, radioallergosorbent test, biopsy result, the number of tissue eosinophils in the biopsy sample. The final result was interpreted according to the above-mentioned data.

A total of 34 patients suffering from EGID (19 with upper and 15 with lower GI involvement), and 23 patients suffering from Gastroesophageal Reflux Disorder (GERD) were enrolled in the study. A healthy control group consisting of 25 age- and sex-matched healthy children were selected, too. All their guardians signed formal consent for their children to participate in the study. These were healthy children referred for routine follow-up visits for their growth

and development. All the laboratory evaluations were performed for them except for the biopsy procedure.

Five to six milliliters of blood were taken from all participants and the serum was separated by centrifugation and remained frozen at -20° C until performing the ELISA test. After completion of the sample collection, the sera were transferred to reach the room temperature of 25° C. Then, the serum level of IL-17 was determined by ELISA (R&D system, USA) and the amount was expressed in pg/mL.

**Demographic and laboratory data**

A questionnaire, including demographic data and allergic signs and symptoms consisting of dermatological (atopic dermatitis), upper and lower respiratory (allergic rhinitis, asthma) and gastrointestinal complaints (blood streaks in stool, food allergy, GERD) in addition to the in vivo and in vitro results were prepared and completed for every patient. A wheel of 3 mm or greater compared with the negative control (seen in skin prick test) was considered positive.

**Statistical analysis**

IBM SPSS V. 23 was used for statistical analysis. Normally distributed variables were presented as Mean±SD and compared by 1-way ANOVA. Non-parametric data were presented as median (minimum-maximum) and compared by the Kruskal-Wallis test. A P-value of less than 0.05 was considered significant.

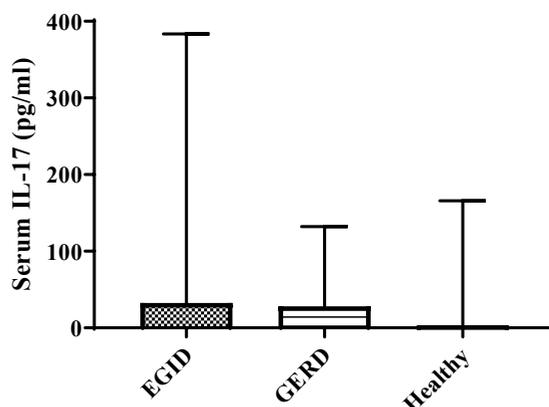
**Results**

Among 82 subjects who participated in this study, 62.2% were male and 37.8% were female. EGID had been diagnosed in 34 patients (19 with upper and 15 with lower GI involvement), while 23 patients suffered from GERD, and 25 persons were healthy subjects. The Mean±SD age of the patients in this study was 8.43 (4.92) years. There was no significant difference in allergic manifestations between the two groups of EGID and GERD patients. The median (minimum-maximum) serum IL-17 level in patients with EGID, GERD and healthy controls were 4.32 (0.00-6.383), 2.82 (0.00-3.231), and 3.6 (0.00-7.165) pg/mL, respectively.

**Table 1.** Median, Minimum, Maximum, Mean±SEM value of interleukin-17 in the patients and control groups

| Group                            | Median  | Min  | Max    | Mean±SEM         |
|----------------------------------|---------|------|--------|------------------|
| Upper gastrointestinal tract     | 67.9090 | 0.00 | 383.63 | 87.3420±21.86305 |
| Lower gastrointestinal tract     | 8.4000  | 0.00 | 165.70 | 15.2994±5.14866  |
| Gastroesophageal reflux disorder | 28.1600 | 0.00 | 132.27 | 35.4084±7.91643  |
| Healthy controls                 | 3.6000  | 0.00 | 165.70 | 31.1449±9.91504  |

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**Figure 1.** Demonstration of serum interleukin-17 in the patients with EGID, GERD and healthy controls  
EGID: Eosinophilic Gastroenteritis Disorder; GERD: Gastroesophageal Reflux Disorder

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The results of the non-parametric analysis revealed no significant difference between the three groups regarding IL-17 ( $P=0.16$ ). However, by classifying EGID in two separate groups with the involvement of upper (eosinophilic esophagitis, eosinophilic gastritis) and lower (eosinophilic enteritis, eosinophilic colitis) GI tracts, a significant difference in serum IL-17 level became evident ( $P=0.004$ ) (Table 1). Figure 1 demonstrates serum IL-17 in different groups of the patients and healthy controls in this study. Table 1 demonstrates median, minimum, maximum, Mean $\pm$ SEM of IL-17 in patients and control groups.

## Discussion

Patients with EGID usually present with failure to thrive, abdominal pain, regurgitation, dysphagia, nausea, and vomiting. These manifestations may be also commonly seen in pure GERD. However, there are differences in the etiology and management of these two gastrointestinal conditions. The most important differences are the IgE-mediated delayed Th2-type responses in EGID and the lack of a pivotal role for the immune system in pure GERD [10]. In this study, we investigated the serum cytokine IL-17 differences in two groups of healthy children and patients with Eosinophilic Esophagitis (EOE).

Gastroesophageal reflux has been regarded as a normal asymptomatic physiological process that is associated with troublesome symptoms or complications that have been defined as GERD [11]. Blanchard et al. evaluated serum levels of CD40L, IL-13, IL-12p70, IL-6, IL-5, IL-4, and EGF in patients with EOE, and showed elevated levels of these cytokines in them compared with the control group. But they did not find a meaningful difference in the serum levels of IL-17 in the two evaluated groups of control and patients [12].

Considering non-IgE-mediated reactions participating in EOE, T cell-mediated hypersensitivity characterized by the activation of antigen-specific T cells and subsequent recruitment of inflammatory T cells becomes more important [13]. Previous studies have shown elevated serum IL-17 levels in inflammatory bowel disease [5]. It has been suggested that increased levels of IL-17 produced by activated T cells [5], including Th17 [4] and eosinophils [14], not only may play an important role in inducing inflammation by the production of pro-inflammatory cytokines [5], but also may enhance nonspecific eosinophilic cytotoxicity in target organs [4]. Th17 subset of T cells which contributes to a significant rise in eosinophilic counts plays an important role in eosinophilic systemic inflammation [4].

The activity of eosinophils is enhanced directly or indirectly by IL-17 derived from activated T cells and monocytes or macrophages [5]. Consequently, the eosinophilic inflammatory response may induce or aggravate the underlying allergic disorder [4, 15-17]. We noted marked changes in IL-17 in our patients with EOE and GERD compared with the healthy controls which were more significant in those with upper GI tract involvement.

However, our study has some limitations. Although the sample size of the study was calculated by an expert in this field, the low number of the patients may influence the accuracy of the results. Therefore, further studies with a larger number of patients are necessary to determine the elevated IL-17 in EGID. Mucosal IL-17 expression seems to represent a more accurate measurement than IL-17 serum level which may be the second limitation of the study because it was not determined in this study. Finding elevated mucosal expression in association with increased serum level could further clarify the usefulness of this investigation in confirming the disease activity.

To conclude, we found a significant relationship between serum IL-17 level and upper GI involvement in EGID. Further studies may characterize the specific role of this cytokine in the pathogenesis of this disorder.

## Ethical Considerations

### Compliance with ethical guidelines

The study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences (Code: IR.SBMU.REC.1394.117). Signed informed consent was obtained from patients' parents or their caregivers following the code of Ethics of the World Medical Association (the Declaration of Helsinki). It is worth mentioning that all patients' information was kept confidential.

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### Authors' contributions

All authors contributed in preparing this paper.

### Conflicts of interest

The authors declared no conflict of interest.

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