

Eosinophil infiltration of the oesophageal mucosa in patients with pollen allergy during the season

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Summary

Background The oesophagus is normally devoid of eosinophils. There are some disorders accompanying with eosinophil infiltration. Food allergy has been reported as a common reason, especially in children but some other studies have also indicated that aeroallergens might have a role in oesophageal eosinophil accumulation.

Objective In this study we investigated whether there is any eosinophil recruitment in the oesophagus of pollen-allergic patients who had respiratory symptoms during the season.

Methods Thirty-eight symptomatic patients (allergic rhinitis (AR) with or without asthma) who had sensitization to grass pollen were included in the study during the pollen season. Controls were composed of 25 healthy non-atopics and 24 patients diagnosed as having gastro-oesophageal reflux disease. Reflux was excluded in allergic and non-atopic groups, whereas the presence of allergy was eliminated in control groups. Gastrointestinal endoscopy was performed in all participants, and biopsy specimens were taken from both the proximal and the distal oesophagus to evaluate eosinophil accumulation. At the same time, blood eosinophil numbers were counted.

Results Oesophageal eosinophil accumulation was found in 10 allergic patients (26%) and in five patients (21%) with gastro-oesophageal reflux disease but none of the healthy controls had eosinophils (0%) ($P < 0.05$). Blood eosinophils were higher in these 10 patients than the rest of the 28 patients without infiltration. In this group, blood eosinophils were also correlated with the number of accumulated eosinophils in the oesophagus ($P < 0.001$). There was more intense eosinophil infiltration at the distal part of the oesophagus in the reflux group when compared with the allergic group (mean 7.6 ± 5.6 vs. 3.2 ± 3.7). Nevertheless, eosinophils were found to be concentrated (mean 5.5 ± 7.3) in the proximal oesophagus of allergic patients, although it was 1.7 ± 1.5 in reflux patients ($P > 0.05$).

Conclusion Our results showed that eosinophil infiltration might be observed in oesophageal tissue of patients with respiratory tract allergy during the symptomatic period. This finding may possibly reflect the systemic and common mucosal aspects of allergic inflammation.

Keywords allergy, eosinophils, oesophagus

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Introduction

Eosinophils are present at different levels in many tissues. Analysed biopsy and autopsy samples have revealed that the organs showing tissue eosinophils at substantial levels were the spleen, lymph nodes, thymus and gastrointestinal tract [1]. Eosinophils are normally detected in the lamina propria of the gastrointestinal (GI) segments from the stomach to the colon probably because of their beneficial functions in host defence [2]. Under baseline condition, constitutive expression

of eotaxin 1 in GI tissue has been provided a signal to promote eosinophil homing into the GI tract [3, 4]. However, in contrast to other parts of the GI tract, the oesophagus normally does not contain resident eosinophils and therefore the finding of eosinophils in the oesophagus always point out medical problems in the patients. Eosinophil infiltration in the oesophagus is accompanied by many disorders such as gastro-oesophageal reflux disease (GERD), eosinophilic gastroenteritis, eosinophilic oesophagitis (EO), IgE-mediated food allergy, parasitic and fungal infections, inflammatory bowel diseases, scleroderma, allergic vasculitis, drug reactions and hypereosinophilic syndrome [5, 6]. These eosinophil-associated oesophageal disorders are mainly classified into primary and secondary subtypes. Although primary eosinophilic oesophagitis (PEO) have been classified as atopic, non-atopic and familial variants by some authors, studies has

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shown that all patients can respond to diet changes [7]. Secondary EE has been classified into two groups: one is a component of systemic eosinophilic disorders like HES and the other group is accompanied by non-eosinophilic disorders such as GERD or scleroderma [8].

PEE (previously called idiopathic EE, atopic oesophagitis and allergic oesophagitis) is an intrinsic chronic inflammatory disorder of the oesophagus. It is characterized by dense eosinophilic infiltration of oesophageal mucosa (>20 – 24 eosinophils/high-power field (hpf)) and involves both proximal and distal parts. Patients with primary EE have intense GI symptoms including dysphagia, vomiting, epigastric or chest pain and respiratory obstructive problems [8, 9]. An other commonly seen disorder, GERD, causes similar but less severe symptoms, and oesophageal intraepithelial eosinophils are accepted as diagnostic criteria for GERD [10, 11]. In GERD, accumulation of eosinophils is mainly in the distal part and typically the number of eosinophils in the oesophageal mucosa is found to be below that of primary EE and reaches at the most 7 eosinophils/hpf.

It is not known what causes the infiltration of eosinophils into the oesophagus while an organ is normally devoid of this cell type. In the last 10 years, as studies revealed a strong atopic background and high incidence of allergic diseases in patients with PEE, it was considered that there might be a possibility of a relationship between PEE and allergy [12, 13]. Initially, this association suggested that food allergy might play a role in the pathogenesis of the disease. Several therapeutic attempts such as elimination of food allergens from the diet, usage of elemental formulas or oral/topical corticosteroids improved symptoms and the elevated eosinophil numbers to normal in biopsies returned [14–18]. Although skin prick tests (SPT) and RAST have identified causative foods of IgE-mediated allergy, further studies revealed non-IgE-mediated reactions by patch testing [19]. PEE has begun to be recognized as a distinct clinical condition in children, and afterwards it was reported in adults [20]. It was remarkable that histologic and immunopathologic features that were found in adult patients with primary EE were very similar to biopsy findings of chronic airway inflammation in patients with bronchial asthma [21, 22].

Eosinophilia is often accompanied by allergic diseases but the role of other allergens except foods is not defined very well in case of oesophageal eosinophil accumulation. In an experimental model, it was demonstrated that intranasal aeroallergen exposure induced marked oesophageal eosinophil infiltration [23]. According to these findings, it is likely that eosinophilic infiltration of the oesophagus and allergic response in the lung might be specifically linked.

Subsequent studies tried to find an explanation for this linkage and demonstrated that eosinophil trafficking to the oesophagus was IL-5 and eotaxin dependent [24]. However, IL-13, another T-helper type 2 (Th2) type cytokine like IL-5 and eotaxin, induced eosinophil accumulation in the oesophagus when it was applied to the lung experimentally [25]. These results point out a possible relationship between Th2-type cytokines and the accumulation of eosinophils in the oesophagus.

Although there are many evidences of food and aeroallergen hypersensitivities as defined by SPT and RAST in patients having EE, we do not know what takes place in

the oesophagus of the symptomatic patients who have respiratory allergy and sensitivity to aeroallergens.

In this study, we aimed to investigate whether there are any eosinophils in the oesophagus of grass pollen-sensitive patients who have symptomatic allergic rhinitis (AR) and/or asthma. The study was performed when the patients were naturally exposed to allergens during the grass pollen season. At the same time that they had respiratory symptoms, it was supposed that all the Th2-type cytokines were overproduced *in vivo*.

Methods

Study population

The study population consisted by allergic patients (group I) and controls. Controls were composed of two groups including non-atopic patients with GERD (group II) and non-atopic healthy volunteers (group III).

Group I constituted 38 patients with seasonal allergic symptoms. Sixteen patients diagnosed as only having AR and the rest of the 22 patients with AR and asthma participated in the study (stage IV 'Severe Persistent Asthmatic' patients were excluded from the study). There were 32 women and six men, with the mean age of 38 ± 10 years (range 18–50). Participants were selected from among the patients who attended the Immunology and Allergy outpatient clinic because of seasonal AR alone or with asthma symptoms. The selection procedure of the patients had been carried out during the season when there was no pollen (from September 2002 to February 2003) according to inclusion criteria. The criteria for inclusion in the study were as follows: (1) a typical history of allergic rhinitis and/or asthma during the peak months of grass pollen, (2) positive results in SPT with grass pollen extracts and in RAST with grass mix panel (Gx1, CAP system Pharmacia, Uppsala, Sweden), (3) exclusion of GERD by using a standard validated reflux questionnaire and (4) exclusion of chronic diseases and parasitic infections. If the patients accepted the procedure, they were requested come to our outpatient clinic to take oesophageal biopsy samples while they had allergic respiratory symptoms. Before the procedure was performed, they were not allowed to use any nasal, inhaler or oral steroid and leukotriene antagonist drugs for at least 2 months. They were only on symptomatic therapy (inhaler β_2 agonists for asthmatic patients and on-demand oral antihistaminic usage for severe symptomatic rhinitis).

From the Gastroenterology Department, 24 patients were included in the study for inclusion of the 'disease control group'. They were diagnosed as having GERD if they had clinical and endoscopic signs of reflux disease and showed abnormal 24-h pH monitoring. There were 17 women and seven men; the mean age was 40 ± 12 years (range 20–57). Volunteers working in different departments of the hospital participated in the study as a 'healthy control group'. These 25 non-atopic controls did not have any GI disorders or complaints. They consisted of 14 women and 11 men, with the mean age of 35 ± 10 years (range 18–60).

The same physician from the Allergy Division took the medical history of the control groups by using a standard questionnaire form to exclude systemic and allergic diseases. SPT with common aeroallergens and foods, serum-specific

IgE (Phadiotop Pharmacia, Uppsala, Sweden) measurement and investigation for parasitic infections were performed in all subjects. Controls were accepted to the study if they had negative parasite tests, SPT, serum-specific IgE and history of allergy. A standard validated reflux questionnaire was filled by non-atopic healthy controls to verify whether if they had any symptoms related to GERD. Heartburn, acid regurgitation, dysphagia and pyrosis are main symptoms of GERD. If allergic patients or healthy controls reported one of the above symptoms at least once a week, they were not included in the study.

Upper GI endoscopy was performed in all participants. Biopsy specimens were taken from four different areas (proximal and distal parts of the oesophagus, antrum, duodenum), and evaluated for eosinophil infiltration by the same pathologist.

At the same time that the biopsy specimens were being taken, the numbers of peripheral blood eosinophils were determined in all participants by using an automated blood count analysis in order to compare with the eosinophil infiltration into the tissues.

All participants were informed about the study, and informed consent forms were obtained from the participants. The Ethics Committee of the Ege University Medical Faculty approved the study.

Skin prick test

Allergic patients and controls underwent SPT with common aeroallergens and foods (Allergopharma, Reinbek/Hamburg, Germany). A panel containing the following allergen extracts was used: five grass species (bermuda grass, ryegrass, blue grass, meadow fescue, timothy), four weed species (English plantain, wall pellitory, mugwort, dandelion), eight tree species (alder, birch, hazel, plane, oak, olive, poplar), mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), three moulds (*alternaria*, *aspergillus*, *cladosporium*) and animal danders (cat, dog, bird, horse and cow).

Positive (histamine phosphate 10 mg/mL) and negative (glycerine) controls were applied. The results were recorded as the largest diameters (in millimetres) of the weal and flare reactions after 15 min. In the presence of erythema, a weal 3 mm greater than the negative control was considered to be a positive response.

Diagnosis of GERD

In order to eliminate GERD, we used a validated reflux questionnaire in allergic patients and healthy controls. This form was derived from Locke et al. [26], and had been previously validated in an English-speaking western culture. The form was translated into Turkish, linguistically validated and adapted to the cultural profile of Turkey [27]. Among the patients and healthy controls, the persons who described acid regurgitation or pyrosis once a week or more common were accepted as having GERD, and were excluded from the study. The GERD was diagnosed based on symptoms. It has been shown in our previous studies that our validated GERD questionnaire has a high sensitivity. The sensitivity of the questionnaire concerning heartburn was strong at 94%; for regurgitation it was 84%; and for GERD it was 81%, in

agreement between gold standard (upper GI endoscopy, 24-h pH monitoring and clinical findings) methodologies and interview reports [27, 28].

Endoscopy and tissue samples

Upper GI endoscopy was performed in all participants by the same gastroenterologist after it was decided that they fulfilled the inclusion criteria. A local anaesthetic was applied as a spray in the throat approximately 5 min before the endoscopy was started. The participants were sedated with 3 mg midazolam intravenously. The endoscope (Olympus video endoscope) was introduced orally and monitored on screen by the gastroenterologist. Biopsy specimens were taken from each of the following four sites: the proximal part of the oesophagus, from 3 to 5 cm above the Z line in the distal oesophagus and from the antrum and duodenum (two pieces from each site and 3–4 mm in size). Biopsies, as regards the allergic patients, were attempted to be taken especially at the peak period of symptoms during the pollen season. Patients with asthma were especially warned to use only bronchodilator drugs, and patients with rhinitis to take only short-acting antihistamines until upper GI endoscopy had been performed.

Histopathology

All the tissue specimens were immediately fixed in 10% phosphate-buffered neutral formaline solution and embedded in paraffin following a minimum of 6 h of fixation and routine tissue process. Paraffin blocks were cut into 4–5 µm sections and stained with haematoxylin–eosin (H&E). All biopsy specimens were evaluated by the same experienced GI system pathologist blinded to the clinical and endoscopic findings of the patients. An Olympus BX 50 microscope ($\times 4$ magnification: 4, 32 mm, $\times 40$ magnification: 0, 54 mm. Ocular: $\times 10$ magnification) was used for evaluation. Biopsies of distal and proximal parts of the oesophagus were evaluated separately with respect to the presence of eosinophils.

In all sections, the epithelium and lamina propria were screened with $\times 10$, $\times 20$ and $\times 40$ magnifications prior to counting. When eosinophils were identified, the cells in the most densely infiltrated area were counted, and data were expressed as the total number of eosinophils in 10 consecutive hpf at $\times 40$ magnification. Antrum and duodenum biopsies were searched for eosinophil infiltration as well to exclude possible eosinophilic gastroenteritis.

Statistical analysis

The statistical analyses were carried out by SPSS (11.0) software program on a computer. Data were presented as mean $\pm$ SEM. Statistical significance comparing different sets of groups was determined by using the χ^2 test. The Mann–Whitney *U*-test was used for comparison of between-group data. Correlation analyses were performed by the Pearson correlation coefficient.

Results

The characteristics of the study groups are shown in Table 1. There were 15 patients who had at least one eosinophil in

Table 1. Characteristics of the groups, oesophageal eosinophil accumulation and mean values according to the proximal and distal parts of oesophagus

	Allergic patients (<i>n</i> = 38)	GERD (<i>n</i> = 24)	Healthy controls (<i>n</i> = 25)	<i>P</i> -value
Mean age	35 ± 10	40 ± 12	38 ± 10	
Sex (F/M)	6/32	7/17	11/14	
Eosinophil Accumulation	10/38 (26%)	5/24 (21%)	0/25 (0%)*	< 0.05
Proximal eosinophilia	<i>n</i> = 6 (16%)	<i>n</i> = 4 (16%)		
Mean number of eosinophils	5.5 ± 7.3	1.7 ± 1.5		NS
Range of eosinophil counts	1–20	1–4		
Distal eosinophilia	<i>n</i> = 9 (23%)	<i>n</i> = 3 (12%)		
Mean number of eosinophils	3.2 ± 3.7	7.7 ± 5.6		NS
Range of eosinophil counts	1–12	3–14		

GERD, gastro-oesophageal reflux disease; NS, non-significant.

In five of 10 allergic patients and two of five patients with GERD, eosinophilia was detected both parts.

**P* < 0.05 compared with the frequencies of the each patient group.

10 × 40 hpf of oesophageal biopsy. Biopsies revealed oesophageal eosinophils in 10 of 38 allergic patients (26%) and in five of 24 patients with GERD (21%) but none of the 25 healthy control had eosinophils in their specimens (0%) (*P* < 0.05). Figures 1a and b demonstrate oesophageal eosinophil infiltration in biopsy sample of a patient with allergy.

However, there was no statistically significant difference between patients with respiratory allergy and GERD with respect to the frequencies of having oesophageal eosinophil accumulation (26 and 21%, respectively).

Eosinophils that resided in the tissues of the antrum or duodenum were not found to be increased in number in the means of eosinophilic gastroenteritis [29] in any biopsy samples.

When eosinophil accumulation had been evaluated according to the distal and proximal parts of the oesophagus, six allergic patients (16%) and four patients with GERD (16%) showed eosinophil infiltration into the proximal part. On the other hand, nine allergic patients (23%) and three patients with GERD (12%) showed eosinophil infiltration at the distal part of biopsies. But interestingly, the mean values of the accumulated eosinophils were clearly different between the two groups. Although it was not significant, in the proximal part of the oesophagus, a higher mean eosinophil number was detected in the allergic group than that of patients with GERD, whereas biopsy samples taken from the distal parts of the oesophagus in GERD patients revealed that the mean eosinophil number was prominently much higher than that of the allergic group. But this difference was also not statistically significant (Table 1). Eosinophil accumulation existed in both the distal and also the proximal parts of the oesophagus in five of 10 allergic patients and in two of five patients with GERD. The distribution of the eosinophils that were counted in biopsy samples taken from distal and proximal parts of the oesophagus in both groups is shown in Fig. 2.

The comparison of peripheral blood eosinophil numbers between two patient groups showed that allergic patients had statistically significantly increased eosinophils in blood. The mean amount of eosinophils was $335 \pm 37 \times 10^6/\text{mm}^3$ in allergic patients and $145 \pm 20 \times 10^6/\text{mm}^3$ in patients with GERD (*P* < 0.001).

When the mean values of blood eosinophil numbers were assessed separately in 10 allergic patients with oesophageal

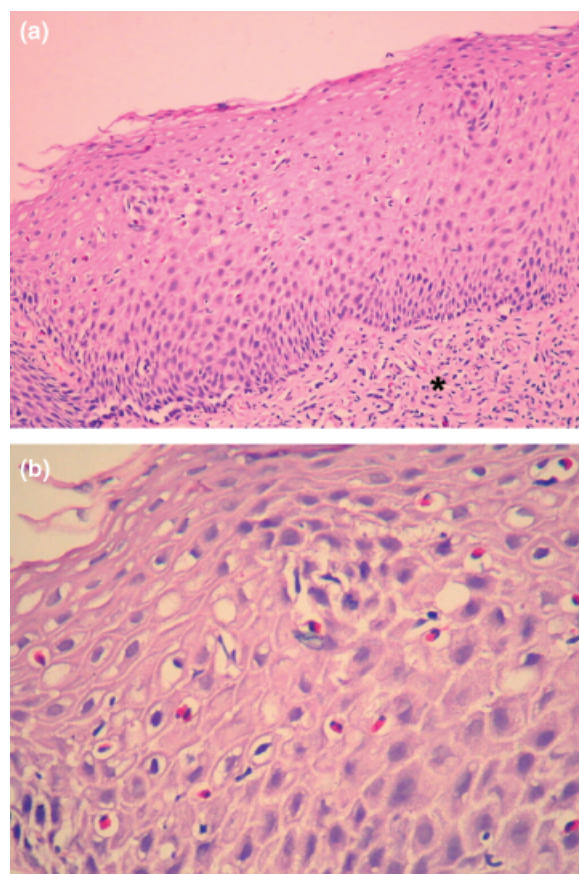


Fig. 1. Eosinophil infiltration in oesophageal biopsy sample of an allergic patient. Eosinophil leucocyte infiltration in squamous epithelium of the oesophagus. Scanty eosinophils are present in the lamina propria marked with (*) (a). (haematoxylin-eosin(H&E) × 100). Eosinophils with typical bilobed nuclei. (b) (H&E, × 400).

eosinophilia and in 28 allergic patients without eosinophil accumulation, the first group had significantly elevated circulating eosinophils ($446 \pm 79 \times 10^6/\text{mm}^3$) than the value ($297 \pm 40 \times 10^6/\text{mm}^3$) measured in the blood of the second group (*P* = 0.012). But comparison of age, gender and duration of allergic disease in these two groups did not reveal significant differences (Table 2). On the other hand, a positive

correlation between peripheral blood eosinophil numbers and the counts of accumulated eosinophils in the oesophagus was found in patients with allergy ($r = 0.872$, $P = 0.001$). This correlation has been shown in Fig. 3. But the same relationship was not detected in the GERD group ($r = -0.371$).

Analysis of allergic patients according to their clinic diagnosis showed that there was no difference in the frequency of finding eosinophil infiltration into oesophageal tissue. Eosinophils were detected in 25% of patients with AR (four of 16) and in 27% of patients diagnosed as having AR concomitant with allergic asthma (six of 22). We compared the mean age, gender, the mean duration of allergic disease and the mean peripheral eosinophil numbers in these subgroups (Table 2). A statistically significant association was found only in mean peripheral eosinophil number of patients with AR and asthma. Patients who were diagnosed as having AR with asthma and had shown eosinophil infiltration in the oesophageal biopsy ($n = 6$) had higher mean blood eosinophil numbers ($485 \pm 130 \times 10^3/\text{mm}^3$) when compared with the values ($268 \pm 50 \times 10^3/\text{mm}^3$) in patients with AR and asthma without oesophageal eosinophil infiltration ($n = 16$) ($P = 0.019$). But mean eosinophil num-

bers in blood did not show any difference among patients with only AR when they were evaluated regarding eosinophil infiltration (Table 2).

All asthmatic patients with or without oesophageal eosinophil infiltration were analysed according to the severity of asthma on the basis of mean blood eosinophil numbers and duration of disease (Table 3). Three of 16 asthmatic patients without eosinophilic infiltration were considered as having intermittent asthma, six had mild persistent and seven had moderate asthma symptoms. It must be pointed out that none of the six asthmatic patients showing oesophageal eosinophil infiltration had intermittent asthma, three patients had mild persistent and the rest of the three asthmatics were considered as having moderate persistent asthma (patients with severe persistent asthma symptoms had already been excluded from the study.) Although it was not statistically

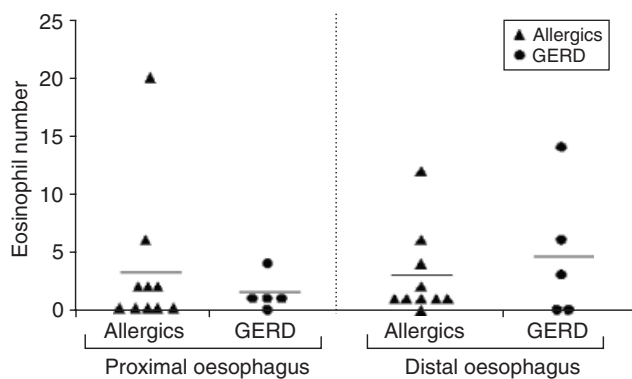


Fig. 2. Distribution of the eosinophils that were counted in the distal and proximal parts of the oesophagus in patients with GERD and allergies.

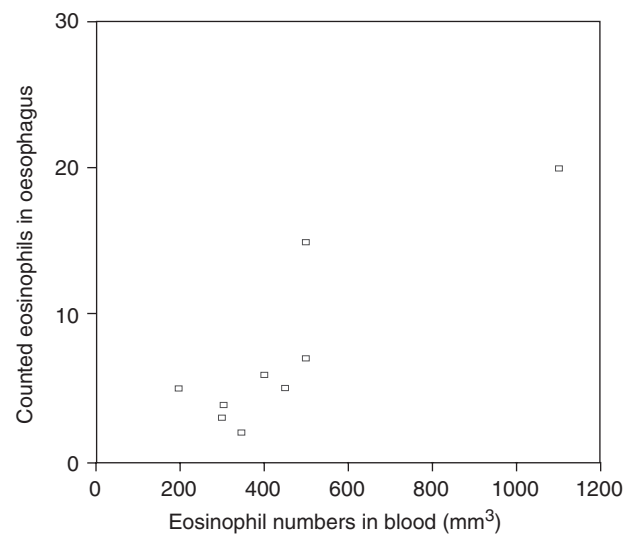


Fig. 3. Positive correlation between the numbers of blood eosinophils and accumulated oesophageal eosinophils in allergic patients. $n = 9$, $r = 0.87$, $P < 0.001$.

Table 2. Analysis of the subgroups of allergic patients according to eosinophil infiltration into oesophageal tissue.

	Mean age years (SEM)	Gender F/M (%)	Mean duration of disease, years (SEM)	Peripheral eosinophils (SEM)
Oesoph. eos. (–) $N = 28$	38.0 ± 2.1	$N = 22/6$ 79%/21%	8.1 ± 1.2	297 ± 40
Oesoph. eos. (+) $N = 10$	38.2 ± 1.9	$N = 10/0$ 100%/0	5.1 ± 1.3	446 ± 79
P -value	NS	NS	NS	0.012
Oesoph. eos. (–) AR+AA $N = 16$	39.3 ± 2.4	$N = 12/4$ 75%/25%	6.8 ± 1.3	268 ± 50
Oesoph. eos. (+) AR+AA $N = 6$	39.2 ± 1.2	$N = 6/0$ 100%/0	5.4 ± 1.1	485 ± 130
P -value	NS	NS	NS	0.019
Oesoph. eos. (–) only AR $N = 12$	36.4 ± 3.8	$N = 10/2$ 83%/17%	9.9 ± 2.1	337 ± 67
Oesoph. eos. (+) only AR $N = 4$	36.5 ± 4.7	$N = 4/0$ 100%/0	5.2 ± 3.2	387 ± 43
P -value	NS	NS	NS	NS

Results are expressed as mean cell numbers in $10^6/\text{mm}^3$.

AR, Patients with allergic rhinitis, AR+AA, Patients with allergic rhinitis and allergic asthma; NS, non significant; oesoph. eos, oesophageal eosinophils.

Table 3. Severity of asthma, blood eosinophil numbers and duration of disease in patients with and without oesophageal eosinophil infiltration.

	Severity of asthma		
	Intermittent	Mild persistent	Moderate persistent
Number (%) and blood eosinophils* (SEM)			
Oesoph. eos. (–) (N = 16)	3/16 % 19 183 ± 60	6/16 37% 266 ± 42	7/16 44% 307 ± 107
Oesoph. eos. (+) (N = 6)	0/6	3/6 50% 419 ± 99	3/6 50% 550 ± 278
P-value	NS	NS	NS
Duration of disease* (years)			
Oesoph. eos. (–) N = 16	3 ± 1.7	6 ± 1.2	9.1 ± 2.7
Oesoph. eos. (+) (N = 6)	–	4 ± 1	6 ± 2
P-value	–	NS	NS

Oesoph. eos, oesophageal eosinophils.

*Data are expressed as mean values (cell numbers in 10⁶/mm³ for eosinophils and mean year (Y) for duration.

significant, asthmatic patients showing eosinophil infiltration in oesophageal tissue had higher eosinophil counts in blood.

The mean duration of allergic diseases of the patients was compared on the basis of whether they had oesophageal eosinophil infiltration or not. Remarkably, it was observed that the duration of disease was found to be shorter in patients with eosinophil infiltration in all subgroup analyses. But the differences were not significant.

Discussion

In this study, we have demonstrated the presence of eosinophil accumulation in the oesophagus of patients with respiratory tract allergy to pollens, while they were symptomatic during the pollen season. The frequency of encountering eosinophils in oesophageal tissues of allergic subjects was similar to that of GERD but significantly higher than that of the healthy group. Under normal conditions, among non-haematopoietic organs, GI segments are unique in that they have tissue-dwelling eosinophils. However, while these cells show a non-homogeneous distribution throughout the GI tract, interestingly, only the oesophagus, was excluded eosinophils presence even under normal conditions [2, 30]. In accordance with this previously documented knowledge, in our study, detected no resident eosinophils in oesophageal tissue of the healthy group. On the other hand, oesophageal specimens obtained from both the disease groups revealed eosinophil infiltrations. Eosinophil infiltration of the oesophageal tissue is observed in some diseases such as PEE, parasitic infections, connective tissue diseases and GERD. In our patient groups GERD, parasitic infections, connective tissue diseases and other secondary reasons had already been excluded.

GERD is one of the most prevalent disorders of GIS and is commonly encountered during clinical practice. In GERD, intraepithelial eosinophils are almost the hallmark of the disease, and can be detected in more than 50% of the reflux

patients [31, 32]. The exact reason why eosinophils accumulate in the oesophagus remains unclear. One explanation is that it may be related to the irritating effect of the strong acid content of the stomach. But because some patients do not respond to anti-acid therapies (H2 blockers or proton pump inhibitors), it is possible that there might be still some unclear factors that may have a role in eosinophil infiltration. Usually, the intensity of eosinophilic infiltration has been proposed as an indicator of failed response to anti-reflux therapy [33]. An allergic component has been claimed by some of the authors in a subgroup of patients with GERD, who especially have higher eosinophil numbers (>7/hpf) in the biopsy [6]. In our study, histopathologic evaluation revealed that 21% of the patients with GERD showed eosinophilic infiltration in oesophageal tissue. Probably because we excluded possible allergic aetiology in our GERD group by using three different methods (taken detailed history, SPT and specific IgE screen), oesophageal eosinophilia was found at a frequency lower than that in the other reports and at a concentration of less than seven cells (except one patient).

PEE is characterized by dense eosinophilia in the oesophageal epithelium (≥24 eosinophils/hpf), accompanied by a typical history such as dysphagia or food impaction and consistent endoscopic abnormalities. There were many studies performed on PEE to search for possible causes of the accumulation of eosinophils in such a 'protected' tissue. Some of these studies revealed that a strong atopic background and allergic factors were responsible for eosinophilic infiltration in PEE [19]. Initial studies demonstrated the association of PEE with food allergy, especially in children. When sensitized individuals were exposed to food allergens, eosinophilic infiltration developed in the oesophagus. When these patients were treated in such a way as to eliminate the foods responsible, oesophageal eosinophilia reduced markedly [14, 18, 34–36].

Afterwards, an important result appeared in a murine model [23]. In this study, Mishra et al. presented a model of EE induced by exposure of mice to respiratory allergens. Interestingly, respiratory allergen challenge caused a significant increase in the levels of eosinophils both in the blood and also broncho-alveolar lavage fluid, together with the oesophageal eosinophil infiltration. In contrast, they did not find any significant change in the stomach and small intestine. There two important outcomes might be obtained from this experimental model. Firstly, the study indicated that under the condition of an experimental respiratory hypersensitivity to aeroallergens, development of pulmonary eosinophilic inflammation occurs simultaneously with eosinophil infiltration into oesophagus and blood eosinophilia. Secondly, these results proved a pathophysiological connection between allergic responses in the lung against inhaled allergens and oesophageal eosinophil infiltration. The results from our human study were very much similar to those of the experimental study and strongly supported this model. Our study group which consisted of patients with upper and/or lower respiratory tract allergy, was already naturally hypersensitive to grass pollens. During the pollen season, while they were exposed to allergens and had allergic respiratory symptoms, we revealed eosinophil accumulation in the oesophageal tissue in one-fourth of the patients, although

none of them reported any gastric or oesophageal complaints (but, in the biopsy samples taken from the healthy controls during the same period, there was no eosinophil at all.) This group of allergic patients showing eosinophil infiltration in oesophageal tissue also had significantly higher blood eosinophil number. Moreover, there was a positive correlation with eosinophils in peripheral blood and eosinophil numbers accumulated in the tissue. We could not examine the respiratory tract of the allergic patients histopathologically because they had the symptoms but it was supposed that there must have been eosinophilic inflammation in their airways. In the literature, there was a case related to pollen allergy and Fogg et al. reported a case of EE, in which GI symptoms worsened during the spring and showed improvement during the winter. The patient was also diagnosed as having AR and asthma against pollen. All biopsies performed during the winter months revealed mild or no oesophageal eosinophilic inflammation, although biopsy samples taken in the pollen season showed moderate or severe eosinophilic inflammation in the oesophagus. This association suggested that EE in this pollen-allergic patient might be related to pollen exposure [37].

Another study showing the pathophysiologic interactions of inflammatory reactions between the mucosa of the respiratory tract and that of the GI tract in patients with birch pollen allergy was reported by Magnusson et al. [38]. They demonstrated a significant increase of eosinophils and IgE-bearing cells (mast cells) in duodenal mucosa of patients with birch pollen allergy during the birch pollen season when compared with that out of the season. Their study group consisted of patients who had seasonal respiratory allergic symptoms. For our patients, we do agree with the fact that control biopsies should have been performed after the pollen season to prove that the reason responsible for eosinophil accumulation in the oesophagus was pollen exposure. However, we could not carry out this because of ethical reasons.

In order to understand the underlying pathophysiologic mechanisms of oesophageal eosinophil accumulation in our cases, we had to look at the studies on PEE because it was a unique model for 'eosinophils in the oesophagus' with unknown mechanisms. These studies revealed an association with Th2-type immune response in an eotaxin-dependent manner [23–25, 39]. Some cytokines such as IL-1, IL-3, IL-4, IL-5, IL-13 and GM-CSF not only have enhancement effects on eosinophil development, migration and function but also regulate eosinophil accumulation together with some chemokines including RANTES, monocyte chemoattractant protein (MCP)-3 and MCP-4, eotaxins, etc. Among these regulating factors, IL-5 is mainly a specific cytokine for eosinophils and eotaxin is accepted as a critical tissue recruitment factor for eosinophils [8]. It was demonstrated that eotaxin-1 is constitutively expressed in the GI tract at variable levels. This expression shows dwelling of eosinophils in the GI mucosa at baseline [2]. But the studies suggested that eotaxin alone was not sufficient for eosinophil accumulation into the oesophagus and IL-5 was required as an additional factor [23, 24, 40]. Another Th2 type-cytokine, IL-13, was also found to have a role in the mechanism of EE. IL-13 delivery to the lung-induced EE by an IL-5, eotaxin-1 and STAT6-dependent mechanism [25]. In their article, Nicholson et al. reported a clinical study demonstrating increased IL-4

expression in the presence of immunopositive mast cells and 'activated' eosinophils in the oesophageal tissues of a patient with PEE as well as secondary EE cases. They suggested a common allergic pathogenesis underlying both conditions [41]. Among the Th2-type cytokines, IL-4 and IL-13 play a role in the recruitment of eosinophils from blood into tissues by enhancing endothelial adhesion molecule VCAM, whose counter ligand (VLA-4) is present on eosinophils. These findings establish that the effective immune mechanisms in pathogenesis of EE also seen to be involved in allergic asthma [42–44]. Thus, the factors responsible for accumulation of eosinophils into the mucosal surfaces with respect to allergic nose, lung or oesophagus might be similar.

In their experimental model, Mishra et al. detected eosinophil infiltration into the oesophagus only when allergen was applied by an intranasal route and not by oral or intragastric exposures [23]. This result might be explained mechanistically. Rothenberg speculated that sensitization might have occurred through the respiratory tract when sensitized individuals subsequently swallow or ingest aeroallergens deposited in the upper GI tract, and then a hypersensitivity response leading to eosinophil infiltration into the oesophagus may develop [6]. In our study, eosinophil infiltration of oesophagus in patients with allergy and those with GERD was demonstrated at both the proximal and distal parts. In the GERD group, this result can be explained by proximal reflux. Patients suffering from GERD may also have proximal reflux, and acid contact may cause proximal eosinophil accumulation. We did not know which patients had this.

But if the mean eosinophil numbers of those parts were compared, our results were found to be supportive of his speculation in a way. Although not significant statistically, allergic patients had more dense eosinophil accumulations in the proximal part, whereas eosinophils were present in higher numbers at the distal oesophagus of the patients with GERD. On the other hand, interesting, it was found that while eosinophilic infiltration had been occurring in a tissue (oesophagus) normally devoid of these cell types, there was no change in the number of resident eosinophils in the antrum or duodenum. This finding was the same as that of the animal model of Mishra et al., suggesting a connection between the upper airways and the upper GI tract. It might be thought that, at first an inflammatory process against aeroallergens in the airways develops and then it might expand to the upper GI tract by the common lymphatic system and macro-environment and shared T cells.

An other and probably most likely reason for oesophageal eosinophil accumulation in allergic patients might be related to the systemic aspect of allergic inflammation in the light of the immunologic data published previously [38, 45–47]. In these reports, it is considered that allergic inflammation is a systemic immune response; cells of this inflammation such as eosinophils and lymphocytes of nasal, bronchial and enteric mucosa might belong to the same re-circulating pool. Our findings showed that eosinophil numbers in peripheral blood were higher in allergic patients who also had oesophageal eosinophil accumulation than that of allergic patients without accumulation. The presence of a strong correlation between the numbers of eosinophils in peripheral blood and in oesophageal tissue agrees with this idea.

However, especially in patients with asthma, increased peripheral eosinophil numbers and severity of asthma appear

to be two important factors that affect eosinophil accumulation in oesophageal tissue. In asthma, while larger mucosal areas may be involved in allergic inflammation, probably with some other additional effects eosinophils may expand towards different mucosal sites. These 'additional effects' might be some individual differences, and may appeared during the early period of allergy.

In summary, an allergic inflammatory response does not seem to be localized to the target organ. Most likely, with the effects of increased Th2 cytokines and chemokines, some changes at neighbouring mucosal sites can be induced. Eosinophil infiltration of oesophageal mucosa might be accompanied by allergic airway disease during the symptomatic period, even though it may not cause any GI complaints.

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