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Levels of eotaxin-3 as a noninvasive biomarker for monitoring the treatment EoE in school age children with food allergy

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Summary

Objective

Eosinophilic esophagitis (EoE) is a chronic disease characterized clincally by symptoms of esophageal dysfunction and histologically by eosinophil-predominant inflammation and typically requires serial invasive endoscopic biopsy examinations to document the characteristic histologic features of the disorder. The INH PubMED review indicates that approximately 25 studies are published in between 2006 and 2014 that propose a number of molecules as biomarkers from tissue biopsies and serum samples of EoE and non EoE patients.

We aimed to identity eotaxin-3 that correlated with activity of EoE as measurmed by esophageal eosinophilia and response to treatment by using elimination diet in children group with food allergy in the course of EoE .

Subject and methods: 5 children (from 12 to 18 age) with EoE were studied for up to 12 weeks of hypo allergic diet therapy and 12 children with normal esophagogastroduodenoscopies with biopsies were enrolled to the control group. The serum level esotaxin-5 were measurement in the study group two times (0, 12 weeks), total IgE and specific IgE, skin prick test, patch test were done at the enrolment times. All samples were processed using eotaxin-3 Human Elize Kit (Ray Bio, USA).

The mean level eotaxin-3/CCL26 in the blood serum was 115,22 pg/ml (median 112,04 CI 95% (81,32-155,5). Total IgE had impact on eotaxin-3 excretion ($p<0,05$). The serum level of eotaxin-3 was correlated with tissue eosinophilia ($r= 0,66$ $p < 0,01$) and peripheral blood level of eosinophilia ($r=0,80$ $p<0,01$). After treatment by using elimination diet of the some foods (milk, wheat, soya bean) the level of the eotaxin -3 was lower ($p<0,001$).

Conclusions: serum levels of eotaxin-3 can be used as a non invasive biomarker for monitoring therapy of EoE by using an elimination diet in school-age children with food allergy.

Introduction:

Eosinophilic esophagitis (EoE) is a severe and chronic, antigen/immune-mediated disease of the esophagus characterized clinically by symptoms related to esophageal dysfunction and histologically by an eosinophil-predominant inflammation that is limited to the esophagus [1]. This disease is now a growing health problem and affects about 50 per 100 000 people in westernized countries [2,3], and at least 10 per 100 000 childrens [4]. Whether this reflects increasing disease incidence or recognition is unclear, this increase also coincides with the increasing of asthma and allergic disease in the industrial world. Clinical manifestation of EoE in children are nonspecific and vary by age such that diagnosis based on the symptoms alone is non faceable. Infants and toddlers often presents with feeding difficulties, irrability,whereas school aged children are more likely to present vomiting, abdominal or chest pain and laryngeal symptom. Dysphagia is more characteristic symptoms in adolescents and oldest aged children[4]. Studies show that two-thirds of patients with EoE suffers or have suffered from allergic conditions such as asthma,eczema, chronic rhinitis [4], and that the vast majority of EoE patients who are sensitized to multiple food allergens [1,5,6] are testimory to the allergic background of the disease. EoE is associated with high levels of cytokines, particularly Il-4,IL-5.Il-13, increased production of eotaxin chemokines vel eotaxin -1,-2 and -3 (reffered in new nomenclature respectively CCL11, CCL24 and CCL26) that attract inflamatory cells, predominatly eosinophils [7]. Several findings for a potiential role of eotaxin-3/CCL26 in EoE. Eotaxin-3 induces chometaxis and activation of eosinophilic granilocytes in vivo [8]. Eotaxin -3 mRNA increased drastically in asthmatic patients after antigen challenge, several studies suggest togother the association of single nucleotide polymorphisms in the gene eotaxin-3 with asthma, rhinitis and EoE [8,9]. In the studies were reported, that mice deficient in the receptor for eotaxin were protected against experimental EoE. These results implicate eotaxin-3 as a clinical effector molecule for EoE and provide insight in to pathogenesis [10]. EoE diagnosis requires endoscopy to demontrate the presence of characteristic histological features, the presence of eosinophils in the mucosa of the esophagus and proliterative changes in the epithelium. Current recommendations for the treatment of EoE include the elimination of food antigens [11] or the inclusion of elemental diet (amino acid-based formula) [4,5] and anti-inflamatory medications.

To our knowledge, for today is not definite highly sensitive and specific non-invasive blood test, which would be useful for monitoring the pathogenesis and treatment of EoE. Therefore, the our aim of the study was to demonstrate the influence of selected factors

(tissue eosinophils, eosinophils in the peripheral blood, total IgE, allergy elimination diet) on the level of eotaxin3-/CCL26 in childrens group with EoE to assess the suitability determinations of eotaxine-3 in the blood serum as an independent factor control EoE in childrens with food allergy.

Methods

Patients

Five childrens ranging in age from 12 to 18 years with EoE and food allergy from the 3 rd Department of Pediatrics, Bytom (Medical University of Silesia, Katowice) was studied for up to 12 weeks of used eliminate food to which the children are sensitized . Biomarker (eotaxin-3 / CCL26) in serum were analyzed as to their use as a noninvasive biomarker for the diagnosis and assessment of EoE disease status. All patients fulfilled the EoE 2011 criteria for EoE [1]. Food allergy were diagnosed in everyone patients from study group by positive prick and/or patch test and/or high level of specific IgE (> 0,7 IU/ml). The mean of age was 15,2 (±2,8), the rates males/girls was 4:1, 2 patients have asthma and allergic rhinitis (AR), 1 patient have asthma, AR, eczema, 1 patients have AR and conjuctivitis and 1 patients have AR and eczema. Symptoms reported by patients from study group were: chest pain (1 children), abdominal pain (1 children), decreased appetite (2 childrens), coughing and nausea (3 childrens) but the physical examination were typically normal. Clinical, laboratory and pathological date were obtained from all patients and chart reviev was performed. Exclusion criteria included any coexisting esophageal condition, Helicobacter pylori infection, inflamatory bowel disease. 12 children (5 males -40%) with nonspecific reasons including recurrent abdominal pain, with normal esophagogastroduenoendoscopies in the biopses were enrolment as controls. The avarage age of this group was similiar to the study group and was 14,8 ±4,1. 3 patients have asthma, 1 patient -AR, 1 patient -AR and conjuctivitis. So we divided the control group on the 2 subgroup: with atopic disease control without EoE (AC) and without atopic diseasy healthy control (HC). Informed consent was obtained from all participants with EoE and control group and parents commencing the study. The study was approved by the local research ethnic committee.

Endoscopic

All patients with EoE were studied by underwent endoscopy and biopsy at the time of enrolment in the study. Biopsy specimens were obtained from the maximal and distal esophagus and were prepared and analyzed as previously described [14] and number of

eosinophils per 400 x helf was recorder.In histological evidance of infilltration of distal or proximal oesophageal mucosa with: 24 or more eosinophils in one 400xhpf vel mean 15 and more in two testing materials used to diagnosis active EoE.

Collection and storage of samples

Each child from the study and control group was examined by the same physician to exclude infection or active allergy symptoms before the serum was obtained in the laboratory. Serum samples of eotaxin-3/CCL26 were collected 2 times in the study group: in the enrolment times and after using hypo-allergic diet treatment by 3 months (0,12 weeks) and immediately stored at - 20 O C.

Atopic measurement

Skin Prick Test SPT (Allergopharma, Germany) for a large (12) panel of foods and environmental (13) allergen was performed at the enrolment times in the study and control group. Patch test was performed for food allergens (Allergopharma, Germany) and also for native food allergens used Finn Chambers. Total IgE and specific IgE were measurement by used UniCAP Pharmacia metods. Results of allergy examination was presents in tab 1.

Eotaxin-5 measurement

Detection of eotaxin-3/CCL26 was performed by ELIZE Kit (Cayman Chemical, Ann Arbor, MI, USA). All measurements were done in duplicate according to the manuphacturer's instructions and the mean value was calculated. The sensitivies of the EliSA's were 7,8 pg/ml for eotaxin -3.

Statistical analysis

Data were analyzed using MedCalc 9.6 (MedCalc, Mariakerke, Belgium). Data were expressed as mean ± SD. We examined the relation among eotaxin 03/CCL26 levels and hypoallergic diet, histological changes, eosinophils peripherial blood levels, serum immunoglobulin E (IgE) levels in the study group. We used test T-Statistica and test Pearsons. $P< 0,05$ was considered to be statistically significant.

Results

Clinical characteristic

Five a school ages patients (4 boys) with EoE were studied. Active EoE was characterized by histological proof (number of eosinophils per 400 x helf). The mean tissua eosinphils were 17,8 ± 2,68 (mediana 18,0) in the study group. Disease manifestation were typical for EoE and equally distribution of asthma disease were similar in EE and AC. 2 from 5 patients in the EE group received inhalant steroid in the low dose for control of allergic asthma, whereas in the AC group 1 patients receiving inhalant steroid. The food allergy were diagnosed in everyones of EE group. The more frequently were diagnosis milk (3/5) and wheat (3/5) allergy. EE group was characterized by higher levels of peripherial eosinophilia and total IgE. The clinical characteristics study and control group are given in table 1.

Serum levels of eotaxin-3 in EoE patients

Eotaxin-3 protein levels in the blood were quantified in EE, AC and HC. Eotaxin-3 protein serum levels of EE was significantly increased compared with AC and HC ($p<0,05$). The serum levels of eotaxin-3 were respectively 114 ±27 pg/mg, 18,54 ±5,4 pg/ml, 8,99±1,16 pg/ml in EE, AC, and HC (mean ±SD, $p<0,001$).

In addition when we compared EE patients before and after treatment by using elimination diet (milk in 3 patients, wheat in 3 patients, soybean in 1 patient) by 12 weeks we found a significantly drop in eotaxin-3/CCl26 levels paralleling clinical improvement (before 114,5 +27 pg/ml vs 26,875+4,84 pg/ml). Ryc.2

Correlation of eotaxin-3 serum level with eosinophilia and other labolatory data

To determination possible correlation of eotaxin-3 levels with blood IgE and eosinophils level we used Pearson's test. Eotaxin-3 levels correlated significantly with all investigated clinical parameters: blood eosinophils levels ($r=0,80, p<0,01$), eosinophils in thissue biopsy ($r=0,66$, $p<0,01$) and IgE levels ($r=0,34, p<0,05$) from EE patients. These finding are depicted in table 2.

Table I. Clinical manifestation of EoE patients (EE), allergy disease controls (AC), healthy controls (HC) pg/ml.

	EE	AC	HC
Patients	**5**	**5**	**7**
Age	**12-18**	**13-18**	**12-16**
Sex M/F	**4/5**	**3/5**	**2/5**
Peripherial blood eosinophils %	18	3	6
IgE IU/ml	160,0	128,5	68,7
Eotaxin-3 pg/ml	112,4	18,5	8,99
Food allergy test (+) (Prick,patch,IgE)	5/5	1/5	0/7
+Milk	3/5	0/5	0/7
+Wheat	3/5	1/5	0/7
+soybean	1/5	0/5	0/7
Aeroalllerge test (+)	2/5	5/5	0/7
+Outdoor	1/5	2/5	0/7
+Indoor	2/5	4/5	0/7
Asthma	3/5	3/5	0/7
Allergic rhinitis	5/5	4/5	0/7
Eczema	2/5	0/5	0/7
Conjuctivitis	1/5	1/5	0/7
Family atopy (+)	3/5	4/5	1/7

Table 2: Correlation coefficient (r) among serum eotaxin-3/ CCL26 and other histology and laboratory date in children with acute EoE (EE)

	Eotaxin-3 / CCL26	
	r	p
Tissue eosinophils	0,66	<0,01
Peripherial blood eosinophils	0,80	<0,01
Total IgE (IU/ml)	0,34	<0,05

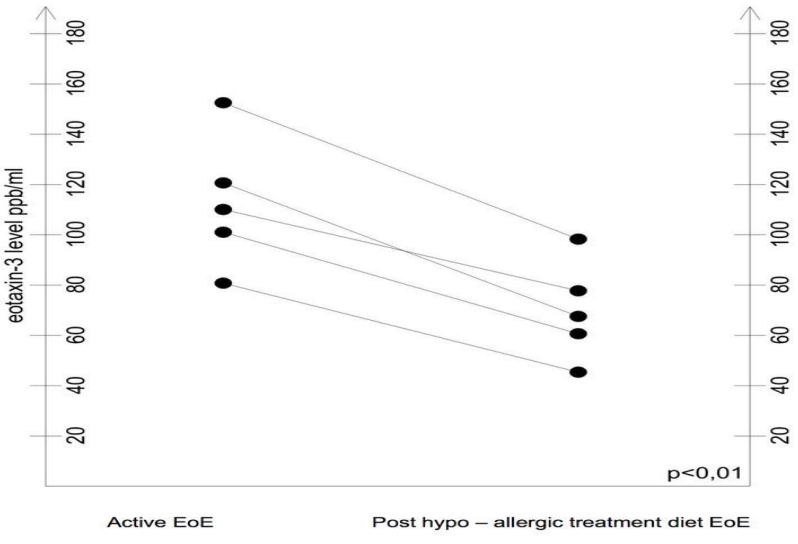

Figure 1. Levels of Eotaxin – 3 /CLL – 23 in active EoE and post – allergic diet.

Discussion

Eosinophilic esophagitis (EoE) diagnosis and follow up response therapy is based on repeated endoscopies and histological examination for eosinophils/hfp. This procedure is invasive and risky on particular for pediatric population, but it is necessary in accordance with the recommendations [1,11,12**].** The INH PubMed review indicates that approximately 26 studies are published in between 2006 and 2014 that propose a number of molecules as biomarkers from tissue biopsies and serum samples of EoE and non EoE patients **[**13**].** These molecules include eosinophil-derived neurotoxin, eotaxin-1, eotaxin-2,oetaxin-3, interleukin -5 (IL-5),IL-6,IL-9,IL13, eosinophil peroxidase, absolute eosinophil count, mast cell, TSLF minor necrosis factor including transcripts of KBP51 and microRNAs21 and 223 [14] [15**].** The study of 3 noninvasive biomarkers collected from children during a prospective trial explored the possibility that EoE may be monitored without as repeated gastroscopy [16]. Similarly, two clinical and experimental analysis reports provide the possibility that the CD274 mRNA and CD274 -expressing eosinophil levels may be novel possible noninvasive biomarkers for EoE [17]. In our study, we show that 1) eotaxin-3 is highly elevated in serum in active EoE patients compared with atopic and non-atopic control children without EoE 2) eotaxin-3 levels correlate with markers of diseasy activity and 3) eotaxin-3 were higher in children with active sites disease and decreased with the 3 months of elimination diet (milk,wheat,soyabean) shown to allergie

The discovery of a chemokine family (the eotaxins) has contributed substantially to the knowledge eosinophil trafficking to inflamatory sites [18]. All eotaxins (-1, -2, -3) is a CC chemokine, a signal is transmitted through the same receptor (CCR), with only 40% homology each other, and the genes for all of these eotaxin are located on different chromosome. Experimental evidence suggests crucial involvement in the perpetuation of tissue eosinophilia. Expression of eotaxin-3 is significantly increased in late stage after allergen challenge in bronchial asthma [19]. Eotaxin- 3 has been linked to the pathogenesis of EoE [9] because the level of eotaxins-3 were strongly elevated in active EoE with peripheriał blood eosinophilia and tissue eosinophilia. Although the correlattion of eotaxin-3 of the serum IgE were lowest but simultaneously may by clinically relevant. In over hand the eotaxin-3 level is the more higher in active EoE than non EoE patients and reduced significantly after treatment EoE patients by used elimination diet. So the eotaxin-3 seems to play a major and specific role in the suistained severe eosinophil infiltration in EoE.

In conclusion we found a significant and specific alteration of eotaxins-3 in active EoE childrens with food allergy.Eotaxin-3 might constitute a pathogenic player in tissue eosinophilia and subsequent esophagus damage, and a biomarker for disease activity and interesting therapeutic target in EoE.

It has become increasingly clear that there is a significant allergic predisposition in the EoE population with the majority of patient having concurrent allergic rhinitis, asthma, eczema, and/or a history of atopy [20]. In examined childrens group with EoE we established ,that the most common clinical manifestation of allergy was AR (100%). Based on allergic pathogenesis, it is likely that EoE represents a new manifestation of atopy[20]. In childrens EoE seems to be primarily a food antigen-driven disease with the majority of responding to the elimination of common dietary antigen and having disease recrudescence upon reinduction of the instigating food antigen [4,5,21,22]. In this study food allergy were diagnosis to the following allergens: milk and wheat (3 childrens), soybean (1 children) and we decided to used elimination diet from this foods. Interestingly, that patients with EoE have also been reported to exhibit seasonal variations in their symptoms and changes in their esophageal eosinophil levels. The mucosal eosinophil counts were elevated during the spring and summer and were suppressed during the winter time indicating a role for aeroallergens. **[**23,24**]** In examined group we also diagnosed hypersenitivity to the outdoor and indoor allergens. Experimental study of the EoE in mice showed that EoE can be induced both by food and aeroallergens [25]. Murine modeling has established also that EoE is a Th2-associated disease [26] with increased antibody production (mainly IgE immunoglobulins) and strong cellular shift towards an increase of Th2 cells that produce Il-4, Il-13 and 5 observed [27,28]. The clinical relevance of Il-5 in humans was demonstrated by clinical trials utilizing a neutralizing anti-Il-5 antibody (mepolizumab), which causes reduction in peripherial blood eosinophils in human brionchial asthma and EoE [29][30]. Human EoE is associated with overproduction of Th2 cytokines Il-4 and Il-13 which indicate the eosinophil-specific chemokines (e.g eotaxin-3) may be the crucial meaning in the pathogenesis EoE [31][32].

More, recently eotaxin-3/CCL26 levels was implicated on eosinophils and its role in allergic disease and EoE. Thus, there is an urgent need to continue with innovative studies to uncover new possibilities for therapeutic monitoring.

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