

Short communication

Increased plasma eotaxin in atopic dermatitis and acute urticaria in infants and children

Background: The previously reported eotaxin overexpression in the lesional skin of atopic dermatitis (AD) led us to the assumption that circulating levels of eotaxin may be elevated too. We sought to investigate the plasma expression of eotaxin in children with skin allergy in relation to clinical activity and type of lesions.

Methods: Plasma eotaxin was assayed in 78 infants and children, of whom 16 had AD, 19 had acute urticaria (AU), and 43 were healthy matched subjects. Seven children in the group of AU were resampled for plasma eotaxin after clinical remission.

Results: The plasma eotaxin levels in AD (median = 158 pg/ml, mean [SD] = 168 [61] pg/ml) were significantly higher than the control values (median = 60 pg/ml, mean [SD] = 59.5 [18.5] pg/ml). Not only did patients with AU demonstrate elevated plasma eotaxin levels (median = 126 pg/ml, mean [SD] = 124 [33] pg/ml), but also a significant decline occurred on follow-up. The coexistence of angioedema with AU did not cause any further increase in plasma eotaxin expression. Plasma eotaxin levels were significantly higher in AD than in AU, probably reflecting the chronic nature of eczematous AD lesions. The plasma eotaxin levels did not correlate with serum total IgE, peripheral blood absolute eosinophil count, or age of the patients. However, there was a positive correlation between age and plasma eotaxin in the control group.

Conclusions: Our findings imply that circulating levels of eotaxin increase in AD and during flares of AU, probably to serve in the recruitment and activation of eosinophils. It may also represent a biomarker of lesional activity.

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Eotaxin is a chemokine that promotes the selective recruitment of eosinophils, the major effector cells in allergic inflammation. Unlike other chemokines, eotaxin is eosinophil specific and does not attract other types of leukocytes (1–3). It may thus explain the tissue eosinophilia that characterizes certain diseases (4). Severe tissue damage ensues as eosinophils release their highly cytotoxic granular proteins (2). CCR3 is the receptor that mediates the majority of the eosinophil chemotactic effect and is the only receptor for eotaxins identified to date (5–7). CCR3 is present on cell types associated with allergy, such as eosinophils (5, 8), basophils (9), and T helper 2 cells (10). Eotaxin mRNA and/or protein was detected in endothelial cells (6), lung epithelial cells (11), macrophages (12), corneal keratocytes (13), and dermal fibroblasts (14).

Eosinophils are thought to play an effector role in the lesional skin of atopic dermatitis (AD) because they migrate from the blood into lesional skin and release cytotoxic mediators such as eosinophil cationic protein and eosinophil protein X (15, 16). High levels of these

proteins were reported in the serum and urine of infants and children with AD, correlating with disease activity (17, 18). The prevalence of AD has been steadily increasing, and in most countries it now affects more than 10% of children at some point during childhood (19). It is an important skin condition with significant costs and morbidity to patients, their families, and society overall (20). Regardless of the patient's age, mild to severe erythema, scaling, and excoriations that reflect the severe itch are generally present (21). Although AD may begin at any age, in 75% of children, the first signs are present by 6 months. Involvement of the whole cutaneous surface may occur, but the predominant areas are the face in infants, extensor aspects of the limbs as the child begins to crawl, and the limb flexures in older children and adults (22). Infants and children with AD are at great risk of developing respiratory allergy later, and this is evident in the natural history of allergy or the allergic march (23, 24).

Another more prevalent allergic disease of the skin is urticaria or hives. It is estimated that up to 20% of the

general population will experience an episode of acute urticaria (AU) (25). It is characterized by well-circumscribed but sometimes coalescent, localized or generalized erythematous skin lesions called wheals or welts. They are thought to result from local subcutaneous or intradermal leakage of plasma. If this leakage involves deeper structures, including dermal and subdermal sites, angioedema occurs (26, 27). AU and angioedema are listed among the causes of eosinophilia (28). Eosinophils are abundant in the skin wheals, and hypereosinophilia was reported in cases of angioedema (29).

The established association between eosinophils and skin allergy and the relation between eotaxin and eosinophil activation and recruitment into the skin suggest that eotaxin may share in the pathogenesis of allergic skin diseases in childhood. We thought that measurement of eotaxin in biologic fluids such as plasma would provide new insight into its place among other markers of allergic inflammation. Research efforts in this field may ultimately lead to adjuvant lines of therapy with eotaxin antagonists.

Material and methods

Study population

The study comprised 78 infants and children of whom 16 had AD, 19 had AU with or without angioedema, and 43 were healthy subjects included as a control group. Seven subjects in the group of AU and angioedema were followed up until their recovery and were then restudied. The patients were enrolled from the Pediatric Allergy and Immunology Unit, and emergency room and outpatient clinics of Ain Shams University Children's Hospital, Cairo, Egypt, over a period of 3 months. Informed consent was obtained from the parents or caregivers of each patient or control subject before enrollment in the study. The sample was a stratified nonrandom one subclassified into three groups:

Atopic dermatitis (AD). The AD group comprised 16 subjects whose ages ranged between 2 months and 7.5 years with a mean age (SD) of 2.6 (2.1) years (median=2.4 years). They were nine boys and seven girls. The inclusion criteria were pruritis, typical eczematous lesions, intermittent course, family history of atopy, and a duration of illness longer than 6 weeks (30). We excluded subjects with history of other allergic diseases, such as asthma, allergic rhinitis, or allergic conjunctivitis, in order to be able to interpret the effect of skin allergy alone. The infants and children were subjected to a detailed history taking and clinical examination to avoid subjects with a concomitant illness. Children clinically suspected to have parasitic infestations were excluded. The patients were evaluated upon recruitment before the start of therapy, and children who were currently receiving systemic corticosteroids were not included.

Acute urticaria (AU). The AU group comprised 19 infants and children. They were 11 boys and eight girls, and their ages ranged from 9 months to 8 years (median=2.5 years, mean [SD]=3.3 [2.3] years). They were recruited from the emergency room while presenting with acute attacks of wheals and itching. The manifestations were accompanied by angioedema in seven subjects (26, 27). The duration of the attack ranged between 1 and 3 h; history of previous attacks was reported in three children only.

Infants and children in this group were not receiving any medication at recruitment, and those with clinical data suggestive of other atopic diseases or parasitic infestations were excluded from the study. Seven subjects agreed to be resampled 1 week after complete cure. Two of them had angioedema at presentation and their demographic data are presented in Table 1.

Control group. The controls comprised 43 healthy infants and children. They were chosen so that for each patient at least two sex- and age- (± 1 month) matched subjects were enrolled. They were 25 boys and 18 girls, and their ages ranged between 2 months and 8 years (median=2.5 years, mean [SD]=3.4 [2.5] years). The inclusion criteria were the absence of personal or first-degree relative history of atopy, personal history or clinical evidence of parasitic infestations, or any chronic illness. They underwent general and systemic clinical examinations to exclude any current illness. Their weight and height (or length in infants) measurements in relation to normal percentiles for age (31) were taken as markers of physical and nutritional well-being. Subjects with weights and/or heights that fell below the 5th or above the 95th percentiles were excluded. We also excluded subjects who had evidence of peripheral blood eosinophilia or elevated serum total IgE for age.

Study measurements

Plasma eotaxin assay. A blood sample (2 ml) was withdrawn from each subject by venipuncture, with EDTA as an anticoagulant. Plasma was separated from cells within 30 min of collection by centrifugation at 1000 *g* for 15 min and stored at -20°C until assayed. Hemolysed samples were excluded. The kit used was a human eotaxin immunoassay (Quantikine, R & D Systems, Minneapolis, MN, USA). The assay employs the quantitative sandwich enzyme immunoassay technique using *E. coli*-expressed recombinant human eotaxin and antibodies raised against the recombinant factor. Its lowest detectable dose of eotaxin is below 5 pg/ml.

Serum total IgE. Serum total IgE was assayed in all subjects by ELISA (Eurogenetics, IgE Quantitative, Tessenderlo, Belgium). Results are expressed in IU/ml. Owing to the variability in serum total IgE levels with age in childhood, we calculated the percentage values from the reference ranges (32) by dividing the subject's level by the highest normal for age $\times 100$. IgE levels used in the correlations were both the measured and the calculated percentage values. Serum total IgE levels that exceeded the highest normals for age were considered elevated.

Complete blood count. The blood count was performed in every subject with the Coulter counter (Coulter MicroDiff 18, USA). The differential leukocytic counts were estimated manually from the blood film and expressed in absolute count values. Infants and children whose absolute eosinophil counts (AEC) exceeded the normal reference values for age (32) were considered to have peripheral blood eosinophilia. Blood sampling of all subjects was performed at the same time (10 am) daily to avoid diurnal variations in eosinophil counts, and aliquots were taken for the different study assays

Statistical analysis

The results were analyzed by a commercially available computer software package (StatView, Abacus Concepts, Inc, Berkeley, CA, USA). The data are presented as median, mean, and standard deviation (SD). For nonparametric comparison of eotaxin levels among different groups, we used the Mann-Whitney U test. The Wilcoxon signed rank test was also used to compare paired eotaxin

Table 1. Demographic and laboratory data of seven patients with acute urticaria who were resampled for eotaxin after cure. * Wilcoxon signed rank test was used in comparison

Patient no.	Age (years)	Sex	Angioedema	AEC ($\times 10^3/\text{mm}^3$)	Eosinophilia	Serum total IgE (IU/ml)	Elevated serum total IgE	Plasma eotaxin (pg/ml)	
								Attack	Remission
1	7	F	+	0.5	+	110	+	129	113
2	6	M	+	0.5	+	14	—	88	73
3	4	M	—	0.2	—	70	+	125	84
4	5.5	M	—	0.7	+	60	+	126	62
5	1.7	F	—	0.2	—	10	—	160	88
6	1.8	F	—	0.1	—	50	+	120	42
7	2.7	M	—	0.6	+	25	+	75	26
Minimum	1.7			0.1		10		75	26
Maximum	7			0.7		110		160	113
Median	4			0.5		50		125	73
Mean	4.1			0.4		48.4		117.6	69.7
SD	2.1			0.23		35.6		28.1	29.3
								$P=0.018^*$	

AEC: absolute eosinophil count; SD: standard deviation.

levels (before and after remission of symptoms). Kruskal-Wallis multivariant and chi-square analyses of the demographic data were performed. The relation between the various numerical parameters was studied by the Pearson correlation coefficient (r) test. Two-tailed P values below 0.05 were considered significant.

Results

The studied sample comprised 35 patients and 43 control subjects. The ages of the patients ranged from 0.17 to 8 years (median = 2.5, mean [SD] = 3 [2.2] years), and the ages of the control group were in the same range with a similar median age (2.5 years) and a mean age of 3.4 (2.5) years ($P=0.65$). The male to female ratio in the patients and controls were 1.33:1 and 1.38:1, respectively ($P=0.93$). The sex distribution among the three studied groups (AD, AU, and controls) was matched by multivariant analysis ($P=0.64$).

The plasma eotaxin levels of the patients with AD ranged between 90 and 300 pg/ml (median = 158 pg/ml, mean [SD] = 168 [61] pg/ml). These values were significantly higher than the corresponding values of the group of AU ($P=0.016$), which ranged between 75 and 212 pg/ml, and the median and mean (SD) values were 126 pg/ml and 124 (33) pg/ml, respectively. The healthy infants and children had much lower plasma eotaxin levels (range = 28–95 pg/ml, median = 60 pg/ml, mean [SD] = 59.5 [18.5] pg/ml) than the previous two groups ($P=0.0001$) (Fig. 1). Within the AU group, seven children had concomitant angioedema. Their plasma eotaxin levels (median = 128 pg/ml, mean [SD] = 124 [20] pg/ml) were comparable to those of the rest of the group (median = 123 pg/ml, mean [SD] = 124 [39] pg/ml; $P=0.55$). Seven infants and children in the same group were reinvestigated for plasma eotaxin after complete remission of the attack (Table 1). Their levels declined, and the difference was statistically significant ($P=0.018$). Values after remis-

sion of AU were comparable to the control values ($P=0.29$). The duration of the urticarial attack did not correlate with the plasma eotaxin expression ($P=0.26$).

Patients (compiled altogether) whose peripheral blood absolute eosinophil count (AEC) exceeded the normal values for age ($n=18$) had plasma eotaxin levels (median = 130 pg/ml, mean [SD] = 140 [42] pg/ml) that were comparable to those of the 17 patients with normal AEC (median = 128 pg/ml, mean [SD] = 149 [61] pg/ml). Similarly, the plasma eotaxin levels did not vary with the elevation of serum total IgE above the highest normal for age. The median and mean (SD) levels were 160 pg/ml and 161 (55) pg/ml, respectively, in nine patients with normal IgE and 128 pg/ml and 138 (50) pg/ml in 26 patients with elevated IgE levels. The plasma eotaxin levels of the 35 patients could not be linked by the Pearson correlation coefficient test to their absolute eosinophil counts ($P=0.6$), serum total IgE concentrations ($P=0.1$), or IgE percentages from normal ($P=0.2$). The same findings were observed on studying the AD and AU groups separately.

The plasma eotaxin levels of the patients did not correlate with their ages, whether compiled together ($P=0.1$) or separated into the two groups, namely, AD ($P=0.3$) and AU ($P=0.4$). However, a positive correlation was elicited between the plasma eotaxin levels and the ages of the healthy control infants and children (Fig. 2).

Discussion

As a potent eosinophil selective chemoattractant, eotaxin is implicated in the causation of the tissue eosinophilia that characterizes allergic disorders. Intranasal eotaxin administration was reported to increase the number of eosinophils in nasal secretions of adults with allergic rhinitis (33). Increased expression of eotaxin was also demonstrated in the bronchoalveo-

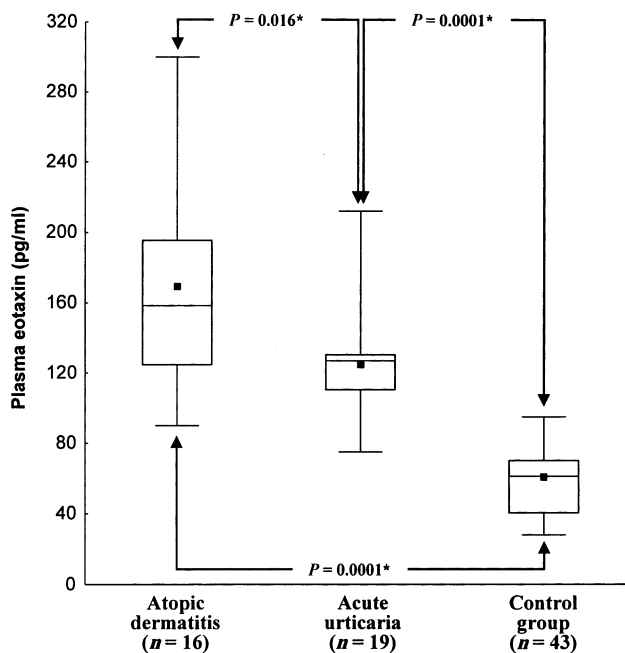


Figure 1. Plasma eotaxin levels in studied groups. Horizontal lines indicate median values and boxes enclose interquartiles (25th and 75th percentiles). Ranges are marked as maximum and minimum, and dots represent mean values. * Mann-Whitney test is used in comparisons.

lar lavage fluid (34), induced sputum (35), and plasma of adult patients with acute asthma (36). Recently, eotaxin was found to be increased in skin biopsy specimens of adults with AD (37).

This study revealed that plasma eotaxin levels were significantly increased in 16 infants and children with AD as compared to a group of 43 age- and sex-matched healthy nonatopic subjects. Not only did the AU group demonstrate elevated median and mean plasma eotaxin, but also a significant decline in the levels occurred after remission of the attack in seven patients (Table 1). It is worth mentioning that plasma eotaxin levels after remission were quite comparable to the control values. The plasma eotaxin levels were significantly higher in the AD group than in subjects with AU (Fig. 1). The difference is probably related to the chronic nature of AD lesions, which may be associated with continuous tissue eosinophilia, unlike the acute bursts of urticaria that resolve to leave a normal skin between the attacks. We did not study cases of chronic urticaria in children due to their rarity, but evaluation of their status would certainly be interesting. The presence of angioedema did not cause any further increase in the plasma eotaxin expression in patients with AU. Angioedema has the same pathogenetic mechanism as AU except that the plasma leakage has a deeper pattern (27). Although the degree of decline of plasma eotaxin in two patients with urticaria plus angioedema in the follow-up group seemed to be less than that of the rest of the group,

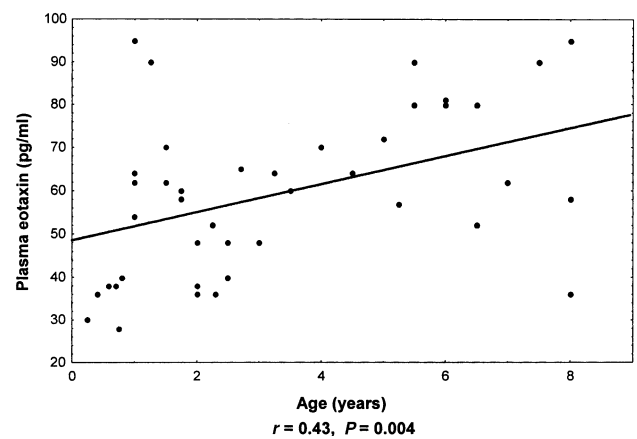


Figure 2. Positive correlation between plasma eotaxin and age in healthy infants and children (control group).

the significance of this observation is limited by the small number of subjects in each category (Table 1, patients 1 and 2).

It was previously mentioned that eotaxin may have a role in regulating the constitutive number of eosinophils in peripheral blood (3). However, the peripheral blood AEC could not be correlated to the plasma eotaxin level in our series. Patients with AD and AU who had eosinophilia were comparable to those with normal AEC as far as the eotaxin expression in their plasma was concerned. It seems that eotaxin is mainly concerned with the recruitment of eosinophils into the tissues rather than their upregulation in the blood. Moreover, blood eosinophil numbers do not always reflect the extent of eosinophil involvement in affected tissues (28). In other words, the activity and releasability of eosinophils are not always correlated with their counts.

The increased expression of plasma eotaxin in our series may reflect the movement of eosinophils into the skin and possibly their activation. The affected skin of patients suffering from AD has been found to contain deposits of eosinophil-derived proteins and sometimes intact eosinophils (15, 16). Eosinophils were also isolated from the skin wheals of patients with urticaria (29). Disruption of eosinophils and their degranulation cause severe tissue destruction and therefore may be important in the inflammatory processes underlying skin allergy. The fact that eosinophil deposits are found mainly in the dermis led to the hypothesis that dermal fibroblasts generate eosinophil attractants. Eotaxin was detected in the supernatants of long-term TNF-stimulated dermal fibroblasts (14). Eotaxin-induced dermal eosinophil influx and an increase in eosinophil peroxidase activity were demonstrated by intradermal eotaxin injection in experimental rats (38). The CC chemokines RANTES, monocyte chemoattractant protein-4 (MCP-4), and eotaxin and their receptor CCR3 were found to be increased in AD skin lesions (39, 40). Recently, Taha et al. (37) reported a close

correlation between the expression of eotaxin mRNA in the epidermis and the presence of eosinophils within the subepithelial skin region, suggesting that eotaxin may play a major role in the pathophysiologic mechanisms of AD. Eosinophilic infiltrates in the lesional skin may be causal, or these cells may have been recruited in response to injury (41). Eotaxin was postulated to have other mechanisms of action beyond chemotaxis. For instance, eosinophil activation by eotaxin was found to prime the production of reactive oxygen species from eosinophils (42). Eotaxin was also found to cooperate with substance P in inducing eosinophil-derived neurotoxin release (43). HayGlass et al. (44) stated that the broadened understanding that chemokines function more than as "traffic directors" has several implications for allergic diseases.

Plasma eotaxin levels in our series could not be correlated to the serum total IgE concentrations or percentage values. Moreover, patients considered to have elevated IgE levels were comparable to those with normal IgE levels in terms of plasma eotaxin expression. However, the number of cases in the normal IgE category was too small to justify a conclusion. Although the plasma eotaxin levels were somewhat positively correlated ($r=0.43$) to the ages of the healthy infants and children (Fig. 2), a similar relation was not observed in the patients. Probably, more influential factors operate in the allergic state such as disease activity or severity. This was particularly evident in the three infants, 2–4 months old, enrolled in the group of AD with oozing, itchy, eczematous facial lesions. Their plasma eotaxin levels (225, 190, and 108 pg/ml) were obviously much higher than those of six age-matched infants in the control group (range 28–40 pg/ml). Disease activity seemed to be the main determinant of eotaxin status in their case. It also seems that eotaxin, unlike many other biomarkers of the allergic response, can reach high levels in early infancy, and its value in the early diagnosis or prediction of allergy could be worthwhile. Moreover, the observation that eotaxin

levels increase in normal subjects with age deserves attention, as it may point to an effect of age or body size on eotaxin expression. However, this finding needs more extensive evaluation, as the number of healthy subjects in our series was relatively small.

The plasma levels in our allergic patients were generally lower than those observed by Lilly et al. (36) in adults with bronchial asthma. The geometric means in cases of acute and stable asthma in their series were 520 and 350 pg/ml, respectively. The mean value of our AD group was 168 pg/ml, and that of the children with AU was 124 pg/ml. The difference could be disease-related or, more probably, age-related. We found no published data on normal or pathologic eotaxin levels in plasma or other biologic fluids of infants or children. However, the much lower mean plasma eotaxin in our 43 control subjects (59.5 pg/ml) allowed interpretation of the results.

This pilot study suggests that plasma eotaxin expression is increased in the chronic eczematous lesions of AD. It was also found to increase during the flares of AU and angioedema and to decline after remission. The plasma levels could not be related to the peripheral blood eosinophil counts. The findings are limited by the relatively small number of subjects in each group. We recommend larger studies on the variations in plasma eotaxin in relation to the severity scores of AD and the conventional therapeutic modalities used. Follow-up studies of eotaxin status in allergy-prone neonates may be of value. Setting pediatric reference ranges for eotaxin levels in biologic fluids would also certainly aid research and investigational purposes.

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