

Influence of age and gender on serum eotaxin concentration in healthy and allergic people

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Summary. *Background:* Eotaxin is one of the important chemokines that modulate allergic inflammation. In many studies a correlation between an elevated serum concentration of eotaxin, allergen exposure and allergic symptoms has been confirmed. Influence of other factors on eotaxin concentration is feebly recognized. We made an attempt to assess the influence of age and gender on the serum eotaxin level in healthy people and in patients with intermittent IgE-mediated rhinoconjunctivitis (AR).

Methods: The serum eotaxin level was measured in 245 healthy people and 241 patients with AR before the pollen season with the ELISA technique (KITS, R&D USA, pg/ml). The parametric tests and linear regression analysis were used in statistical calculations.

Results: There were no differences between the allergic group and the healthy one in the mean age (accordingly: 31.3 ± 11.6 yrs. vs. 31.6 ± 12.5 yrs.; $p=0.1$) and the mean serum eotaxin content (118.1 ± 44.9 pg/ml vs. 116.3 ± 34.8 pg/ml; $p=0.3$). A significant relationship between the serum eotaxin level, gender and age was revealed in both groups and regression models were derived. A linear correlation between age (semi-partial correlation $\beta = 0.47$, $p = 0.0000001$) and gender (semi-partial correlation $\beta = 0.3$, $p = 0.0000001$), on the one side, and the serum eotaxin level, on the other, was found for the allergic people. In the control group a similar relationship between the serum eotaxin level and age (semi-partial correlation coefficient $\beta = 0.63$, $p = 0.0000001$) and gender (semi-partial correlation factor $\beta = 0.23$, $p = 0.000006$) was observed.

Conclusions: Age and sex significantly influence the serum eotaxin content in healthy people and patients with IgE-mediated rhinoconjunctivitis.

Key words: eotaxin, allergy, allergic inflammation, CC-chemokines

Introduction

Eotaxin is a unique selective chemokine, that has been implicated in the pathogenesis of allergic inflammation [1-3]. In many studies a correlation between an elevated concentration of the serum eotaxin and allergen exposure has been confirmed [4,5]. On the other hand, the influence of other factors on eotaxin concentration is feebly recognized. Taking into account reports on sex-related differences and age-connected modifications in allergic and immune response, we made an attempt to assess the influence of age and sex on the serum eotaxin level in healthy people and patients with IgE-mediated intermittent allergic rhinoconjunctivitis.

Methods

Two hundred and forty one patients with at least a two-year positive history of intermittent IgE-mediated rhinoconjunctivitis due to tree or grass/cereal pollen were recruited (Table 1). Sensitization to tree or grass/cereal pollen in each case was confirmed by positive skin prick tests according to Nordic standardization (class ++ or more, Allergopharma, Germany) and a detectable level of specific IgE (≥ 0.35 kU/l, Pharmacia CAP, Uppsala, Sweden) [6]. None of the patients had symptoms of allergic rhinoconjunctivitis. None of them had taken immunosuppressive or anti-histamine drugs during the last four weeks preceding the recruitment;

none had received immunotherapy during the last year. The control group consisted of 245 healthy people with a negative history of allergy, confirmed by negative results of skin prick tests (less than class ++, Allergopharma, Germany) and the normal level of total serum IgE (less than 85 kU/l, Pharmacia CAP, Uppsala, Sweden). None of the participants had ever suffered from immunodeficiencies, malignancies or psychiatric diseases. Subjects from both groups were non-smokers and had not had any parasitic diseases or respiratory tract infection during three months before the study. All females included into the study were pre-menopausal (understood as presence of menstrual bleeding during last month), not pregnant or breast-feeding and without hormonal therapy. For all male participants, the age of 65 years was arbitrarily adopted as an upper age-limit of inclusion.

In order to eliminate the influence of aeroallergens on the serum eotaxin level in people with rhinoconjunctivitis all the measurements were done beyond the pollen season. Two blood samples (at 2 days intervals) for evaluation of the eotaxin concentration were obtained from each of the subjects. Blood drawing, handling and storage were performed by the same researcher. During 30 minutes after drawing, the blood samples were centrifuged at 1300 x g at 4°C for 10 min., and then they were kept frozen at -70°C until eotaxin measurements. Levels of serum eotaxin were measured by ELISA method (Quantikine, Human Eotaxin/CCL11 Immunoassay, pg/ml, R&D kits, USA). The detection threshold of the method is 5.0 pg/ml [7]. The mean value of the eotaxin level was calculated for each subject from two serum samples. The coefficient of variation was calculated for evaluation of inter-assay precision of the eotaxin measurements.

The study protocol was approved by the Ethics

Committee of the Military University School of Medicine, Warsaw, Poland, and written informed consent was obtained from all participants.

After the assessment of normality distribution (Kolmogorov-Smirnov test) quantitative data were analyzed by the ANOVA or t-test for non-paired parameters. For *post hoc* comparison the Newman-Keul test was used. A multiple linear regression model was established for influence of age and sex on the serum level of eotaxin in the allergic and healthy subjects. P values below 0.05 were considered significant.

Results

There were no significant differences between the allergic group and the healthy one in the mean age and the serum eotaxin level (Table 1). The coefficient of variation of the serum eotaxin analysis was 12.7%.

In order to assess the relationship between age and the serum eotaxin level, the subjects from both groups were arbitrarily divided into 3 age-subgroups: below 25 years of life, 25-34 years of life and above 34 years of life (Table 2). The comparative analysis revealed that younger people from the allergic and healthy groups had a significantly lower serum eotaxin level than older participants.

Moreover, it was observed that the healthy and allergic females, irrespective of age, had a significantly lower serum eotaxin level than the males from the corresponding groups (Table 3).

A multiple linear regression model was derived for both the healthy and allergic subjects to establish the relationship between the serum eotaxin level, age and sex in the healthy people and the patients with Ig-E mediated rhinoconjunctivitis.

Table 1. Mean values of age and serum eotaxin level in allergic and healthy people (ANOVA test, differences are significant with $p < 0.05$).

Group	Age (yrs); mean (SD*)	Eotaxin level (pg/ml); mean (SD)
ALLERGIC		
All (n [#] = 241)	31.4 (11.6)	118.1 (44.9)
Birch (n = 79)	28.9 (10.7)	121.7 (46.8)
Hazel/alder (n = 54)	31.5 (9.7)	109.3 (46.6)
Grasses/cereals (n = 108)	33.1 (12.8)	119.9 (42.4)
CONTROL (n = 245)	31.6 (12.5)	116.3 (34.8)
	p = 0.1	p = 0.3

SD* - standard deviation, n[#] - number of subjects

Table 2. Sex-and age-related differences in mean serum eotaxin level (ANOVA results, differences are significant with $p < 0.05$).

Group		<25 years	25-34 years	>34 years	p
ALLERGIC					
All		n = 85	n = 76	n = 80	
age	mean (SD) (years)	20.1 (3.4)	29.3 (3.0)	45.3 (7.1)	
eotaxin level	mean (SD) (pg/ml)	98.3 (44.1) [^]	114.4 (39.5) [*]	142.7 (39.2) [#]	[^] vs [*] = 0.02 [^] vs [#] = 0.00002 [*] vs [#] = 0.00002
Females					
		n = 43	n = 40	n = 40	
age	mean (SD) (years)	19.5 (3.3)	29.3 (3.4)	45.7 (6.4)	
eotaxin level	mean (SD) (pg/ml)	82.4 (37.0) [^]	100.8 (31.8) [*]	133.0 (36.2) [#]	[^] vs [*] = 0.02 [^] vs [#] = 0.0001 [*] vs [#] = 0.0002
Males					
		n = 42	n = 36	n = 40	
age	mean (SD) (years)	20.6 (3.4)	29.3 (2.6)	45.0 (7.6)	
eotaxin level	mean (SD) (pg/ml)	114.6 (45.2) [^]	129.4 (42.1) [*]	152.5 (40.0) [#]	[^] vs [*] = 0.1 [^] vs [#] = 0.0005 [*] vs [#] = 0.02
CONTROL					
All		n = 82	n = 81	n = 82	
age	mean (SD) (years)	19.2 (2.7)	29.2 (3.1)	46.5 (8.0)	
eotaxin level	mean (SD) (pg/ml)	93.8 (26.1) [^]	115.1 (32.7) [*]	139.9 (29.0) [#]	[^] vs [*] = 0.00001 [^] vs [#] = 0.00002 [*] vs [#] = 0.000009
Females					
		n = 41	n = 41	n = 44	
age	mean (SD) (years)	18.7 (2.8)	28.6 (3.4)	45.3 (7.6)	
eotaxin level	mean (SD) (pg/ml)	83.4 (26.1) [^]	106.6 (35.0) [*]	131.3 (26.3) [#]	[^] vs [*] = 0.0003 [^] vs [#] = 0.00002 [*] vs [#] = 0.0001
Males					
		n = 41	n = 40	n = 38	
age	mean (SD) (years)	19.7 (2.7)	29.9 (2.7)	47.9 (8.3)	
eotaxin level	mean (SD) (pg/ml)	104.3 (21.7) [^]	123.8 (28.0) [*]	149.8 (29.1) [#]	[^] vs [*] = 0.001 [^] vs [#] = 0.0001 [*] vs [#] = 0.0001

Table 3. Mean values of age and serum eotaxin level in gender subgroups (Results of t-test for non-paired variables, differences are significant with $p < 0.05$).

Group			Females	Males	p value
ALLERGIC		n = 241	n = 123	n = 118	
age	mean (SD)	(years)	31.2 (11.8)	31.5 (SD 11.5)	p = 0.8
eotaxin level	mean (SD)	(pg/ml)	104.8 (40.7)	132.0 (SD 45.1)	p = 0.000002
CONTROL		n = 245	n = 126	n = 119	
age	mean (SD)	(years)	31.2 (12.2)	32.4 (SD 12.8)	p = 0.6
eotaxin level	mean (SD)	(pg/ml)	107.7 (35.2)	125.4 (SD 32.1)	p = 0.00005

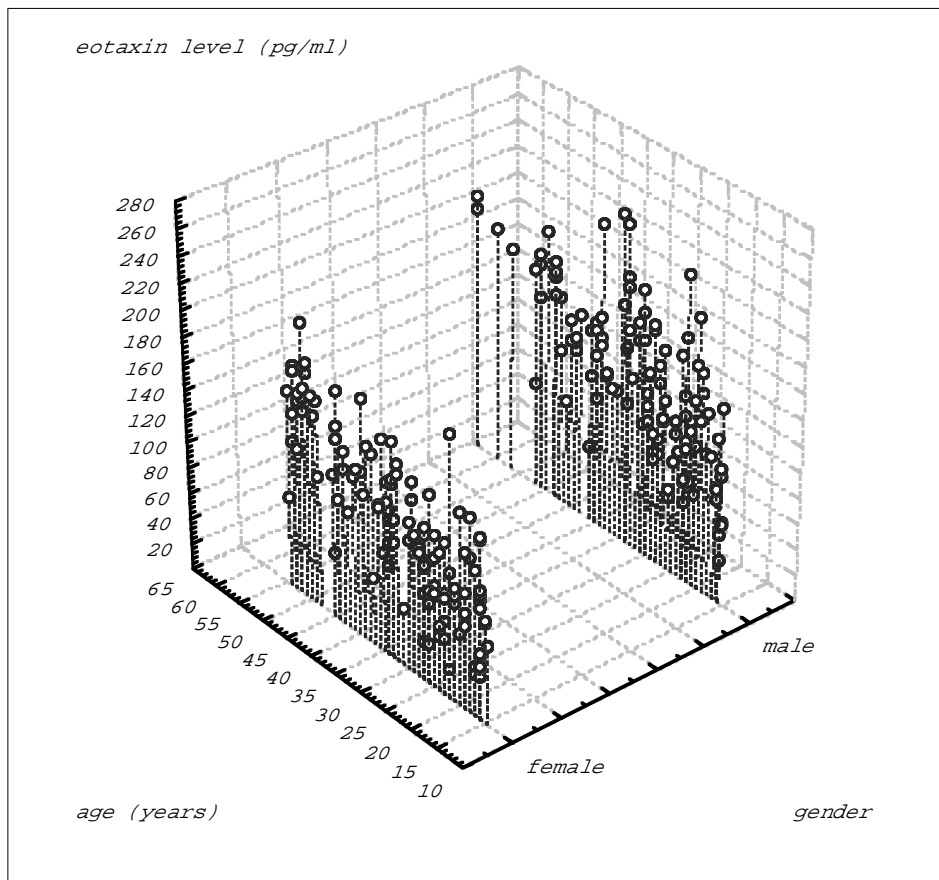


Figure 1. Regression model describing influence of age and sex on serum eotaxin level in patients with Ig-E mediated rhinoconjunctivitis (SE - standard error, SEE - standard error estimation).

$$\text{EOTAXIN} = 21.5 \text{ (10.0) } + 1.8 \text{ (0.2) } \times \text{AGE} + 26.4 \text{ (4.8) } \times \text{GENDER} \pm 37.5 \text{ (SE)}$$

(R = 0.56, $p < 0.000001$).

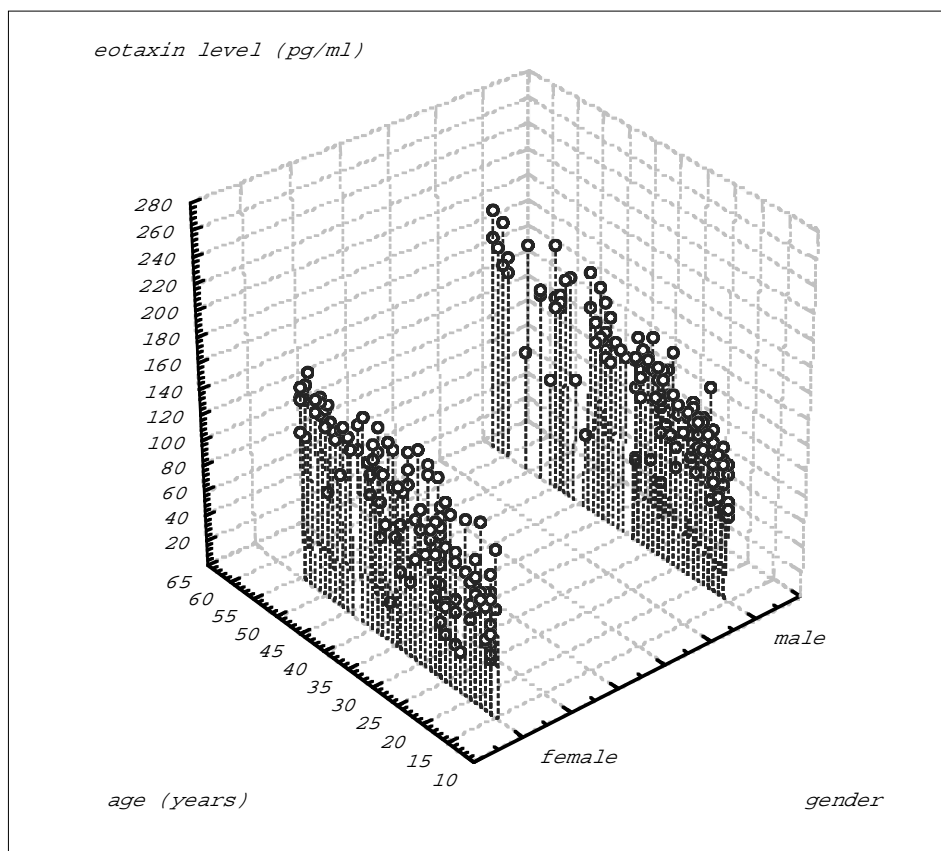
A linear correlation between age (semi-partial correlation coefficient $\beta = 0.47$, $p = 0.0000001$) and gender (semi-partial correlation coefficient $\beta = 0.3$, $p = 0.0000001$) on the one side and the serum eotaxin level, on the other, was found in the allergic group. A multiple regression coefficient was well marked (R = 0.56) and distinctly significant ($p = 0.0000001$). The final

regression model for the allergic group is shown by Figure 1.

In the healthy control group a linear correlation between age and eotaxin level (semi-partial correlation coefficient $\beta = 0.58$, $p = 0.0000001$) and gender and eotaxin level (semi-partial correlation factor $\beta = 0.23$, $p = 0.000006$) was also observed (Figure 2).

Figure 2. Regression model describing influence of age and sex on serum eotaxin level in healthy people (SE - standard error, SEE - standard error estimation).

$$\begin{aligned} \text{EOTAXIN} &= 40.7 (6.9) + 1.6 \\ & (0.14) \times \text{AGE} + 16.2 (3.5) \times \\ & \text{SEE} \\ & \text{GENDER} \pm 27.0 \\ (R = 0.63, p < 0.000001). \end{aligned}$$



Discussion

A late inflammatory response characterized by an influx of inflammatory cells such as eosinophils and lymphocytes after exposure to allergen is one of the important phenomena that contribute to the allergic process. As an eosinophil selective chemoattractant, eotaxin is a significant contributor of a late-phase of allergic response [7-10]. Increased mucosal output of eotaxin was demonstrated after allergen exposure in patients with allergic seasonal rhinitis [11].

The published data concerning variations of serum eotaxin levels in subjects with allergic diseases is scarce. It was documented that plasma levels of eotaxin increase in active atopic dermatitis and acute urticaria [12]. It was also revealed that bronchial asthma incidence increases with the higher plasma eotaxin level [13]. Other findings indicate that the plasma eotaxin concentration is significantly higher in asthmatics seeking emergency treatment for exacerbations of symptoms than in control subjects, with stable disease and suggest that plasma eotaxin levels are directly related to the asthma severity [14]. It is well known that age has been implicated in the prevalence and severity of allergic inflammation [15]. Hossny et al. [12] found a positive correlation of serum eotaxin levels and age in healthy infants and children, however they did not find

a similar relationship in children with active atopic dermatitis or acute urticaria. A significant linear correlation between serum eotaxin levels and age of healthy people and patients with non-symptomatic IgE-mediated rhinoconjunctivitis was revealed in this study. Gender was also revealed to similarly influence the serum eotaxin level in allergic and healthy people. Current evidence does not allow identification of the responsible sex hormone or hormones or changes in hormone levels, which are most important for modulation of concentration of serum eotaxin. Both predominating female sex hormones (estradiol and progesterone) modulate the synthesis, release, and action of cytokines involved in immune responses, such as interferon-gamma (IFN), interleukins (IL -1, -4, -5, -6) and many others [16,17]. Some of these cytokines cause upregulation or downregulation of eotaxin expression [18]. A few years ago it was observed that TNF and cytokines derived from Th₂ lymphocytes (i.e. IL-4, IL-10) can induce eotaxin production, while Th₁-type cytokines (i.e. IFN) have an inhibitory effect on it [19]. In several *in vitro* studies, increasing effect of estradiol and progesterone on Th₂-type lymphocyte cytokines release, on the one hand, and suppressing influence of androgens on Th₁-type cytokines production, on the other, was noted [16,17,20,21]. It was also proved that beta-estradiol and progesterone (opposite to testosterone) enhance eosinophil adhesion to human

mucosal endothelial cells and can induce degranulation of eosinophils [22]. So, finding an immune mechanism that explains why males have higher serum eotaxin levels than females, will be very complicated.

Summarising, this study revealed that age and sex are important determinant factors of the serum content of eotaxin in healthy people and patients with IgE-mediated intermittent rhinoconjunctivitis. The mechanism of sex-related differences in the serum concentration of eotaxin is unclear and should be elucidated in the future.

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