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Serum eosinophil cationic protein (S-ECP) in a population with low prevalence of atopy

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Abstract The study is a part of the European Community Respiratory Health Survey. A random sample (n=351) of 20–44-year olds and persons of the same age with asthma-like symptoms or current asthma medication according to a postal questionnaire (n=95) were studied. Interview was taken, methacholine challenge was done and ECP, total and specific IgE were measured from serum. The median S-ECP value was 8.0 μg/l in the random sample. The geometric mean of S-ECP was higher in subjects with, than without atopy (10.2. *vs* 8.9 μg/l, $P<0.01$) and in subjects with bronchial hyperresponsiveness (BHR) than in subjects without BHR (9.9 *vs* 8.0 μg/l, $P<0.01$). The levels correlated weakly to forced expiratory volume in one second (FEV_1) (r=0.13, $P<0.01$) and were not independently correlated with respiratory symptoms, asthma or FEV_1 after adjusting for BHR, IgE, sensitisation and smoking. Our results indicate that the level of eosinophil activation is low in a population with a low prevalence of atopy, even when BHR is common.

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Keywords S-ECP; epidemiology; asthma; bronchial hyperresponsiveness; atopy.

INTRODUCTION

In the last two decades, the conception of asthma has changed from regarding it as a disease of the bronchial muscles to an inflammatory airway disease (1). Epidemiological asthma research has, however, usually been based on questionnaires and lung function data, while methods for assessing airway inflammation have only been used to a limited degree (2).

The number of eosinophil granulocytes in peripheral blood (B-Eos) is traditionally the most common method for assessing inflammation in epidemiological research (3–7). More recently, measurements of eosinophil degranulation products, such as eosinophil cationic protein (ECP) in peripheral blood has been introduced as a method to assess and monitor inflammation in asthmatic patients (8–10). There is, however, limited experience from epidemiological research and that only from Scandinavia (11,12).

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Furthermore, difficulties in defining asthma in epidemiological studies have enforced to find objective measures with relative stability over time. Bronchial hyperresponsiveness (BHR) has mostly been used as an objective marker of asthma in epidemiological studies. Generally, the prevalence of BHR parallels that of self-reported asthma and wheeze (13,14). Previous analyses of population samples in Estonia have, however, led to contradictory results in low prevalence of self-reported asthma and atopy contrasting to high prevalence of BHR and respiratory symptoms including wheeze both in children (15,16) and in adults (17,18). These results could reflect either low awareness with concurrent high prevalence of nonatopic asthma or high prevalence of other respiratory conditions resulting in high prevalence of BHR in this region. In this context, high S-ECP levels in the population would support the first and low levels the latter hypothesis.

The aim of this investigation was to describe the distribution of S-ECP in a population with a low prevalence of atopy and allergic respiratory disorders and to study the relationship between levels of S-ECP and variables related to asthma and allergy.

MATERIAL AND METHODS

Study areas and subjects

The subjects were enrolled as part of the European Community Respiratory Health Survey (ECRHS)(19). In the first phase of the study, a questionnaire including seven questions on asthma-related symptoms was mailed to 3000 individuals between 20 and 44 years of age, with equal number from either sex(20). The study population was randomly selected from the Civil Register. To avoid linguistic problems, only people of Estonian nationality were selected. From this cohort, a further random sample of 800 persons was selected to the clinical phase of the study. Only individuals who had answered to the mailed questionnaire were invited to participate (n=723). In order to enrich the sample with symptomatic individuals, all persons from the original study group who reported use of asthma medication, attacks of asthma, or awakening because of shortness of breath and who were not already included in the random sample were invited to participate (n=197).

Questionnaire

The ECRHS questionnaire was used both for screening and interview (19–21). The questionnaires were translated from English into Estonian and then back-translated into English by an independent person. The interview included questions on symptoms, diagnoses, smoking history, parental smoking history, family structure, family history, early environment, education, employment, home environment, diet, use of medicines, attitudes to medicines and the use of health services.

S-ECP

The blood samples that were collected for ECP measurements were kept at 24°C for 60 min before they were centrifuged. Sera were stored at −20°C. The concentration of S-ECP was assayed with a double antibody radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden).

Serum IgE levels and circulating IgE antibodies

Serum IgE levels and IgE antibodies against *D. pteronyssinus*, timothy, birch, cat and *Cladosporium* were determined by Pharmacia CAP™ system (Pharmacia Diagnostics, Uppsala, Sweden).

Spirometry and methacholine challenge

Forced expiratory volume in one second (FEV_1) was measured with Jäeger Flowscreen (Erich JÄEGER GmbH & CoKG, Würzburg, Germany). The predicted value was calculated for each subject(22). In this analysis, FEV_1 was expressed as a percentage of the predicted value. Methacholine challenge was carried out using a dosimeter Spira Elektro 2 (Respiratory Care Centre, Hämeenlinna, Finland). The results were expressed as slope using the method described by Chinn *et al*. (13). In addition, subjects with a fall of FEV_1 by at least 20% with an accumulated methacholine dose of 2 mg was categorised as BHR.

Definitions

Atopy was defined as the presence of serum IgE antibodies $\geq$0.35 kU/l against at least one allergen.

Wheeze was defined as answering "yes" to the following question: "Have you had wheezing or whistling in your chest at any time in the last 12 months?"

Asthma was defined as an affirmative answer to the question: "Have you ever had asthma?"

Allergic rhinitis was defined as a positive answer to the question: "Do you have any nasal allergies, including hay fever?"

Skin allergy was defined as answering "yes" to the question: "Have you ever had eczema or any kind of skin allergy?"

Ethical considerations

All participants gave their informed consent. The study was approved by the Ethics Committee of Tartu University.

Statistical analysis

The statistical analysis was performed with the StatView 5 software package (SAS Institute Inc, Cary, NC, U.S.A.). Chi-square- and t-tests were used to analyse differences between groups. Linear regression was used to correlate two continuous variables and logistic and multiple linear regression for multiple variables. Log-transformation was performed to achieve a normal distribution of S-ECP and S-IgE. S-ECP values are presented as geometric mean ($\pm$SD). A P-value $<$0.05 was regarded as statistically significant.

RESULTS

S-ECP was obtained from 351 subjects from the random sample and 95 subjects from the enriched sample. The characteristics of subjects from the random sample is presented in Table 1. When the symptomatic sample was also included the prevalence of atopy, BHR and asthma increased to 22, 22 and 4%, respectively.

Table 1. Characteristics of the random population (%) and mean (range) in the clinical phase of ECRHS in Tartu, Estonia

	Random population (n=351)
Women	55.7
Age	32 (21–45)
Current smokers	35.0
Atopy	19.1
BHR	18.6
Self-reported asthma	2.0
Nasal allergy	16.7
Skin allergy	45.6
Wheeze	21.8
FEV_1 (% predicted)	108 (58–157)

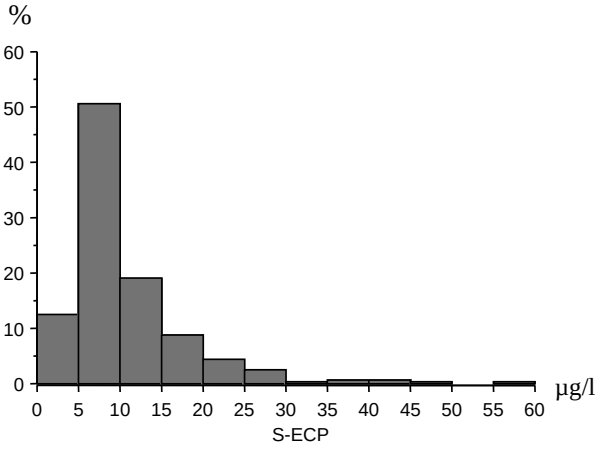

Fig. 1. Distribution of S-ECP levels in a random population sample of 20–44 year olds in Tartu, Estonia.

The prevalence of self-reported nasal allergies in the postal questionnaire was higher among persons subject to S-ECP measurements than among those who did not participate in the clinical phase of the study (19 *vs* 10%; $P<0.01$). Respiratory symptoms associated with wheezing and night cough were also more prevalent among participants in the second phase of the study.

The S-ECP levels in the random population sample were clearly skewed, with a median value of 8.0 µg/l and an interquartile range of 6.0–12.0 µg/l (Fig. 1). The levels were not significantly related to gender or age but they were significantly higher in current smokers than in non-smokers (9.0 (4.8–16.9) *vs* 7.9 (4.6–13.6) µg/l, $P<0.05$).

Further, the S-ECP levels were significantly higher in atopic than in non-atopic subjects (10.2 (5.8–17.0) *vs* 8.2 (4.6–14.9) µg/l, $P<0.01$) and higher in subjects with, than without BHR (9.9 (5.6–17.4) *vs* 8.0 (4.5–14.3) µg/l, $P<0.01$). S-ECP also correlated weakly with the IgE levels (r=0.19, $P<0.001$) and with bronchial responsiveness (r=0.11, $P<0.05$). Furthermore, the S-ECP levels were higher in subjects with self-reported asthma than in subjects without asthma and in wheezing as compared to non-wheezing subjects (10.6 (6.2–18.2) *vs* 8.0 (4.4–14.4) µg/l, $P<0.001$), while there was no relationship between S-ECP and either nasal or skin allergy (Fig. 2).

The specificity and sensitivity of an S-ECP value above the interquartile range (12.0 µg/l) for atopy were 32 and 80%, respectively, for BHR 30 and 80%, for self-reported asthma 63 and 97% and for wheeze 36 and 76%.

Logistic regression analysis revealed that wheeze was independently related to atopy, BHR and current smoking, while asthma was independently related to atopy. No independent significant correlation was found between S-ECP above the 75% interquartile range (12.0 µg/l) and wheeze or asthma (Table 2). Expressing S-ECP as a continuous variable did not change the result of the analysis.

Although S-ECP was significantly related to FEV_1 in linear regression (r=−0.13, $P<0.01$), this correlation did not remain significant after adjusting for age, gender, smoking, total IgE and bronchial responsiveness (partial r-value = −0.05, P=0.32).

DISCUSSION

The main finding in this population study is that although S-ECP correlated to self-reported asthma, atopy, BHR, asthma symptoms and lung function, it had no independent predictive value when other asthma-related variables were taken into account.

Tobacco smoking was associated with elevated S-ECP levels which concords to a previous study (23). Epidemiological studies indicate that high blood eosinophil counts may be independently related to the decline in FEV_1 (7,24). We were unable to confirm any independent relationship between FEV_1 and S-ECP.

The results correspond with those of a previous Swedish study using an identical protocol. The S-ECP levels were, however, considerably lower in Tartu than those in Sweden (11). The median S-ECP level in Uppsala, Sweden was 11.9 µg/l compared to 8.0 µg/l in Tartu. Although the analyses were done in different laboratories, the protocol for S-ECP measurements was identical in the two studies. We cannot entirely exclude technical differences since in Swedish study the blood was allowed to coagulate at room temperature before centrifugation. This would, however, not change our results of lower levels of S-ECP in Tartu, as the level of ECP rises with increasing temperatures and the room temperature is generally lower than 24°C, thus diminishing the difference that we revealed (25). It is therefore more likely that the results reflect a difference in eosinophil activation between these two populations.

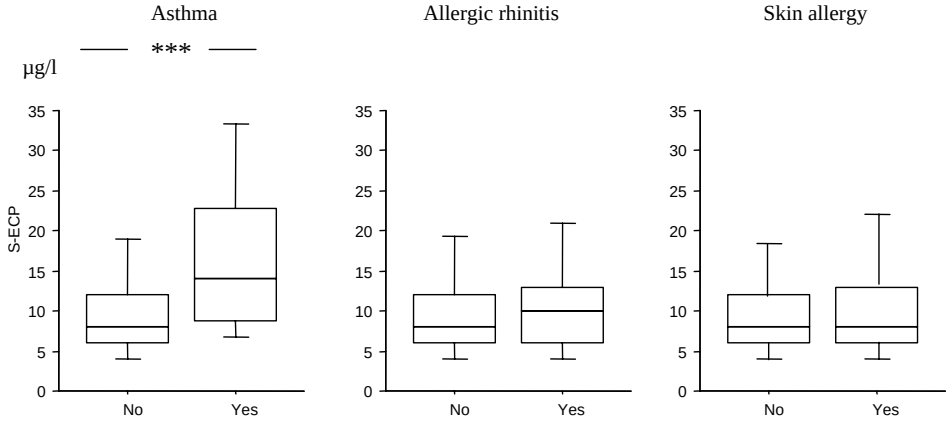

Fig. 2. Levels of S-ECP in subjects with and without self-reported asthma, nasal allergy and skin allergy. The box plots show the values of median, interquartile range and the 10th (⊥) and the 90th percentiles (T).

Table 2. Independent relationships between asthma symptoms, S-ECP, atopy, BHR and smoking, as adjusted for age and gender (OR and 95% CI).

	Wheeze	Asthma
S-ECP above 12 µg/l	1.4 (0.7–2.7)	2.4 (0.5–11)
Atopy	3.8 (1.9–7.3)	8.6 (1.8–41)
BHR	2.5 (1.3–5.0)	3.9 (0.9–18)
Current smoking	3.5 (1.9–6.6)	0.5 (0.1–2.3)

The lack of gold standard in epidemiological diagnosis of asthma abates the possibility to estimate the value of any certain parameter in measuring asthma in epidemiological studies. Furthermore, atopy, elevated S-ECP and BHR may be on causal pathway to asthma: atopic sensitisation being the prerequisite to allergic inflammation, the latter marked by elevated S-ECP. Activation of eosinophils in its turn can result in increased BHR. It is therefore not surprising that after controlling for the effect of atopy and BHR, S-ECP no longer remains associated with asthma symptoms and self-reported asthma. Thus, inflammatory markers such as S-ECP, despite not having independent predictive value for asthma, can be useful in international asthma surveys, particularly, when comparing populations with large differences in the prevalence of respiratory diseases other than asthma which are associated with BHR. Determination of S-ECP levels may also be useful when comparing populations with high and low awareness of asthma. Previous analyses of the study group has revealed high prevalence of BHR (18) and respiratory symptoms, including wheeze (20), while the prevalence of atopy, diagnosed asthma and asthma treatment were low as compared to other centres participating in ECRHS (17,20,21). The corresponding prevalence of atopy in Uppsala, Sweden was 32.3% (21) and Tartu had the second lowest prevalence of atopy among all the centres in the ECRHS (26). The low prevalence of eosinophil activation would support our previous suggestions that the prevalence of asthma is low in Estonia (20,21).

We conclude that in this Estonian population sample S-ECP was elevated in subjects with either atopy or BHR, but the levels were not related with asthma symptoms or lung function when other asthma-related variables were controlled for. Our results indicate that the level of eosinophil activation is low in a population with a low prevalence of atopy, even when bronchial hyperresponsiveness is common.

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