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Original article

Urinary eosinophil protein X in children: the relationship to asthma and atopy and normal values

Background: In epidemiologic studies, it may be difficult to identify children with bronchial asthma. Since this is the most common chronic respiratory disease in childhood, and its prevalence is still increasing, reliable methods for identification of asthmatic children are required. This study evaluates the use of urinary eosinophil protein X (U-EPX) in epidemiologic studies in identifying atopic and asthmatic children.
Methods: U-EPX was measured in 877 Austrian schoolchildren. The skin prick test (SPT) was performed with eight common aeroallergens, and established questionnaires were used to assess respiratory symptoms.
Results: Of our cohort, 2.8% reported physician-diagnosed asthma, 5.1% reported wheezing within the last 12 months, and 24.1% were found to be atopic. In children with physician-diagnosed asthma, as well as in atopic children (positive SPT), median U-EPX levels were significantly higher than in healthy subjects (142.8 and 89.6 *vs* 63.9 μg/mmol creatinine, $P<0.0001$ and $P<0.0001$, respectively). In addition, perennial sensitization to inhalant allergens resulted in higher U-EPX levels than did seasonal sensitization. The odds ratio for U-EPX levels over the 90th percentile was significantly elevated for asthma, for wheezing, for nocturnal cough, and for breathlessness at exercise, as well as for seasonal and perennial sensitization. Pulmonary function was negatively related to U-EPX levels.
Conclusions: Measurement of U-EPX, which can be obtained easily, may be helpful in diagnosing both asthma and atopy in children. However, there is a great overlap between controls and symptomatics, a fact which reduces the sensitivity of U-EPX in determination of the prevalence of asthma in epidemiologic studies.

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Numerous epidemiologic studies in different countries have demonstrated that bronchial asthma is now the most common chronic disease in children. A prevalence of 3–8% has been assumed (1–4), whereas the prevalence of self-reported asthma symptoms is as high as 36.8% (5). In addition, the prevalence of asthma has continued to rise within the last decade (6).

Bronchial asthma is defined as chronic or recurrent wheeze and cough, bronchial hyperresponsiveness, and airway inflammation (7). In epidemiologic studies, recurrent wheeze can be evaluated by questionnaires, which may have the disadvantage of not being objective. In addition, in comparison of epidemiologic studies from different countries, the different languages used can influence results, a fact which led to the development of video questionnaires (5). The testing of bronchial hyperresponsiveness, a quite common instrument in epidemiologic studies, is time-consuming, and is not specific for bronchial asthma only, and a great overlap between asthmatics and healthy control subjects has been demonstrated in most of the studies (8–10). In clinical studies, it has been demonstrated that markers of eosinophil activation, such as eosinophil cationic protein (ECP) or eosinophil protein X (EPX), are increased in childhood asthma (11–13). Furthermore, they are related to disease activity and are assumed to be helpful in monitoring treatment. They can be determined in serum, nasal lavage fluid, sputum, and, as recently shown, in urine (14, 15). Urinary EPX (U-EPX) can be obtained easily and in a noninvasive way in children of all ages. Therefore, we investigated whether measurement of U-EPX can be used in epidemiologic studies to detect bronchial asthma and atopy in children. In addition, we wanted to establish normal values.

Material and methods

Study design and subjects

A cross-sectional study was carried out in 877 school-

children (mean age 11 years, range 10–12). They were visited in their schools for spirometry, urine sample, and skin prick test (SPT) on one day. The participants' parents were asked to fill out a questionnaire and to return it by mail. All of the children were members of a cohort of a longitudinal study dealing with lung growth, as described elsewhere (16). This investigation took place in September and October 1997 from 0800 to 1300. Informed consent was obtained from the parents, and the study protocol was approved by Vienna University's ethics committee.

Urine analysis

Urine was collected from each child as a spot sample. Whenever leukocytes in urine samples were detected by urine sticks, these samples were withdrawn. Urine was transported into our laboratory in a cooled box, and was frozen at $-30^{\circ}C$ immediately after arrival. The mean lag time between sampling and freezing was 3 h. Urine samples were diluted 11 times in a phosphate buffer containing 0.15 mol/l NaCl, 1% bovine serum albumin, 0.1% Tween-20, 10 mmol/l ethylenediaminetetraacetic acid, and 0.2% *N*-cetyltrimethylammonium bromide, as previously described (14). Urinary EPX was measured with a sensitive and specific radioimmunoassay (Pharmacia & Upjohn AB, Uppsala, Sweden). Results were expressed as μg EPX/mmol creatinine (CREA).

SPT

SPT was performed on the volar forearm of 784 children with extracts of seven common aeroallergens (*Dermatophagoides farinae*, *D. pteronyssinus*, cat, dog, grass pollen mixture, hazel, and birch (ALK Abelló, Hørsholm, Denmark). A negative control (saline) and a positive control (histamine 10 mg/ml) were included. Wheals were considered positive if they had a diameter of at least 3 mm. In none of the cases did the negative control give a wheal reaction.

Spirometry

Pulmonary function tests were performed on 877 participants as follows. Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), and maximum expiratory flow at 50% vital capacity (MEF_{50}) were recorded in the form of a maximum flow volume curve (Jaeger, Germany) according to the American Thoracic Society standard (17). The best of two efforts was used for calculations.

Questionnaire

To evaluate respiratory symptoms within the last 12 months, parents were asked to complete a modified questionnaire of the respiratory part of the International Study on Asthma and Allergy in Childhood (ISAAC) (4). The presence of wheeze, shortness of breath, cough apart from colds, nocturnal cough, and cough during exercise, as well as the use of medication 5 days before testing, were assessed. The questionnaires were handed out to the children to be filled out by their parents.

Statistical analysis

In view of the skewed distribution of urinary EPX, we used the nonparametric Wilcoxon rank-sum test to determine whether various strata were significantly different or not. Logistic regression models on the log-scaled variable were calculated to derive estimators for odds ratios, confidence intervals, sensitivity, and specificity. All statistical analyses were carried out with the SAS package, release 6.11.

Results

Normal values for U-EPX

Children who reported no respiratory symptoms within the last year and who had a negative skin prick test were assumed to be healthy. In these subjects ($n = 580$), the median U-EPX level was 63.6 μg/mmol CREA, 10th percentile was 28.76 μg/mmol CREA, and 90th percentile was 140.96 μg/mmol CRE. Boys had significantly higher values than girls ($P < 0.001$) (Table 1, Fig. 1).

U-EPX and atopy

Of 784 children, 189 (24.1%) had a positive SPT for at least one allergen, and were therefore assumed to be atopic. U-EPX levels were significantly higher in atopic than nonatopic children (median 89.6 μg/mmol CREA *vs* 62.5 μg/mmol CREA; $P < 0.0001$) (Table 1) (Fig. 2). In addition, SPT demonstrated that 146 (77.3%) out of 189 atopic subjects were sensitized to perennial allergens. U-EPX levels were also significantly higher than in

Table 1. Urinary EPX (μg/mmol creatinine) in healthy and symptomatic children, expressed as median and 5th–95th percentiles

		Urinary EPX (μg EPX/μmol creatinine)			
		n	Median	5–95%	*P* value*
Total		877	68.2	23.4–208.5	
Girls		372	61.7	21.6–192.8	
Boys		505	72.6	26.1–214.2	<0.0001
Atopy	Yes	189	89.6	27.6–280.3	
	No	595	62.5	22.2–174.8	<0.0001
Perennial sensitization	Yes	146	100.0	33.0–295.6	
	No	638	62.8	22.2–173.4	<0.0001
Seasonal sensitization	Yes	129	85.9	28.5–264.1	
	No	655	60.4	22.3–199.4	<0.0001
Bronchial asthma	Yes	21	142.8	32.8–338.2	
	No	730	65.7	29.4–205.9	<0.001
Wheezing	Yes	38	96.4	21.1–413.6	
	No	705	65.0	23.2–203.6	<0.05

* Chi square.

Table 2. Odds ratios (OR) and 95% confidence interval (CI), as well as sensitivity, specificity, and positive and negative predictive values (PPV and NPV) for U-EPX higher than 90th percentile (140.1 μg EPX/mmol creatinine)

	OR	95% CI	Sensitivity	Specificity	PPV	NPV
Asthma	6.10	2.44–15.24	10%	88%	54%	79%
Wheezing	4.00	1.90–8.24	26%	91%	14%	95%
Atopy	4.44	2.75–7.19	22%	94%	55%	78%
Nocturnal cough	2.55	1.34–4.86	19%	94%	18%	92%
Breathlessness	2.32	1.03–5.23	20%	91%	11%	95%
Seasonal sensitization	2.56	1.51–4.32	19%	92%	31%	85%
Perennial sensitization	5.61	3.44–9.15	27%	94%	51%	85%

those children without perennial sensitization (median 100 μg/mmol CREA *vs* 62.8 μg/mmol CREA; $P<0.0001$) (Table 1).

The prediction of atopy by high U-EPX was associated with acceptable specificity, but positive predictive value and sensitivity were low (Table 2). However, sensitivity still increased with the numbers of allergens given in a positive SPT result. Sensitization to one allergen was associated with a sensitivity of 17% ($n=53$); to two allergens, with 23% ($n=41$); to three allergens, with 24% ($n=22$), to four allergens, with 14% ($n=19$); to five allergens, with 56% ($n=4$); and to six allergens, with 33% ($n=6$). Only one child was sensitized to all allergens and that child's U-EPX was lower than the 90th percentile. Of the boys, 73% were atopic, as were 79% of the girls.

Respiratory symptoms and their relationship to U-EPX

A total of 751 questionnaires assessing respiratory symptoms were returned by the children's carers. Exactly 38 (5.1%) out of 743 children reported wheezing within the last 12 months. These children demonstrated significantly higher U-EPX levels than nonwheezing subjects (median 96.4 μg/mmol CREA *vs* 65.06 μg/mmol CREA; $P<0.05$) (Table 1). Twenty-one (2.8%) out of 751 children had been diagnosed as having bronchial asthma by a physician. Their U-EPX levels were significantly higher than those of nonasthmatic subjects (median 142.8 μg/mmol CREA *vs* 65.7 μg/mmol CREA; $P<0.0001$) (Table 1). Polysensitized subjects did not report asthma or wheezing more often than monosensitized subjects.

Table 3. Frequency count: subjects with high U-EPX and physician-diagnosed asthma or wheezing within last 12 months

Asthma	Wheezing	% of subjects having U-EPX >90th percentile	% of subjects having U-EPX <90th percentile
No	No	9	91
No	Yes	23	77
Yes	No	25	75
Yes	Yes	42	58

As shown for atopy, specificity for asthma or wheezing was high, but sensitivity was low (Table 2). Frequency counts for physician-diagnosed asthma, wheezing within the last year, and high-U-EPX are shown in Table 3. If we take into account high U-EPX as well as reported wheezing, the sensitivity to detect asthma was 42%, compared to 9% if we take high U-EPX alone.

U-EPX and pulmonary function

When we adjusted for sex, height, atopy, and asthma, FVC was negatively related to U-EPX ($\beta=-0.02$, $P=0.44$). The same was true for FEV_1 ($\beta=-0.25$, $P=0.02$) and MEF_{50} ($\beta=-0.05$, $P=0.01$).

Medication and physical examination before urinary sampling

Ninety-eight children reported the use of drugs within 5

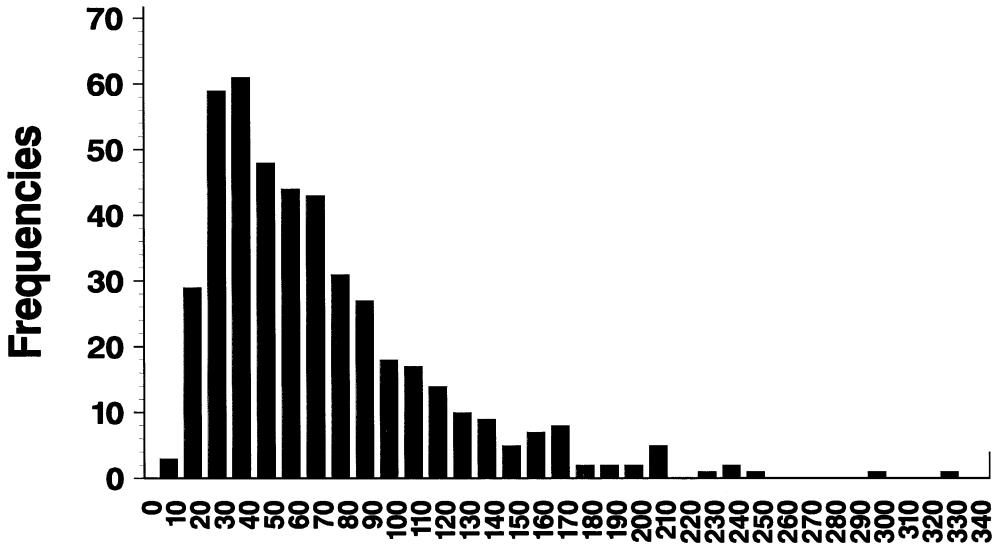

Figure 1. Number of subjects with respect to U-EPX levels in healthy children ($n=580$).

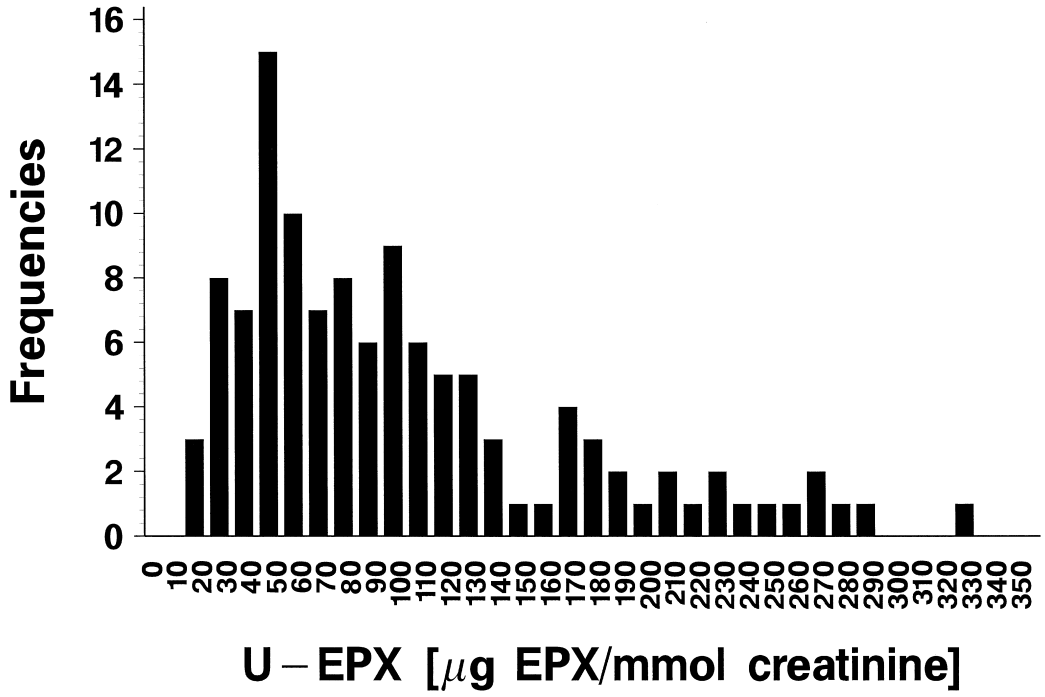

Figure 2. Number of subjects with respect to U-EPX-levels in atopic children ($n = 189$).

days before urinary sampling. In particular, 10 had taken inhaled β_2 agonists, 11 had taken inhaled steroids, three had taken inhaled disodium cromoglycate (DSCG), nine had taken oral antihistamines, and 43 had taken acetylsalicylic acid or paracetamol. Thirty-two children did not remember their medication. There was no statistically significant effect of drugs on U-EPX.

Discussion

Previous clinical studies in small numbers of patients have shown a strong relationship between markers of eosinophil activation and both bronchial asthma and atopy (12, 14, 15). Those markers showed associations with disease activity, and were influenced by treatment. In epidemiologic studies, measurement of U-EPX seems to be promising as a tool to detect asthmatic children. In detail, assessment of U-EPX is easy to perform in all age groups without the need for cooperation, and is free of unpleasant investigative procedures. Furthermore, costs and time per study subject are low, a factor which is very important in epidemiologic studies of hundreds or thousands of probands. In our study, children who suffered from respiratory symptoms, or had been diagnosed with bronchial asthma, had significantly higher U-EPX levels than controls. A physician's diagnosis of bronchial asthma had the highest OR for high U-EPX, but wheezing, nocturnal cough, and breathlessness had significantly increased ORs, too. However, there was an overlap between the groups. The specificity for asthma was fair (88%), but the sensitivity was very low (10%). Both values were somewhat higher for atopy (specificity 94%, sensitivity 22%). Therefore, we assessed whether atopy had influenced our data for asthma, and after statistical adjustment for atopy, the OR for asthma was no longer elevated significantly (Table 4). However, after adjustment for atopy, wheezing within the last year still had significantly increased OR. Those findings suggest a stronger relationship between U-EPX and atopy than between U-EPX and asthma. However, respiratory symptoms, i.e., wheezing, were highly significantly related to high levels of U-EPX, even in those subjects without allergic sensitization. Thus, childhood asthma is mostly attributable to allergy. In addition, the strong negative relationship between pulmonary function and U-EPX might reflect higher levels of bronchial inflammation and hence airways obstruction in children with higher levels of U-EPX.

Table 4. Odds ratios (OR) and 95% confidence interval (CI) for U-EPX higher than 90th percentile, adjusted for atopy

	OR	95% CI
Asthma	2.6	0.9–7.46
Wheezing	3.32	1.38–7.98

Subdivision of atopics according to their allergens showed perennial sensitization to be associated with higher U-EPX-levels and higher OR for high U-EPX than sensitization to seasonal allergens. One explanation for that might be that our assessment took place in the late autumn, and therefore outside the pollen season. Polysensitized individuals had higher ORs for high

Table 5. Comparison of different techniques of inhalation challenge

Authors	Method	Sensitivity	Specificity	PPV	NPV
Frischer et al. (18)	Ultrasonic nebulized distilled water	36%	92%	11%	98%
Jenkins et al. (19)	Hypertonic saline	54%	89%	64%	85%
	Saline challenge and respiratory symptoms	47%	94%	74%	84%
Pattemore et al. (9)	Histamine	52%	90%	47%	92%

PPV: positive predictive value; NPV: negative predictive value.

U-EPX than monosensitized subjects. This might be due to a higher probability of contact with relevant allergens and consecutive eosinophilic activation.

The data of epidemiologic studies using different approaches to testing of bronchial hyperresponsiveness are shown in Table 5, demonstrating higher sensitivity for these techniques than assessment of U-EPX. Nevertheless, there are disadvantages in testing bronchial hyperresponsiveness, such as the need for time- and money-consuming measurement procedures and potential hazards due to bronchial challenge. Still, testing of bronchial hyperresponsiveness will remain a stronger tool in evaluating asthma prevalence than assessment of U-EPX. In contrast, the low sensitivity of U-EPX to detect asthmatics out of a cohort of a normal population will not hinder its application to monitoring disease in patients with bronchial asthma. Especially in smaller children, where there may often be limited ability to measure pulmonary function or to use other, more invasive procedures to assess bronchial inflammation, these normal values and values for atopics and asthmatics could be helpful to clinicians in daily practice. As previously demonstrated, the relatively simple and painless sampling procedure to obtain U-EPX gives the same information on disease activity as serum measurement of ECP or EPX (20).

In epidemiologic studies, high levels of U-EPX will often contribute to asthma or respiratory symptoms, confirming the role of eosinophilic inflammation in childhood asthma. Nevertheless, high levels of U-EPX seem to reflect atopy rather more than asthma. Because of the great degree of overlap, determination of U-EPX alone will not allow us to distinguish between asthmatic and healthy children in epidemiologic studies. Currently, there are many techniques under evaluation for measurement of airway inflammation. Above all, assessment of ECP in nasal lavage or induced sputum, and exhaled nitric oxide may be used in future epidemiologic studies successfully (20, 21).

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