Eur Respir J 2000; 16: 588–592
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European Respiratory Journal
ISSN 0903-1936

Urinary excretion of leukotriene E_4 and eosinophil protein X in children with atopic asthma

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Urinary excretion of leukotriene E_4 and eosinophil protein X in children with atopic asthma. C. Severien, A. Artlich, S. Jonas, G. Becher. ©ERS Journals Ltd 2000.

ABSTRACT: Measurement of leukotriene E_4 (LTE_4) in urine is a noninvasive method for assessing changes in the rate of total body cysteinyl leukotriene production. Eosinophil protein X (EPX) has been used to assess eosinophil activity and monitor inflammation in bronchial asthma. The aim of the study was to look for differences in urinary LTE_4 and EPX concentrations between children with stable atopic asthma and healthy controls and to compare asthmatic children with different disease severity. In addition the relationship was evaluated between urinary LTE_4 amd EPX levels and lung function.

LTE_4 was also measured (enzyme immunoassay) together with EPX (radioimmunoassay) in urine and lung function tests were carried out in children with mild asthma (steroid-naive) (n=49), moderate to severe asthma (using inhaled steroids) (n=31) and healthy control subjects (n=28).

Urinary leukotriene E_4 (LTE_4) was significantly higher in children with asthma than in controls (median [25–75 percentile] 238.5 (126.5–375.7) SD 191.8 *versus* 189 (51–253.2) SD 131.7 pg·mg^{-1} creatinine; p=0.021). Urinary EPX was also significantly increased in asthmatic children compared with controls (85.5 [64–131.5] SD 76.2 *versus* 48.5 [43.2–90] 112.1 μg·mmol^{-1} creatinine; p=0.006). There were no differences in urinary LTE_4 and EPX between the group of mild and the group of moderate to severe asthmatic children. There were significant associations between the urinary LTE_4 and intrathoracic gas volume (ITGV), residual volume (RV), forced expiratory volume in one second (FEV_1), forced expiratory capacity (FVC) and maximum expiratory flow rate at 25% of vital capacity (MEF_{25}).

Urinary EPX was only correlated with maximum expiratory flow rate at 75% of vital capacity (MEF_{75}). Thus measurement of urinary LTE_4 may predict the degree of airflow obstruction in asthmatic children. Urinary LTE_4 and EPX are useful markers of airway inflammation and can be helpful in guiding asthma management. There was no correlation between LTE_4 and EPX levels.

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Keywords: Asthma
children
leukotriene
eosinophil protein X

Received: September 8 1999
Accepted after revision May 18 2000

This study was partially sponsored by Glaxo Welcome.

Cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4), synthesized from arachidonic acid through a 5-lipoxygenase pathway play an important role in asthma. These mediators can increase vascular permeability, enhance nonspecific bronchial hyperresponsiveness and are potent constrictors of bronchial smooth muscle [6–8]. LTC_4 is the dominant arachidonic acid metabolite released in lung tissue. This mediator is very unstable and rapidly converted to LTD_4. Finally LTD_4 may undergo further bioconversion to a less potent metabolite LTE_4, which is the most stable of the these three compounds and is excreted in the urine [9, 10].

The measurement of LTE_4 in urine is a useful noninvasive method to assess changes in the rate of total body cysteinyl leukotriene production. It has been shown that urinary LTE_4 concentration is significantly raised with an acute exacerbation of asthma, following allergen challenge or after aspirin challenge in aspirin sensitive individuals [10–12]. However, it has not been determined whether baseline measurements of urinary LTE_4 in the absence of challenge can provide useful information in asthmatic children.

Eosinophil protein X (EPX) is released from eosinophil granulocytes and has been used to assess eosinophil activity and monitor inflammation in bronchial asthma [13, 14]. It is a chemically stable protein that can be detected in the urine of healthy subjects and has been found to be significantly increased in children with acute asthma [15]. However it has not been examined whether baseline measurement of urinary EPX concentrations can be a useful marker in asymptomatic children with asthma.

Therefore the authors postulated that it may be possible to distinguish between healthy children and children with bronchial asthma and to assess disease severity by measuring LTE_4 and EPX in urine during a stable, asymptomatic interval. They examined the urinary leukotriene E_4 and the EPX concentrations in children with mild, moderate to severe asthma and healthy controls.

Furthermore they evaluated the relationship between urinary LTE_4 and EPX concentrations and diverse lung function variables.

Methods

Subjects

Asthma was defined as a clinical history with recurrent obstructive bronchial symptoms, evidence of at least 20% reversibility after inhalation of a short acting beta-sympaticomimetic and after other conditions had been excluded. Eighty children with asthma (58 male children, 22 females children) with an age of 10.5±2.5 yrs (mean±SD) from our asthma outpatient clinic entered in this study. Atopy was confirmed in all of them with a positive Prick test (induration >3 mm) and/or positive radioallergosorbent test (RAST) (Specific IgE ≥class 3) to one or more common inhalant allergens or elevated total serum immunoglobulin (IgE) level, with respect to age related normal values. Pollen atopic patients were balanced between the two groups of asthmatic children.

Forty nine patients were classified as having mild asthma (Group 1) and 31 patients had moderate to severe asthma (Group 2) according to the International Consensus Report on Diagnosis and Treatment of Asthma. [5]. At the time of the study, the prescribed asthma medication of patient Group 1 was limited to an inhaled short acting β_2-agonist alone or in combination with a parasympaticomimetic drug (Ipratropium bromide), as needed and all patients of Group 2 were taking inhaled steroids (fluticasone 200–400 $\mu g \cdot day^{-1}$, Budesonide 400–800 $\mu g \cdot day^{-1}$) daily on a regular basis for at least 12 weeks. Only clinically stable asthmatic children were considered for the study, therefore subjects were excluded if they had other illnesses based upon history, physical examination or had a respiratory infection within 4 weeks prior to the study visit. During the study, throughout July and August from 13:00h–16:00h a urine sample was taken from the subjects followed by a physical examination and a lung function test.

The control group consisted of 28 healthy children, matched for sex and age, with no history of infection during the last 4 weeks and no personal or familial history of asthma and atopy.

Spirometry measurements. Pulmonary function tests were carried out by body plethysmography (Masterlab, Jaeger, Germany) after a physical examination and obtaining the urine sample. Peak expiratory flow rate (PEF), maximum expiratory flow at 75% (MEF_{75}), 50% (MEF_{50}) and 25% (MEF_{25}) of vital capacity (VC), forced expiratory volume in one second (FEV_1) and forced expiratory vital capacity (FVC) were measured and a flow-volume curve recorded. Furthermore resistance (*r*), VC, intrathoracic gas volume (ITGV) and residual volume (RV) were determined. The best of three attempts was taken for analysis as recommended by the American Thoracic Society [3]. The results presented are given in % of predicted value according to accepted reference values [4].

Collection and storage. Urine was obtained from each child in the laboratory prior to the performance of the lung function test. The samples were immediately centrifuged to remove cellular debris at 10,000×g for 8 mins, the supernatant was then removed, coded and stored in aliquots of 5 mL at -70°C until analysis.

Analysis of leukotriene E_4, eosinophil protein X and creatinine. LTE_4 in urine was measured by ACE™ Enzyme Immunoassay Kit (Cayman Chemical, Ann Arbor, MI, USA) a sensitive and specific enzyme immunoassay. This assay is based on the competition between free LTE_4 and a LTE_4 tracer (LTE_4 linked to an acetycholinesterase molecule) for a limited number of LTE_4 - specific rabbit antiserum binding sites [1]. All measurements were done in duplicate and the mean value was calculated. The detection limit in the assay was <8 $pg \cdot mL^{-1}$. The interassay coefficient of variation was <10% in our laboratory. Urinary LTE_4 concentrations were expressed as $pg \cdot mg^{-1}$ creatinine. Before assessment of EPX the urine was diluted 11 times in a phosphate buffer containing 0.15 mM NaCl, 1% BSA, 0.1% Tween 20, 10 mM ethylenediamine tetraacetic acid (EDTA) and 0.2% N-cetyl-trimethyammoniumbromide.

EPX was measured by a sensitive and specific double-antibody radioimmunoassay (Pharamacia, Sweden) [2]. All measurements were done in duplicate and the mean value was calculated. The detection limit in the assay was <3 $\mu g \cdot L^{-1}$. This method showed an interassay coefficient of variation of <10% in our laboratory. Urinary EPX concentrations were expressed as $\mu g \cdot mmol^{-1}$ creatinine. Urinary creatinine concentrations were measured by Kodak Ektachem Clinical Chemistry Slides (Eastman Kodak Company, USA) a colorimetric test was performed with a Kodak Ektachem 700 analyser.

Statistics. Results are expressed as medians (25–75 percentile unless mentioned otherwise). Comparison of urinary LTE_4 and EPX concentrations, age and pulmonary function parameters between the groups were performed using the Mann-Whitney U-test. All other correlations were performed using Spearman′s rank correlation. Values of p <5% were considered significant.

Results

Urinary LTE_4 concentrations were significantly higher in children with asthma than in controls (238.5 (126.5–375.7) SD 191.8 *versus* 189 (51–253.2) SD 131.7 $pg \cdot mg^{-1}$ creatinine; p=0.021). However, only the subgroup of moderate to severe asthmatic children demonstrated significantly higher urinary LTE_4 levels compared to controls. Comparison between the group of mild and moderate to severe asthmatics revealed no significant difference in urinary LTE_4 concentrations (216 (101.5–382.5) *versus* 272 (153–370) $pg \cdot mg^{-1}$ creatinine) (fig. 1). Urinary EPX levels were also significantly increased in asthmatic children compared with controls (85.5 (64–131.5) SD 76.2 *versus* 48.5 (43.2–90) SD 112.1 $\mu g \cdot mmol^{-1}$ creatinine; p=0.006).

Furthermore, both subgroups of subjects with mild and moderate to severe asthma showed higher urinary EPX concentrations compared to healthy controls. Subgroups of mild and moderate to severe asthmatic children had similar EPX levels (84 (54.5–131.5) *versus* 91 (65–158) $\mu g \cdot mmol^{-1}$ creatinine) (fig. 2). Of interest, there was no correlation between urinary LTE_4 and EPX concentrations in asthmatic

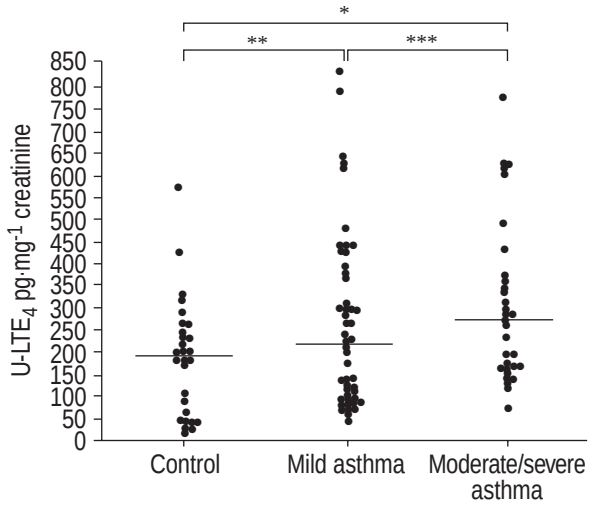

Fig. 1. – Urinary leukotriene E_4 (U-LTE_4) concentration in asthmatic and healthy children. Control n=28; mild asthmatic n=49; and moderate/severe asthmatics n=31. The horizontal bar represents the median. *: p=0.021; **: p=0.075; ***: p=0.19.

children (p=0.226). Pulmonary function variables of children with asthma showed significant differences in R, FEV1, and MEF75, MEF50, MEF25 compared to control (table 1). Urinary LTE_4 concentrations of asthmatic children demonstrated a positive correlation with ITGV and RV (fig. 3) and a negative correlation with FEV1 (fig. 4), FVC and MEF25 (table 2). Urinary EPX concentration showed a negative correlation with MEF75 (p= 0.002) only.

Discussion

This study showed that urinary LTE_4 was significantly higher in children with stable asthma than in healthy controls, providing further evidence that cysteinyl leukotrienes are involved in the chronic inflammation of children with asthma. However, only the subjects with moderate to severe asthma had significantly increased urinary LTE_4 levels compared with controls, indicating that there is a considerable overlap of the LTE_4 concentrations between mild asthmatics and controls. There was a clear relationship between concentrations of urinary LTE_4 levels and (FEV1), FVC, MEF25, ITGV and RV indicating that concentrations of urinary LTE_4 may be a valuable predictor of small airway disease in asthmatic children.

This finding may be important for the management of asthma in children, especially under the age of 5 yrs, because in this age group no reliable lung function test can be performed. In contrast to the present results, some investigators found baseline LTE_4 levels in adults with asthma to be no different from those measured in normal subjects [11, 16]. No difference was found in urinary LTE_4 levels between mild and moderate to severe asthmatic children, suggesting that there may be no influence of steroid use on the cysteinyl leukotriene production. Similar findings have been reported in adults with asthma by other authors [16, 17]. It is interesting that therapy with inhaled steroids did not seem to influence LTE_4 levels, because this may

Table 1. – Pulmonary function parameter in children with asthma and healthy control subjects

Lung Function % pred	Asthma	Controls	p-value
Subjects n	80	25	
R	152 (115.5–185.7)	127 (106.2–149.7)	0.022
VC	103 (92–113)	97.5 (88.7–108.2)	NS
ITGV	96 (83.5–116)	95.5 (80.5–105.7)	NS
RV	127.5 (98–149.5)	112.5 (84.5–138.7)	NS
FVC	101.5 (91–108)	98.5 (92.2–108.7)	NS
FEV1	100 (90.2–109.7)	107 (99.5–117.7)	0.009
PEF	92 (80.2–101.7)	90 (83–100.2)	NS
MEF75	86 (75–97)	107 (94.5–107)	0.014
MEF50	76 (64.2–89)	96 (78.7–115.7)	0.001
MEF25	63 (47.2–77.5)	99.5 (79–114.7)	0.001

Values presented as % (range). p-value indicates statistical difference between asthmatic children and control subjects. NS: not significant; *R*: resistance; VC: vital capacity; ITGV: intrathoracic gas volume; RV: residual volume; FVC: forced expiratory vital capacity; FEV1: forced expiratory volume in one second; PEF: peak expiratory flow; MEF75, MEF50, MEF25: maximum expiratory flow rate at 75% 50% 25% of VC.

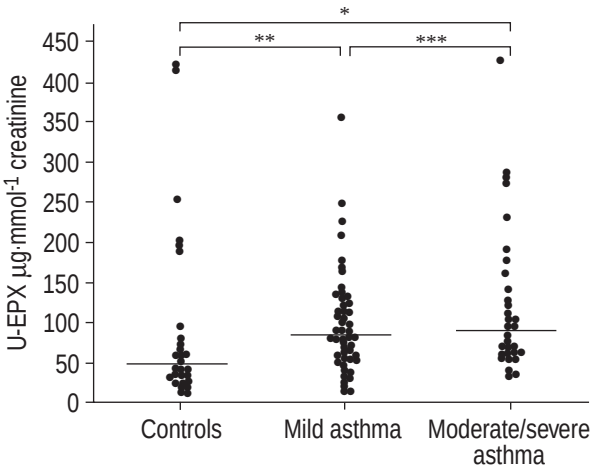

Fig. 2. – Urinary eosinophil protein X (U-EPX) concentrations in asthmatic and healthy children. Control n=28; mild asthma n=49; and moderate/severe asthmatic n=31. The horizontal bar represents the median. *: p=0.003; **: p=0.034; ***: p=0.199.

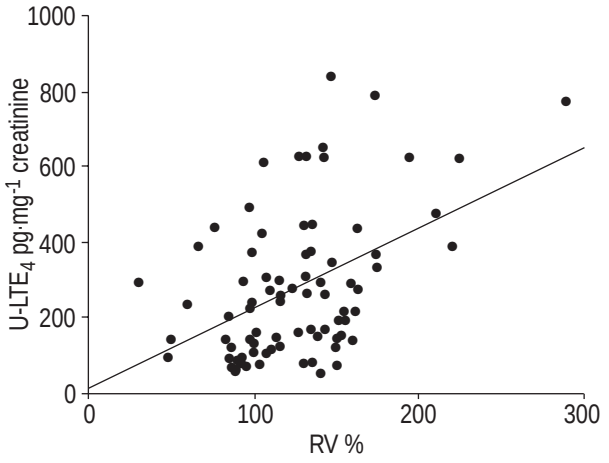

Fig. 3. – Correlation between urinary leukotriene E_4 levels (U-LTE_4) and residual volume as percentage predicted (RV %) in 80 children with asthma. r=0.388, p=0.01.

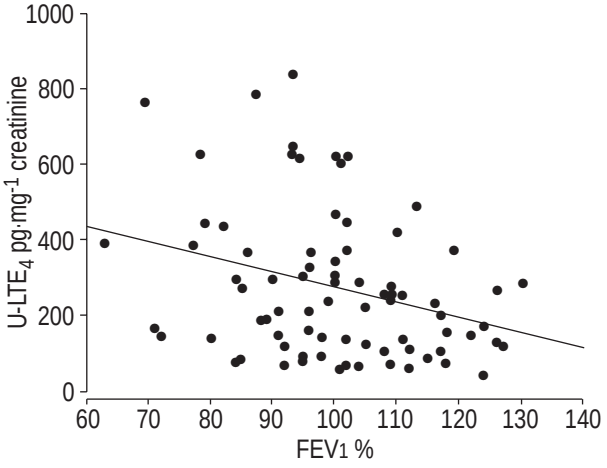

Fig. 4. – Correlation between urinary leukotriene E_4 levels (U-LTE_4) and forced expiratory volume in one second as percentage predicted (FEV_1 %) in 80 children with asthma. r=0.286, p=0.010.

be important in relationship to different alternatives for asthma treatment. However, impaired compliance of taking medication, especially in children, needs to be considered, before the absence of a relationship between glucocorticoid use and urinary LTE_4 concentration can be confirmed [18].

The measurement of LTE_4 in urine in this study was performed by an enzyme immunoassay (EIA). LTE_4 has been shown to be stable in urine samples stored at -20°C for months without the addition of preservatives. A correlation has been shown between analysis of LTE_4 in crude urine samples by EIA and measurements after purification on solid phase extraction followed by separation on reversed-phase high performance liquid chromatography, considered to be the most accurate method [19]. Previous studies have shown, that urinary EPX concentrations are increased in children with acute asthma compared with healthy control subjects [2, 15]. However, correlation between pulmonary function variables and urinary EPX levels are not consistently found in children with symptomatic asthma [20, 15].

Table 2. – Coefficients of correlation between urinary leukotriene E_4 (U-LTE_4) concentrations and variables of lung function in 80 children with asthma

Lung Function % pred	U-LTE_4	
	Correlation Coefficient	p-value
R	-0.018	NS
VC	0.171	NS
ITGV	0.221	0.049
RV	0.383	0.001
FVC	-0.245	0.029
FEV_1	-0.286	0.010
PEF	-0.107	NS
MEF_{75}	-0.118	NS
MEF_{50}	-0.211	NS
MEF_{25}	-0.225	0.045

All values are expressed as % predicted. *R*: resistance; VC: vital capacity; ITGV: intrathoracic gas volume; RV: residual volume; FVC: forced expiratory vital capacity, FEV_1: forced expiratory volume in one second; PEF: peak expiratory flow; MEF_{75}, MEF_{50}, MEF_{25}: maximum expiratory flow rate at 75%, 50% and 25% of VC; NS: not significant.

The results showed that children with asymptomatic (stable) asthma, whether they were treated with inhalative steroids or not, had increased urinary EPX levels compared with healthy controls. This confirms the results of previous studies, where the urinary excretion of EPX in asthmatics was not inhibited by treatment with glucocorticoids [2, 17]. This may be explained by a incompletely suppressed inflammation in the bronchial mucosa in asthmatics treated with steroids [21] or by an inadequate compliance, which is known to occur frequently in anti-inflammatory treatment [22]. Although urinary LTE_4 and EPX levels increased in children with asthma, LTE_4 concentrations did not correlate with EPX levels. This may suggest that release, metabolism and clearance of the two compounds may be different within the group of asthmatic children.

In conclusion, the measurement of leukotriene and eosinophil protein X in urine, avoids blood sampling or even more invasive methods like bronchial biopsy or bronchoalveolar lavage, and may be useful in assessing airway inflammation; and, as a complement to lung function tests and asthmatic symptoms, may help to find the optimal therapy for children with chronic asthma. However, longitudinal studies are needed to clarify that these inflammatory markers of airway inflammation will prove helpful in guiding asthma management.

Acknowledgements. The authors thank K. Grywatz and G. Pauch for excellent technical assistance.

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