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Urinary leukotriene E4 at 12 months and influencing factors

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Summary

Objective

LTE_4 is the end point of cysLTs pathway and its only stable product. Its role was discussed in asthma and AEDS.

We aimed to investigate the impact of genetic and environmental factors (sex, maternal positive family atopy history, breastfeeding, passive smoking and pet exposure).

60 newborns (including 30 boys) were enrolled in the study. Each child was examined at 12 months and urine samples for urinary LTE_4 measurement were collected. All samples were processed using ACE^{TM} Enzyme Immunoassay Kit (Cayman Chemical, Ann Arbor, MI, USA).

The mean level of urinary LTE_4 at 12 months was 186,99 pg/ml (median: 159,0; CI 95%: 157,79- 221,59). The Shapiro- Wilk test showed that the distribution of the levels of urinary LTE_4 were abnormal. 12- month- old girls had higher urinary LTE_4 levels than boys (mean: 270,50 vs. 193,55), but maternal positive atopy history, pet exposure, tobacco smoking or length of breastfeeding had no impact on urinary LTE_4 excretion.

Conclusions

In conclusion, most of genetic or environmental factors do not change levels of urinary LTE_4 in infants.

CysLTs are potent mediators of airway narrowing, edema, smooth muscle proliferation, and increased mucus production during asthmatic attack. LTE_4 is a potent bronchoconstrictor in human [3] and asthmatic airways might be selectively hyperresponsive to LTE_4 in contrast to other cysLTs [11].

CysLTs also are involved in the inflammation of the skin in AEDS, possibly through chemotaxis of inflammatory cells, vasodilatation and oedema [16]. Urinary LTE_4 is significantly elevated in children with AD [5]. It was also shown that urinary LTE_4 concentration was correlated with SCORAD.

LTE_4 is an effect of leukotriene C4 convertion through sequential enzymatic removal of glutamic acid and then glycine. So far, LTE_4 has received little attention because it binds poorly to the classical type 1 and 2 cysLT receptors and is much less active on normal airways than LTC4 or LTD4. Recent studies have begun to uncover receptors selective for LTE4: PRY_{12}, an adenosine diphosphate receptor, and $CysLT_ER$.

The past three decades have been characterized by an increase in the prevalence of allergic diseases, particularly in childhood [18]. While the increase of asthma in developed countries seems to stabilise, at the same time we observe the rapid increase in atopic eczema prevalence [1]. The burst of allergies in the western countries, including Poland, is suggested to be provoked to due to both genetic and environmental factors including sex, positive maternal and family atopy history, cigarette smoking, pets, social and economic status.

As far as we known, there are no previous studies aiming to investigate the correlation between the levels of urinary LTE_4 and sex, cigarette smoking, breastfeeding, pets and positive maternal history of allergy among the Polish infants. Therefore, the aim of our study was to demonstrate the influence of selected factors (sex, cigarette smoking, breastfeeding,

pets and positive maternal history of allergy) on the levels of urinary LTE_4 in 12- months-old children.

Methods

Subjects

60 newborns born vaginally at term were enrolled in the study after the parental informed consent was obtained. The exclusion criteria were: newborns born from multiple pregnancies or complicated pregnancies (maternal chronic disease, PIH, GDM, GBS), newborns with congenital defects or intrauterine infection.

At the time of delivery all parents completed a standardized questionnaire, including information on pregnancy, birth, sex, birth weight, social, economical and maternal factors, family atopy history, passive smoking. These children have been followed at the age of 3, 6, 12 months. At every follow- up, detailed questionnaires were completed with the parents for each child regarding breastfeeding, passive smoking, infections and allergic symptoms.

Family allergy score (FAS) system was used for assessment of family allergy history [10].

The study was approved by the local research ethics committee.

Collection and storage of samples

At the age of 12 months, each child was examined by the same physician to exclude infection or present allergic symptoms before the urine was obtained in the laboratory. The samples were immediately centrifuged to remove cellular debris at 10,00x g for 10 min, the supernatant was then removed, coded and stored in aliquots of 5 ml at $-70^{o}C$ until analysis.

LTE_4 measurement

LTE_4 in urine was measured using ACE^{TM} Enzyme Immunoassay Kit (Cayman Chemical, Ann Arbor, MI, USA). All measurements were done in duplicate and the mean value was calculated. This assay is based on the competition between LTE4 and an LTE4-acetylcholinesterase conjugate (LTE4 tracer) for a limited amount of LTE4 antiserum. Because the concentration of LTE4 tracer is held constant while the concentration of LTE4 varies, the amount of LTE4 tracer that is able to bind to the LTE4 antiserum will be inversely proportional to the concentration of LTE4 in the well. The U- LTE4 concentration is expressed as picograms per milligram and the detection limit in the assay was < 8 pg/ml.

Statistical analysis

Data were analyzed using MedCalc 9.6 (MedCalc, Mariakerke, Belgium). Data were expressed as mean ± SD. Urinary LTE4 concentration was log-transformed (log-LTE4) before analysis because its distribution was not normal. Logistic regression analysis was used to assess the influence of analyzed factors on urinary LTE_4 at 12 months. P< 0,05 was considered to be statistically significant.

Results

Sixty newborns (30 boys) were recruited with a mean level of urinary LTE_4 at 12 months of 186,99 pg/ml (median: 159,0; CI 95%: 157,79- 221,59). The Shapiro- Wilk test showed that the distribution of the levels of urinary LTE_4 were abnormal.

Table I presents the mean levels of urinary LTE_4 detected depending on sex, positive maternal atopy history, passive smoking, breastfeeding and pets.

The levels of urinary LTE_4 differ between boys (1) and girls (2) at the of 12 months (p<0,05).(Table I), (Fig. 1).

Table I. The mean levels of urinary LTE_4 depending on genetic and environmental factors (sex, positive maternal atopy history, passive smoking, breastfeeding and pets); pg/ml.

	Number	Mean level of urinary LTE_4	p
Sex **Boys** **Girls**	**60** **30** **30**	**186,99** **193,55** **270,50**	**0,03**
Maternal atopy history Yes No	60 18 42	186,99 208,94 178,30	0,39
Passive smoking Yes No	60 23 38	186,99 196,51 254,22	0,19
Breastfeeding* Yes No	60 23 37	186,99 168,85 220,76	0,12
Pets Yes No	60 13 47	186,99 196,21 184,51	0,76

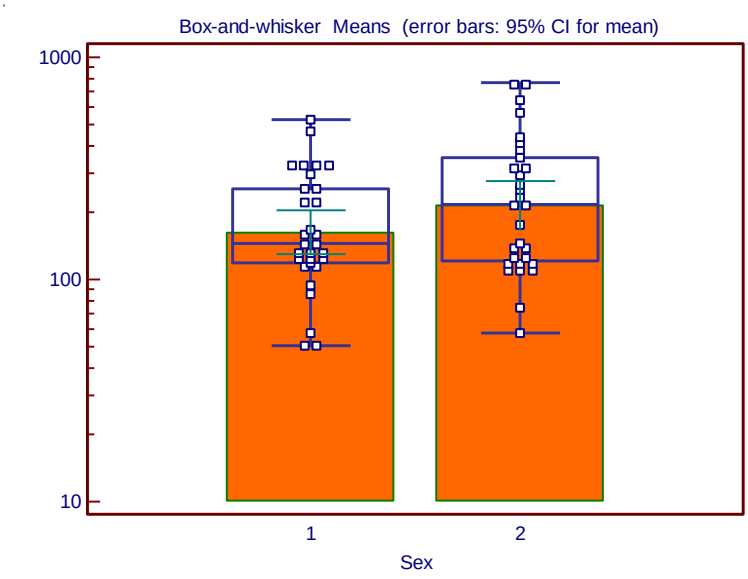

Fig. 1. Levels of urinary LTE_4 at 12-months old infants (1 boys, 2 girls); p<0,05; pg/ml.

Discussion

In this study, we demonstrated that levels of urinary LTE_4 were higher in 12-months old girls than boys. We did not observe any difference in urinary LTE_4 levels depending on cigarette smoking, the length of breastfeeding or presence of pet at home.

It is surprising that exposure to cigarette smoking have not influenced urinary LTE_4 excretion. Previous reports showed that, beside of RSV, secondhand cigarette smoking increases urinary LTE_4 both in healthy infants and infants with RSV- bronchiolitis [8]. The study by Kott et al. noted the increase of urinary LTE_4 only in children exposed to cigarette smoke without parental positive history of asthma. Additionally, their observation is limited to high exposure to cigarette smoke. This may explain the difference in our study. Firstly, we had not divided the exposed group of infants into further subgroups according to the amount of cigarettes smoked a day by parents. Secondly, we had not divided children according to both passive smoking and parental atopy family history. Our assumption that only high exposure of tobacco smoke can produce subsequent elevation of urinary LTE_4 in infants might be sustained by earlier studies [4, 13]. Piedimonte et al. observed higher levels of urinary LTE_4 in children with bronchiolitis and with an atopic/asthmatic family background which is inconsistent with our observation that family atopy history did not influence leukotriene synthesis. In our opinion, bronchiolitis itself was triggering mast cells and eosinophils to produce leukotriens. Atopic background was only additional minor factor that came into importance only because of airways irritation with RSV.

Although beneficial effect of breastfeeding was widely reported [6,17], we have not observed any effect of breastfeeding longer than 3 months on urinary LTE_4 levels at 12-months old children. It is hard to debate whether our results are acceptable because there are no previous studies that might be compared to our results. Similarly, pet exposure have no impact on urinary LTE_4 excretion at 12 months. Recent study reported exposure to cat

allergens early in life increased the risk of late childhood asthma and bronchial hyperresponsiveness, but not the risk of allergic sensitization [2]. They did not observe, however, any impact of early exposure to dog allergens. As we mentioned above, this might be due to lack of subgroup selection eg. infants exposed to cat or dog or guinea pig. It seems that this problem should be further investigated in larger population study because previous studies have shown that the urinary LTE_4 concentration is useful in demonstrating cysLTs release in vivo during allergen challenge [9,15]. Additionally, LTE_4 is a potent bronchoconstrictor in human [3] and it was shown that asthmatic airways might be selectively hyperresponsive to LTE_4 in contrast to other cysLTs [11]. It might be reasonable to enlarge the studied group with selection of infants with wheezing or recurrent bronchitis and to investigate pet exposure in this selected group.

We demonstrated a significant dependence of urinary LTE_4 on sex. Girls had higher mean levels of urinary LTE_4 than boys. The trend to higher levels of urinary LTE_4 in girls was previously observed by Rabinovitch et al [14]. On the other hand, other studies performed at the older age group had showed no association between sex and urinary LTE_4 [7,12].

The ability to predict the risk for having allergic disease in later childhood is enormously helpful in terms of offering an accurate prognosis to parents and identifying children for investigation of prevention strategies. Thus, it was proven that urinary LTE_4 is helpful as pediatric biomarker in asthma or atopic eczema. It seems it is especially important in selecting high- risk infant girls but further studied should be performed.

What is already known on this topic is a little information about levels of urinary LTE4 in children, especially in infants. Additionally, it should be mentioned that most of the previous studies reported levels of urinary LTE4 in children with asthma exacerbation or AEDS.

What this study adds is that levels of urinary LTE4 are different between boys and girls in Polish population. It is noteworthy, that these children were followed for the first year of their life and were not recruited as sick children.

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