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Original Paper

Urinary Leukotriene Level in Children with Asthma

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REZUMAT

Nivelul leucotrienelor urinare la copiii cu astm

Obiectiv: Evaluarea corelației dintre valoarea leucotrienelor urinare și atopie, expunerea la fumul de țigară, severitatea astmului, valoarea fracției de oxid nitric exhalat (FeNO), tratamentul controller cu corticosteroizi inhalatori (CSI) sau antagoniști ai receptorilor pentru leucotriene (LTRA) și controlul astmului.
Material și metodă: A fost inițiat un studiu prospectiv incluzând 42 copii cu vârsta între 5-18 ani diagnosticați cu astm și derulat între august-septembrie 2014 în Clinica de Pediatrie, Spitalul Clinic de Copii "Dr. Victor Gomoiu". S-au documentat atopia, expunerea la fumul de țigară, FeNO, tratamentul cu CSI sau LTRA, severitatea și controlul astmului și s-au măsurat leucotrienele urinare.
Rezultate: Dintre cei 42 de copii incluși în studiu 16 au avut valori crescute ale leucotrienelor urinare (>88,3 pg/μg creatinină), iar 26 au avut valori normale. Evaluarea corelației statistice dintre atopie, expunerea la fumul de țigară, FeNO, tratamentul cu CSI sau LTRA, severitatea și controlul astmului și leucotrienele urinare a furnizat o valoare semnificativă statistic doar în cazul corelației dintre leucotrienele urinare și severitatea astmului (p=0,049).
Concluzii: La copiii cu astm valoarea leucotrienelor urinare se corelează cu severitatea bolii, dar nu cu atopia, expunerea la fumul de țigara, FeNO, tratamentul cu CSI sau LTRA sau cu controlul bolii.

Cuvinte cheie: astm bronsic, severitate astm, leucotriene urinare

ABSTRACT

Objective: To evaluate the correlation between urinary leukotrienes and atopy, tobacco smoke exposure, asthma severity, fractional exhaled nitric oxide (FeNO) and controller treatment with inhaled corticosteroids (CSI) or leukotriene receptor antagonist (LTRA) and asthma control.
Material and method: A prospective study including 42 children diagnosed with asthma aged 5 to 18 years was initiated. The study was conducted from August until September 2014 in the Department of Pediatrics of „Victor Gomoiu" Children Clinical Hospital. Atopy, tobacco smoke exposure, FeNO, treatment with CSI or LTRA and asthma control were assessed and urinary leukotrienes were measured.
Results: Among those 42 included children, 16 had increased urinary leukotriene (>88,3 pg/μg creatinine); the other 26 children had normal urinary leukotrienes. The statistic assessment of the correlation between atopy, tobacco smoke exposure, asthma severity, FeNO, treatment with CSI or LTRA and asthma control provided statistically significant result only regarding the correlation between urinary leukotriene level and asthma severity (p=0,049).

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Conclusion: In children with asthma urinary leukotriene level is correlated with asthma severity, but it is not correlated with atopy, tobacco smoke exposure, FeNO, treatment with CSI or LTRA or asthma control.

Key words: asthma, asthma severity, urinary leukotriene

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways and it represents a major cause of morbidity and mortality, having a rising incidence in the entire world (1).

The physiopathological features of asthma are chronic inflammation of the bronchial mucosa and bronchial hyperreactivity which is expressed as bronchoconstriction in response to various triggers (2).

Leukotrienes (cysteinyl-leucotrienes) represent a group of local mediators of bronchial inflammation which originate in the metabolism of arachidonic acid via lipoxygenase pathway in macrophages, mast cells, eosinophils, neutrophils and bronchial epithelial cells. The leukotrienes A4, B4, C4, D4 and E4 promote bronchial inflammation and bronchoconstriction (3).

The leukotriene E4 (LTE4) is the final product of the leukotrienes metabolism pathway. LTE4 is a chemical stable compound and can be measured in the condensed exhaled air and in urine by using the mass spectroscopy as recommended method (4, 5).

LTE4 is considered a bronchial asthma biomarker. It is the most stable among the products resulted from arachidonic acid metabolism and it has the longest bronchoconstriction effect. Through chemotactic activity LTE4 increase the number of eosinophils and neutrophils in bronchial mucosa. For this reason LTE4 might be considered an acute phase reactant during asthma exacerbations (4, 6).

Until the present time many studies have been conducted having as objective to evaluate the role that urinary leukotriene might have played in asthma management, but often their results have been contradictory. Many of these studies have proven that LTE4 level is higher in children with asthma than in healthy children (7, 8, 9).

Urinary LTE4 is an indirect marker of pulmonary cysteinyl-leukotriene activity. LTE4 levels increases in asthma exacerbations, in aspirin or other allergens exposure, in severe asthma and during the night in patients with nocturne symptoms asthma (4).

Normal urinary LTE4 levels measured using mass spectrometry method are ranging between 50-88,3 pg/μg creatinine. LTE4 levels increase in asthma exacerbations or after triggers exposure and decrease after initiating therapy with lipoxygenase inhibitors or reaching asthma control (10, 11).

The increase of urinary LTE 4 level is correlated with the lung function decrease in children with uncontrolled asthma. In their case asthma phenotype is at least in part determined by one subtype of the gene ALOX5 (arachidonate 5 lipoxygenase) – the gene that encode lipoxygenase (12, 13, 14).

In spite of all these clinical and biological correlations between urinary leukotriene levels and asthma, urinary leukotriene assessment is not yet been protruded as a routine assessment method in asthma management plan.

For these reasons the objective of this study was to determine the urinary leukotriene levels in children with asthma and to evaluate the correlation between these levels and the presence of atopy, tobacco smoke exposure, asthma severity, fractional exhaled nitric oxide (FeNO) value, the use of inhaled corticosteroids or leukotriene receptor antagonist as controller treatment and asthma control.

MATERIAL AND METHOD

A prospective study including 42 patients diagnosed with asthma and monitored in „Victor Gomoiu" Children Clinical Hospital from August until September 2014 was initiated.

Inclusion criteria were: age between 5 and 18 years old; asthma diagnosis established using specific history, clinical and spirometric criteria through demonstrating a FEV1 reversibility of at least 12% after salbutamol inhaling.

Exclusion criteria were other coexistent comorbidities beside allergic rhinitis or overweighting.

For each patient tobacco smoke exposure, atopic status, asthma severity, FeNO value (fractional exhaled nitric oxide), controller treatment and asthma control were assessed.

The atopic status was demonstrated through the measurement of total and specific IgE against commune airway allergens or through skin prick test using standardized airways allergens (used when IgE measurement was not available) or through both of them (when both evaluations were made before the inclusion in this study).

By recalling each patient history of disease from the last month have been documented the day and night time symptoms, the degree of activity limitation and the use of rescue medication (inhaling bronchodilator, salbutamol). By that the degree of asthma control was established, recording at each moment if the patient was controlled, partially controlled or uncontrolled. History data were requested from the patient himself only when the patient was older than 12 years. In patients younger than 12 years

history data were requested from both the patients as well as from their parents and their first degree adult relatives.

FeNO value was measured by using NIOX-MINO with mouth piece, chemiluminescence analyzer approved for FeNO measurement. Normal FeNO value in children ranges between 5-20 ppb in children under 12 years and between 5-25 ppb in children older than 12 years. Using these parameters FeNO values were divided in normal or increased in the statistically analyses used in this study.

Asthma severity was established according to the step of controller therapy needed to obtain asthma control: mild (step 1 and 2), moderate (step 3) and severe (step 4 and 5).

Urinary leukotrienes were measured using spontaneous urine samples obtained at 12 o'clock for each included patient. The reason for choosing this hour was the challenge not to interfere with the school schedule of the patients. By collecting all the urine samples at the same moment of the day we have formally excluded the differences between the leukotriene values that might comes out from the daily variability of urinary leukotrienes levels. It is well known that urinary leukotriene tend to vary very much along the day. The urine samples were refrigerated at -80˚IC according to the instructions from the ELISA kit producer and the measured values of urinary leukotriene were related to the normal urinary creatinine value. Increased urinary leukotriene values were considered those above 88,3 pg/mg creatinine.

For the statistical analyze in this study Pearson Chi-Square Test was used and $p<0,05$ was considered as threshold for statistical significance.

RESULTS

Among included children, urinary leukotriene levels ranging between 10,1 and 554,3 pgμg creatinine were recorded. 26 patients had normal urinary leukotriene levels and the other 16 had increased urinary leukotriene levels (>88,3 pg/μg creatinine).

The correlation between urinary leukotriene level and the presence of atopy

Among those 16 patients with increased level of urinary leukotriene, in 14 patients the atopy is present and the other 2 patients are non atopic. Among those 26 patients with normal urinary leukotriene level, 21 patients are atopic and the other 5 patients are non atopic as depicted in **Table 1**.

Using the Chi-Square test in order to assess the statistical correlation between urinary leukotriene level, considered as qualitative variable (normal and increased), and the presence of atopy we have obtained a p value=0,569 (not statistically significant). This result demonstrates that urinary leukotriene levels have not a significant variation according to the presence of atopy.

Table 1. The distribution of urinary leukotriene values in relation to the presence of atopy

Leukotriene/Atopy	yes	no	Total
Increased	14	2	16
Normal	21	5	26
Total	35	7	42

The correlation between urinary leukotriene level and tobacco smoke exposure

Among those 16 patients with increased level of urinary leukotriene, 10 patients have tobacco smoke exposure and the other 6 patients do not have. Among those 26 patients with normal urinary leukotriene level, 14 patients have tobacco smoke exposure and the other 12 patients do not have, as depicted in **Table 2**.

Using the Chi-Square test in order to assess the statistical correlation between urinary leukotriene level, considered as qualitative variable (normal and increased), and the tobacco smoke exposure we have obtained a p value=0,582 (not statistically significant). This result demonstrates that urinary leukotriene levels have not a significant variation according to the tobacco smoke exposure.

The correlation between urinary leukotriene level and FENO value

Among those 16 patients with increased level of urinary leukotriene, 10 patients had increased FeNO value and the other 6 patients had normal FeNO value. Among those 26 patients with normal urinary leukotriene level, 15 patients had increased FeNO value and the other 11 patients had normal FeNO value, as depicted in **Table 3**.

Using the Chi-Square test in order to assess the statistical correlation between urinary leukotriene level, considered as qualitative variable (normal and increased), and the FeNO value we have obtained a p value=0,757

Table 2. The distribution of urinary leukotriene values in relation to the tobacco smoke exposure

Leukotriene/Tobacco exposure	yes	no	Total
Increased	10	6	16
Normal	14	12	26
Total	24	18	42

Table 3. The distribution of urinary leukotriene values in relation to the FeNO value

Leukotriene/FeNO	Increased	Normal	Total
Increased	10	6	16
Normal	15	11	26
Total	25	17	42

(not statistically significant). This result demonstrates that the probability for a patient with increased FeNO value to have at the same time an increased urinary leukotriene level is not significantly higher than the possibility that he might have a normal urinary leukotriene level.

The correlation between urinary leukotriene level and asthma severity

Among those 16 patients with increased level of urinary leukotriene, 6 patients had mild asthma, 9 patients had moderate asthma and 1 patient had severe asthma. Among those 26 patients with normal urinary leukotriene level, 19 patients had mild asthma and 7 patients had moderate asthma, as depicted in **Table 4**.

Using the Chi-Square test in order to assess the statistical correlation between urinary leukotriene level, considered as qualitative variable (normal and increased), and asthma severity we have obtained a p value=0,049 (statistically significant). This result demonstrates that the probability for a patient with increased urinary leukotriene level to have severe asthma is significantly higher than the probability of a patient with normal urinary leukotriene level to have severe asthma (**Fig. 1**).

The correlation between urinary leukotriene level and LTRA treatment

Among the patients with increased level of urinary leukotriene (16), none of them is receiving LTRA treatment. Among the patients with normal urinary leukotriene level (26), 4 are receiving LTRA treatment and 22 do not receive LTRA treatment, as depicted in **Table 5**.

Using the Chi-Square test in order to assess the correlation between urinary leukotriene level, considered as qualitative variable (normal and increased), and LTRA treatment we have obtained a p value=0,099 (not statistically significant). This result demonstrates that the probability of a patient who receives LTRA treatment to have an increased urinary leukotriene level is not significantly higher than the probability of a patient who is not receiving LTRA treatment to have increased urinary leukotriene.

Table 4. The distribution of urinary leukotriene values in relation to asthma severity

Leukotriene/ Asthma severity	Mild	Moderate	Severe	Total
Increased	6	9	1	16
Normal	19	7	0	26
Total	25	16	1	42

Table 5. The distribution of urinary leukotriene values in relation to LTRA treatment

Leukotriene/LTRA treatment	yes	no	Total
Increased	0	16	16
Normal	4	22	26
Total	4	38	42

Table 6. The distribution of urinary leukotriene values in relation to CSI treatment

Leukotriene/CSI treatment	yes	no	Total
Increased	10	6	16
Normal	12	14	26
Total	22	20	42

The correlation between urinary leukotriene level and CSI treatment

Among the patients with increased level of urinary leukotriene (16), 10 patients are receiving CSI treatment and 6 patients do not receive CSI treatment. Among those 26 patients with normal urinary leukotriene level, 12 patients are receiving CSI treatment and 14 patients do not receive CSI treatment, as depicted in **Table 6**.

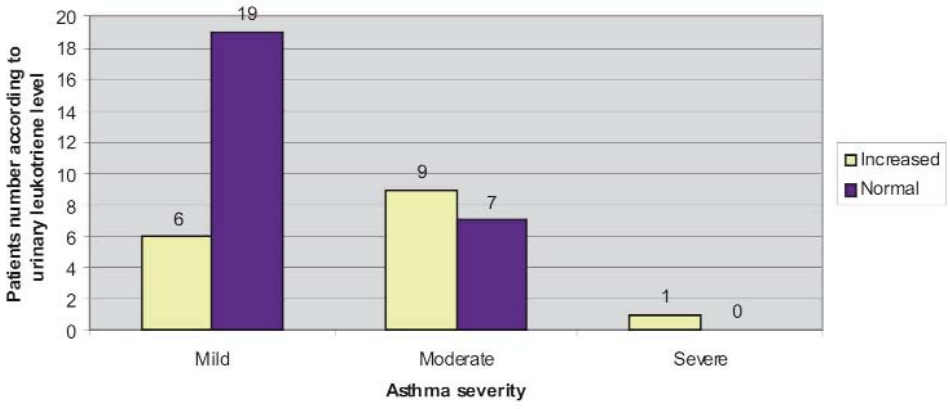

Figure 1. The correlation between asthma severity and urinary leukotriene level

Table 7. The distribution of urinary leukotriene values in relation to asthma control

Leukotriene/Asthma control	Controlled	Partial controlled	Uncontrolledat	Total
Increased	10	1	5	16
Normal	19	3	4	26
Total	29	4	9	42

Using the Chi-Square test in order to assess the statistical correlation between urinary leukotriene level, considered as qualitative variable (normal and increased), and CSI treatment we have obtained a p value=0,303 (not statistically significant). This result demonstrates that the probability of a patient who receive CSI treatment to have an increased urinary leukotriene level is not significantly higher than the probability of a patient who is not receiving CSI treatment to have increased urinary leukotriene.

The correlation between urinary leukotriene level and asthma control

Among those 16 patients with increased level of urinary leukotrienes, 10 patients had controlled asthma, 1 patient had partial controlled asthma and 5 patients had uncontrolled asthma. Among those 26 patients with normal urinary leukotrienes level 19 patients had controlled asthma, 3 patients had partial controlled asthma and 4 patients had uncontrolled asthma, as depicted in **Table 7**.

Using the Chi-Square test in order to assess the statistical correlation between urinary leukotriene level, considered as qualitative variable (normal and increased), and asthma control we have obtained a p value=0,446 (not statistically significant).

The statistical analyze Column Test which takes into account all individual values of the two variables (urinary leukotriene and asthma control) provides a p value=0,929 (also not statistically significant).

This result demonstrates that the probability for a patient with uncontrolled asthma to have an increased urinary leukotriene level is not significantly higher than the probability for a patient with controlled or partial controlled asthma to have increased urinary leukotrienes.

DISCUTIONS

Leukotrienes are markers of bronchial inflammation. Their value was correlated in many studies with different other asthma features: the presence of atopy, tobacco smoke exposure, FeNO value, controller treatment with CSI and LTRA, asthma severity and asthma control.

In this study the urinary leukotriene level was not correlated with the presence of atopy, tobacco exposure, FeNO value, controller treatment with CSI and LTRA or asthma control.

This result is probably related to the fact that urinary leukotriene level tends to vary considerably from one patient to another according to the phenotype features regarding the lipoxygenase expression. At the same time urinary leukotriene level tend also to vary in the same patient from a moment to another in relation with the bronchial inflammation, triggers exposure, the moment of controller medication administration, etc.

Urinary leukotriene level is correlated with asthma severity probably in relation with the fact that asthma severity is correlated with the severity of bronchial inflammation and leukotrienes are markers of bronchial inflammation.

CONCLUSIONS

Urinary leukotriene level is not correlated with the presence of atopy, tobacco smoke exposure, FeNO value, controller treatment with LTRA or CSI or asthma control.

Urinary leukotriene level is correlated with asthma severity.

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