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Urinary levels of leukotriene E4 in acute asthmatic children

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Abstract:

Background and Aims: Increased production of cysteinyl leukotrienes (CysLTs) within the airways causes acute asthma. Leukotriene E4 (LTE4) is a potent constricting mediator and is excreted in urine. This study hypothesized that urinary LTE4 (uLTE4) levels would be increased in acute asthma in Egyptian children. The study measured uLTE4 in children with acute asthma and compared them to a matched healthy control group.

Material and Methods: The study included 40 acute Egyptian asthmatic children and 40 age-and sex-matched controls. All candidates were subjected to a complete clinical study (thorough history and physical examination); with emphasis on severity of asthma attack according to Global Initiative for Asthma Guidelines. Measurement of urinary creatinine was performed for all study candidates. Measurement of uLTE4 (pg/mg creatinine) was performed using commercial ELISA kit.

Results: Levels of uLTE4 were significantly higher in cases compared to controls (305.48 ± 34 pg/mg creatinine versus 175.55 ± 79 pg/mg creatinine respectively, 95% CI (17.7; 242.1), $p=0.024$). Levels of uLTE4 were significantly higher in cases with moderate and severe attacks in comparison to those with mild attacks. There was a significant positive correlation between severity of the attack and uLTE4 levels (Spearman's $\rho = 0.446$, $p=0.004$).

Conclusion: Levels of uLTE4 are significantly elevated during acute asthma episodes in children. The significant direct correlation between severity of these attacks and uLTE4 levels make uLTE4 a possible marker for monitoring acute asthma exacerbations in children.

Keywords: Asthma, acute, uLTE4, children

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Introduction

Asthma is considered the most common inflammatory chronic disease in childhood [1-3]. Its exacerbations are one of the most common causes of hospitalization in children and account for approximately 10,000 intensive care unit (ICU) admissions per year in the United States. Factors associated with the development of these acute

exacerbations are still poorly understood [4]. Increased production of cysteinyl leukotrienes (CysLTs) within the airways causes bronchoconstriction. Leukotriene E4 (LTE4) is considered a potent constricting mediator [5]. LTE4 is excreted in the urine, and total urinary LTE4 (uLTE4) represents about 5% of the pulmonary production [6-7]. An association between uLTE4 and the degree of airflow obstruction in stable asthmatic children has been

previously described [8]. However, published studies investigating uLTE4 excretion in acute asthma are still few, and were performed mostly on adults [9]. Therefore, investigations are needed on children in this field in order to build reliable evidence.

Hypothesis

The present study hypothesized that uLTE4 levels would be increased during acute asthma exacerbations in Egyptian children.

Aim of the work

In order to test this hypothesis, measurement of urinary levels of LTE4 of Egyptian children suffering from acute asthma was performed, and was compared to a matched healthy control group. The study also investigated the relations between clinical and laboratory parameters of acute asthma and uLTE4.

Material and Methods

Study design

This is a case-controlled study that was conducted at University Children's Hospital, Faculty of Medicine-Cairo University, Egypt. It was held from May to December 2011. It included 40 acute Egyptian asthmatic children and 40 age-and sex-matched controls. Sample size was calculated using Power and sample size calculator version 3.0.34 [10] based on the following parameters: Study power 83%, case /control ratio of 1:1, standard deviation of 0.3 and type-I error of 5%.

Cases were outpatients suffering from acute asthma and presented to the emergency department of the hospital for management. They were included if they fulfilled these inclusion criteria: aged from 2 to 16 years, had a clinical diagnosis of acute asthma (mild, moderate or severe) according to Global Initiative for Asthma (GINA) Guidelines [11] and were not on corticosteroids or anti-leukotriene medications for 2 weeks before inclusion. Those who did not fulfill the inclusion criteria of refused to participate in the study were excluded. Forty-three healthy non-asthmatic children were included in this study. They were matched to the cases as regards age and sex. Their personal and family medical history and clinical presentation were free of any atopic or allergic diseases.

Clinical and laboratory methods

All candidates were subjected to a complete clinical study (thorough history and physical examination), with emphasis on severity of asthma attack according to GINA Guidelines. Urine samples were taken from each patient at

the onset of admission to the emergency room and before using any medication. Five milliliters of midstream urine were collected in plastic tubes and stored in -20 °C until the end of the study. The same was performed for the controls. Measurement of urinary creatinine was performed for all study candidates. Measurement of uLTE4 (pg/mg creatinine) was performed using a commercial kit (Glory Science Co., Ltd®, USA) using enzyme-linked immunosorbent assay (ELISA) method.

Ethical considerations

The aim and nature of the study was explained for each candidate and / or parent before inclusion. An informed written consent was obtained from parents / surrogates before enrollment. Children old enough were asked for consent. The study design conformed to the requirements of Revised Helsinki Declaration of Bioethics [12].

Statistical analysis

Statistical analysis was performed using the Minitab 16 software (MINITAB® Inc., USA). The following tests were used: Frequency distributions, percentage distributions, means \pm standard deviation, t- test, chi-square test and one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) as a post-hoc test. Spearman's test of correlation was implemented for ranked data. *P*-values less than 0.05 were considered significant, confidence intervals (95% CI) were presented when appropriate.

Results

Cases and controls were matched as regards age, sex and residence. Cases in acute asthma had their heart rate and respiratory rate significantly higher than controls. Sixteen cases had mild acute attacks, 16 had moderate attacks and 8 had severe attacks (Table 1). Levels of uLTE4 were significantly higher in cases compared to controls (305.48 ± 34 pg/mg creatinine versus 175.55 ± 79 pg/mg creatinine respectively, 95% CI (17.7; 242.1), $p=0.024$) (Figure 1). Cases were further stratified according to the severity of asthma attack. Respiratory rate and uLTE4 were significantly higher in cases with moderate and severe attacks in comparison to those with mild attacks (157.50 ± 71.91 pg / mg creatinine in mild attacks versus 312.70 ± 93.40 pg / mg creatinine in moderate attacks and 587.00 ± 696.70 pg / mg creatinine in severe attacks, $p = 0.011$ between mild group and both moderate and severe groups) (Table 2). There was a significant positive correlation between severity of the attack and uLTE4 levels (Spearman's $\rho = 0.446$, $p=0.004$) (Figure 2).

Table 1. Demographic and clinical data of cases and controls

	Cases (40)	Controls (43)	95% CI ^b	P-value
Age (yrs)	5.43 ± 2.97 ^a	6.64 ± 2.82 ^a	(- 2.47 ; 0.06)	0.063
Sex (male/female)	25 /15	24 /19	-	0.536
Residence (urban/suburban/rural)	19 / 6 /15	15 / 8 / 20	-	0.506
Seasonal variation (yes/no)	17 / 23	-	-	-
Family history of asthma (yes/no)	16 / 24	-	-	-
Atopic manifestations (yes/no)	19 / 21	-	-	-
Heart rate (per minute)	97.45 ± 13.53 ^a	81.14 ± 5.81 ^a	(11.66 ; 20.96)	< 0.001
Respiratory rate (per minute)	30.10 ± 6.07 ^a	20.95 ± 2.46 ^a	(7.08 ; 11.22)	< 0.001
Temperature (°C)	37.01 ± 0.22 ^a	36.99 ± 0.33 ^a	(- 0.10 ; 0.13)	0.811
Severity of asthma attack ^c	16 / 16 / 8	-	-	-

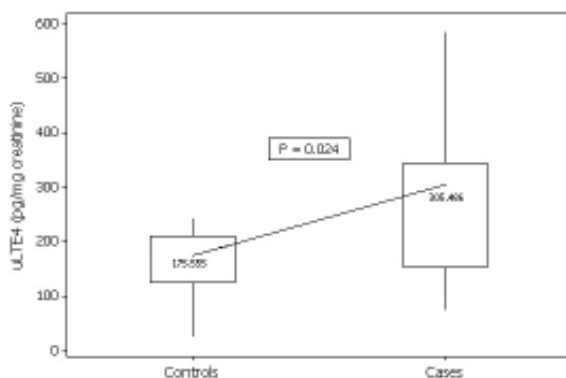
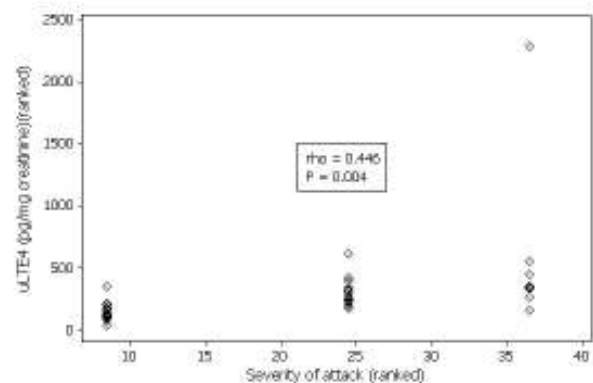
^a Mean ± standard deviation, ^b Confidence intervals at 95 %, ^c Mild, moderate, severe,

^d uLTE4: Urinary leukotriene E4

Table 2. Comparison of cases according to severity of asthma attack

	Mild (16) ^a	Moderate (16) ^a	Severe (8) ^a	P-value
Age (yrs)	5.34 ± 3.09	5.15 ± 2.43	6.18 ± 3.89	0.726
Heart rate (per minute)	94.19 ± 12.63	99.44 ± 12.54	100.00 ± 17.32	0.470
Respiratory rate (per minute)	25.62 ± 4.01	32.18 ± 4.50	34.87 ± 6.77	< 0.001 ^b
Temperature (°C)	37.03 ± 0.23	37.00 ± 0.23	36.97 ± 0.18	0.809
uLTE4 (pg/ mg creatinine) ^d	157.50 ± 71.91	312.70 ± 93.40	587.00 ± 696.70	0.011 ^c

^a Mean ± standard deviation, ^{b, c} Fisher's LSD test showed the significant difference to be between the mild group and both moderate and severe groups. ^d uLTE4: Urinary leukotriene E4.

**Figure 1. uLTE4 levels in cases and controls****Figure 2. Spearman's correlation between severity of attack and uLTE4**

Discussion

We have demonstrated that uLTE4 levels were significantly higher in acute asthmatic children when compared to healthy controls ($p = 0.024$). This agrees with the results of He et al [13] who reported that uLTE4 levels in asthmatic children at the acute and the convalescence phases were significantly higher than those in the control group ($p < 0.01$). Another study [6] investigated uLTE4 levels in preschool children with persistent viral wheeze during an acute attack and in convalescent phase and compared them with normal controls. They reported that levels of uLTE4 were significantly increased in acute persistent viral wheeze. Again, Bizzantino et al [14] studied 205 children aged 2-16 years recruited during an asthma attack and uLTE4 was measured in acute and convalescent samples. They found that uLTE4 levels were higher acutely compared with convalescence ($p = 0.003$). Our findings combined with results of these studies suggest a role for uLTE4 as a marker of asthma exacerbation in children.

We attempted to further determine uLTE4 levels in cases according to severity of their acute attack. Levels were significantly higher in patients having moderate and severe attacks when compared to those experiencing mild attacks ($p = 0.011$). Moreover, there was a significant positive correlation between severity of the attack and uLTE4 levels ($p = 0.004$). Up to the authors' knowledge, these findings were not reported in the previous published studies investigating uLTE4 in acute pediatric asthma. Severien et al results [8] that reported no differences in uLTE4 between the group of mild and the group of moderate to severe asthmatic children are not contradicting with our results, because it was performed on stable asthmatics not in acute exacerbations, and those children were on treatment at the time of inclusion. If further larger studies were conducted and showed similar results, then it would be safe to suggest that uLTE4 levels can be used to monitor the exacerbation and resolution of acute asthma, much like an acute phase reactant.

CysLTs are eicosanoids that have a major role in acute asthmatic exacerbations and in stable asthma [15, 16]. LTC₄, LTD₄ and LTE₄ (formerly the slow reacting substance of anaphylaxis or SRS-A) are formed and secreted during allergic inflammation and induce prolonged, slow contraction of bronchial smooth muscles [17]. Inflammatory cells (mainly mast cells, eosinophils and basophils) aggregate in the airways of asthmatics and synthesize and secrete CysLTs from arachidonic acid by

the enzyme 5-lipoxygenase [18]. LTD₄ and LTE₄ are the extracellular metabolites of LTC₄ and LTE₄ is the most stable and has the longest constrictor effect on smooth muscles [19]. LTE₄ is the only CysLT reliably detectable in biologic fluids, such as plasma, urine or bronchoalveolar lavage of asthmatics. LTE₄ received little attention previously because of its poor binding to "classical" CysLT receptors types 1 and 2 (CysLT 1 R and CysLT 2 R), which led to the assumption that LTE₄ probably was an ineffective end metabolite [20]. However, studies began to discover new receptors selective for LTE₄ e.g. a purinergic receptor (designated P2Y₁₂) and CysLT E receptor (CysLT E R), observed functionally in skin of mice lacking CysLT 1 R and CysLT 2 R. These new discoveries rekindled interest in LTE₄ and its receptors as therapeutic targets not targeted by current CysLT receptor antagonists [20, 21].

The present study supported the relatively few works on uLTE4 in acute asthmatic children, and found a direct correlation between uLTE4 and severity of attacks. However, it had its points of weaknesses like the small sample size, lack of pulmonary function correlations and lack of follow-up to compare between uLTE4 at the acute attack and during convalescence. Nevertheless, the authors think that these would be covered in an upcoming research at the same setting but on a larger scale and with a longitudinal design.

In conclusion, uLTE4 levels are significantly increased during acute asthma episodes in children, with a significant direct correlation between severity of attacks and uLTE4 levels. These make uLTE4 a possible candidate marker for monitoring acute asthma exacerbations in the future.

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