Study of urinary leukotriene E4 levels and total serum IgE levels in children with acute exacerbations of asthma

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Abstract

Background: Cysteinyl leukotrienes are important in asthma pathogenesis. Leukotriene (LT) E4, which is the most stable cysteinyl LT, is excreted in the urine and its concentration is raised in acute exacerbation of asthma. Estimation of urinary levels of LTE4 can thus assess changes in total body cysteinyl LT production.

Objectives: To assess the variation of urinary LTE4 levels in children with asthma during acute exacerbations of varying severity and to see its correlation with the serum IgE levels.

Method: This was a prospective analytical study in which 60 children aged between 3 and 18 years with acute asthma exacerbation formed the study group; 24 age and sex matched children without asthma formed the control group. The urinary leukotriene E4 levels and serum IgE levels were estimated in both groups.

Results: The difference in urinary leukotriene E4 levels between the acute asthma group (288.35pg/mg) and control group (85.71pg/mg) was statistically significant. Among the study group, urinary LTE4 levels in mild, moderate, and severe exacerbations of asthma showed a significant difference ($p<0.0001$). Serum IgE levels in the acute asthma group (541.21 IU/mL) and control group (132.15 IU/mL) showed a statistically significant difference ($p<0.0001$) and it was more in the severe group ($p<0.0001$). A linear correlation was present between urinary leukotriene E4 levels and serum IgE levels among children with acute asthma exacerbation.

Conclusions: In acute exacerbations of asthma, urinary LTE4 and total serum IgE levels were significantly elevated and there was a significant positive correlation between the two.

(Key words: Acute asthma, Urinary leukotriene E4, Total serum IgE, Non-invasive marker)

Introduction

Globally, over the last two decades, incidence of asthma has increased, and asthmatics are at risk for acute exacerbations requiring emergency care[1]. Cysteinyl leukotrienes (cys-LTs) C4, D4, and E4 play an important role in asthma pathogenesis[2,3]. They increase bronchial hyperreactivity and constrict bronchial smooth muscle[4]. LTC4 metabolite is highly unstable and is quickly converted to LTD4. which is further converted to a less potent LTE4, the most stable of the three metabolites and this is excreted in the urine[5,6]. In acute exacerbations of asthma, following allergen/aspirin challenge in aspirin-sensitive individuals, it is reported that urinary LTE4 concentration is significantly raised[6]. Therefore, to assess changes in the rate of total body cys-LT production, estimation of urinary levels of LTE4 can be done[5]. In children with asthma, there is a lot of scope for non-invasive markers like urinary LTE4 levels for assessment of inflammation[2,6]. Various studies have shown that urinary LTE4 levels were significantly higher in subjects with asthma compared to controls[1,2,4,6,7]. However, there are only a few studies regarding urinary LTE4 levels in acute exacerbations and their correlation with total serum IgE levels[2].

Objectives

To assess urinary LTE4 levels in children with asthma during acute exacerbations and to correlate urinary LTE4 levels with total serum IgE levels during asthma exacerbations.

Method

This prospective analytical study was conducted from October 2017 to March 2019 in the paediatric department, JSS Hospital, Mysuru, India. All children aged between 3 and 18 years, meeting the criteria for acute asthma exacerbation according to

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GINA guidelines 2017[8], were included in the study group. Children with other cardiovascular, neurological, respiratory illnesses like pneumonia, bronchiectasis, bronchiolitis and children on leukotriene inhibitors were excluded from the study. Age and sex matched children without asthma formed the control group.

Sampling size:The following formuka was used:
$n = z^2pq/d^2$ where z=1.96 for 95%CI, p=expected true proportion, d=desired precision, q=1-p
Prevalence was obtained from previous community-based studies in children under 12 years, simplified version of the ISAAC questionnaire where prevalence was found to be 5%. Precision 5%.
Estimated sample size n=73.

This was a purposive sampling study consisting of 60 acute exacerbations of asthma and 24 controls, as there were limited studies done on this context concerning the paediatric population. A complete medical history with an emphasis on the symptoms of asthma exacerbation and a detailed drug use history was entered in the proforma. Initial assessment of the cases with acute exacerbration was done using the paediatric asthma severity (PAS) score but children were diagnosed to have asthma based on GINA 2017-2018 guidelines.

All of them were subjected to complete clinical examination and were divided into mild, moderate, and severe exacerbations according to the PAS score[9]. A questionnaire was used to assess asthma control and it was in accordance with the GINA 2017-2018 guidelines. Examination was done by a Paediatric Resident, trained in assessment with PAS score and GINA guidelines. Cases were assessed with targeted medical history based on the questionnaire at the time of arrival in the paediatric emergency department. Allergy history was taken based on questionnaire and no allergy or atopy testing was done. Lung function tests were not part of our protocol and peak expiratory flow rate was not used for the study. Both groups were subjected to the following investigations: At the time of admission, measurement of urinary LTE4 assay was done by sandwich ELISA technique (Cayman Chemicals Ltd) from 5 ml of urine collected in plastic tubes and stored at 5^0C. The total serum IgE levels were measured using the Quantitative ELISA Kit (Bharath chemicals). Laboratory tests were analysed in both medical college accredited laboratory (total serum IgE) and private NABL accredited laboratory (urinary leukotriene E4 levels). Cold chain and storage was done properly. Control children were hospitalised children with non respiratory diseases like typhoid or viral fever and none had atopy or asthma. None of study children were on montelukast as per our exclusion criteria.

Ethical issues: Ethical approval was obtained from the JSS Institutional Ethical Committee (No. JSSMC/PG/4700/2017-18). Assent was obtained from the older children and informed written consent was taken from the parents of the study and control groups of children.

***Statistical analysis*:** Data collected were analysed using SPSS software version 2.1 and values were entered in an Excel sheet. Descriptive statistical analysis was done using mean, median and standard deviation. Inferential statistical tests like independent sample t-test, Mann Whitney test, and one way ANOVA test were applied. The Pearson correlation analysis measured the association between the quantitative variables.

Results

Demographic characteristics of enrolled children are shown in Table 1.

Table 1: ***Demographic characteristics of enrolled children***

Characteristic	Asthma group *(n=60)* Number (%)	Controls *(n=24)* Number (%)	p-value
Age group (years)			
<5	21 (35.0)	05 (20.8)	
5-10	28 (46.7)	12 (50.0)	0.6
>10	11 (18.3)	07 (29.2)	
Gender			
Male	33 (55.0)	13 (54.2)	0.9
Female	27 (45.0)	11(45.8)	

In our study, 47.6% of the children were in the age group of 5-10 years. Males constituted 55% of cases. Half of our cases were on budesonide inhalers. Median levels of urinary LTE4 in the asthma group was 288.35pg/mg and the control group was 85.71pg/mg. There was a statistically significant difference between the two groups (Table 2 and Figure 1).

Table 2: ***Comparision of urinary leukotriene E4 levels and total serum IgE levels among acute asthma in children with exacerbations and controls***

Groups	Urinary LTE4 Pg/Mg					
	Mean	**SD**	**Median**	**Percentile 25**	**Percentile 75**	**P value**
Asthma	378.47	232.22	288.35	200.57	521.31	<0.0001
Controls	88.76	41.31	85.71	65.76	122.25	
	Total Serum IgE Levels IU/ML					
Asthma	561.74	201.36	541.21	289.16	676.15	<0.001
Controls	128.36	40.59	132.15	94.62	161.60	

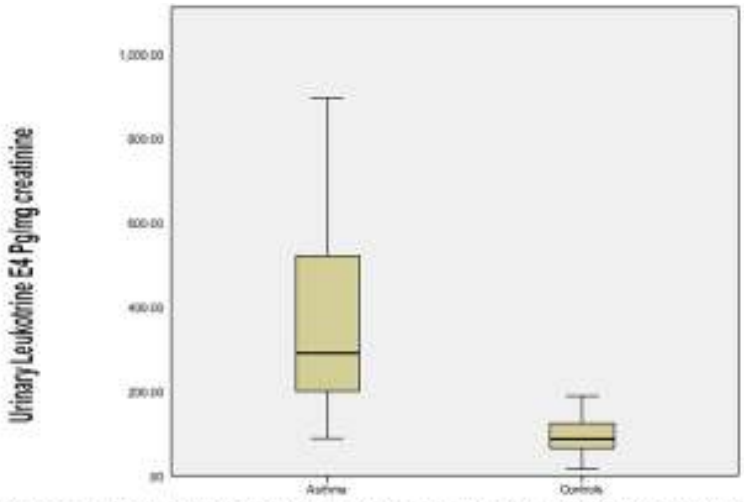

Figure 1: ***Box whisker plot depicting urinary leukotriene E4 levels in both study and control groups***

There was a wide range of variability between the subjects in both groups and the asthma group had more inter-subject variability when compared to the control group (Figure 1).

Out of 60 asthma children, 19 (32%) had a mild exacerbation, 23 (38%) had moderate exacerbation and 18 (30%) had a severe exacerbation. There was a difference in urinary LTE4 levels between mild, moderate, and severe exacerbation groups and the differences were significant (p=<0.0001) [Table 3].

Table 3: ***Urinary leukotriene E4 levels and total serum IgE levels among acute asthma exacerbations in children according to severity***

Acute asthma exacerbations (n=60)	**Urinary leukotriene E4 levels pg/mg creatinine**			**Total serum IgE Levels (IU/mL)**		
Severity	**Mean**	**SD**	**P value**	**Mean**	**SD**	**P value**
Mild (19)	171.91	38.53	<0.0001	340.17	81.68	<0.0001
Moderate (23)	304.81	50.99		501.71	124.30	
Severe (18)	690.63	154.95		983.00	195.71	

There was a significant difference in urinary LTE4 levels between asthmatic group (n=30) on inhaled steroids (263.24±159.78pg/mg) and those who were not on inhaled steroids (n=30) (493.70 ±238.130pg/mg) during acute exacerbation (p<0.0001). Median levels of total serum IgE levels in the asthma group were 541.21 IU/mL and the control group was132.15 IU/mL. There was a statistically significant difference between the two groups (p<0.0001). There was a significant difference in total serum IgE levels between mild, moderate, and severe exacerbation groups and it was more in the severe group. (p<0.0001) [Table 3]. There was a linear correlation between urinary LTE4 levels and serum IgE levels among cases of acute exacerbation of asthma. (Pearson correlation=0.934) (p-value <0.0001) (Figure 2). However, there was no linear correlation between urinary LTE4 levels and serum IgE levels among the controls (p =0.34).

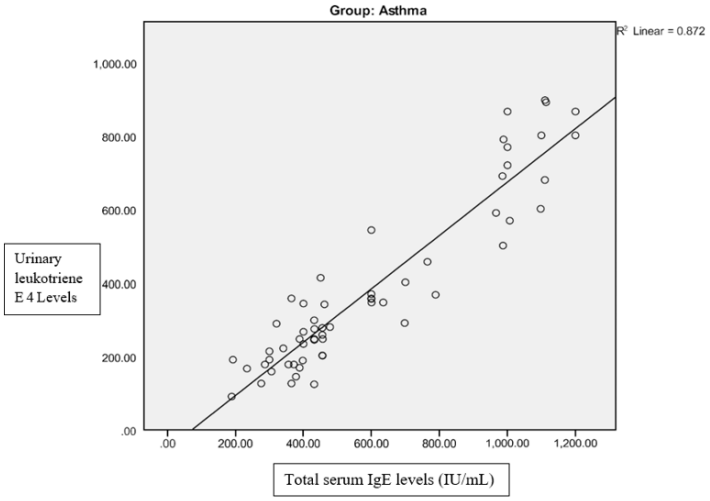

Figure 2: ***Showing linear correlation between urinary leukotriene E 4 levels and serum IgE levels among cases of acute exacerbration of asthma (Pearson correlation=0.934) (p <0.0001)***

Discussion

In young children, diagnosis and treatment of asthma are primarily based on symptomatology and clinical examination[2]. In the present study, out of 60 children with acute asthma, 19 (32%) had mild exacerbation, 23 (38%) had moderate exacerbation and 18 (30%) had severe exacerbation. In a study from Egypt, mild exacerbation of asthma was the most common (55.6%), followed by moderate in 31.7% and severe exacerbation in 12.7%[2]. We found a statistically significant difference in urinary LTE4 levels between acute asthma and control groups and a significant difference in LTE4 levels among mild, moderate, and severe exacerbation groups. Similar observations were made by other workers[1,2,4,6,7,10]. Studies have shown associations between urinary LTE4 levels and acute asthma exacerbations in children and adults[5]. It has been found in a previous study that urinary LTE4 concentrations were significantly higher in asthmatic children than in controls[6]. However, only children with moderate to severe asthma showed significantly higher urinary LTE4 levels compared to controls[6]. Study from Egyptian children has revealed that urinary LTE4 levels were significantly higher in acute asthma exacerbation compared to controls[2]. Another study from Egypt found similar findings in acute asthma children and concluded that urinary LTE4 is a possible marker for monitoring acute asthma exacerbations in children[7].

In a large study involving 146 adults with acute exacerbations of asthma, urinary LTE4 levels were increased during asthma exacerbations followed by a significant decrease during resolution[1]. They also found a significant correlation between the degree of airflow limitation with urinary LTE4 levels during both the exacerbation and resolution periods. This finding suggests that the leukotriene pathway is activated during acute asthma and that leukotrienes play an important role in the pathophysiology of acute asthma[1]. Oosaki *et al*[11] found out that urinary LTE4 levels were significantly high during the acute attack in adults and returned to control levels. Yoshikawa *et al*[4] found that urinary LTE4 levels during an acute attack and stable asthma children were significantly higher than those of the controls. In a study of 60 children with acute asthma, urinary LTE4 levels at the convalescence phase were significantly reduced when compared to the acute phase in asthmatic children[10]. They also noticed a more significant decrease in urinary LTE4 levels in the montelukast treatment group when compared to the conventional treatment group at the convalescence phase[10].

Few of the studies suggested an increase in urinary LTE4 levels are associated with decreased asthma control[5]. However, Sampson *et al*[12] have observed a persistent increase in urinary LTE4 concentrations one month after a severe asthmatic episode and opined that leukotriene production is related to chronic inflammation rather than to acute bronchoconstriction. In a study on chronic cough, there was no significant difference in urinary LTE4 levels between children with chronic cough and controls[13]. Studies have shown that increased urinary LTE4 levels are seen with a variety of asthma triggers like particulate air pollution, tobacco smoke exposure, and respiratory infections[5]. Rabinovitch *et al*[3] observed that, urinary LTE4 was a significant predictor of exacerbation risk in children with asthma exposed to second-hand tobacco smoke. In their study urinary LTE4 levels at or above 106 pg/mg had 67% sensitivity and 100% specificity for predicting children who required emergency care due to exacerbations[3]. Oommen and Grigg[14] observed that preschool children with viral wheeze had higher urinary LTE4 compared to normal controls. They opined that increased cysteinyl leukotriene production is associated with high serum IgE in preschool children with acute wheeze due to virus[14].

Severin C, *et al*[6] found a clear relationship between urinary LTE4 levels and (FEV1), FVC, MEF25, ITGV, and RV. The authors concluded that urinary LTE4 levels may be a valuable predictor of small airway disease in asthmatic children[6]. However, we could not do lung function tests as most of the children in our group were not able to perform lung function tests properly. They also suggested using urinary LTE4 levels as a complement to lung function tests, so that it may help to find the optimal therapy for asthmatic children[6]. In the present study, there is a statistically significant difference in total serum IgE levels between acute asthma and control groups. There was a significant difference in IgE levels between mild, moderate, and severe exacerbation groups and it was more in the severe group. Similar to our observations, a study from Egypt also noticed a significant increase in total serum IgE levels in acute asthma cases when compared to the controls. Total serum IgE levels positively correlated with asthma severity[2].

The present study revealed a positive correlation between total serum IgE levels and LTE4 levels among asthmatic children during an acute exacerbation. Similar observations were noted by a study from Egypt[2]. Chiu C-Y *et al*[15] observed that, out of 182 children increased level of total serum IgE or urinary LTE4 was significantly associated with the risk of developing allergic rhinitis and asthma at 3 years of age. They concluded that urinary LTE4 levels appear to be highly associated with IgE sensitization and its related allergic airway diseases after the age of 2 years[15].

Urinary LTE4 is a biomarker of exposure triggers of atopic and nonatopic asthma, new asthma exacerbations, aspirin-exacerbations and early development of childhood atopy[5]. Urinary levels of LTE4 were not altered by treatment with corticosteroids in acute asthma or by inhaled corticosteroids in moderate to severe asthma[1]. However, we observed a significant difference in urinary LTE4 levels between the asthmatics group on steroid inhalers for at least 3 months and those who were not on corticosteroids. Vachier *et al*[16] recorded higher levels of LTE4 in chronic severe asthma patients compared to mild, moderate asthma and healthy controls despite chronic systemic treatment with glucocorticoids. Hoffman and Rabinovitch *et al* opined that we can use increased urinary LTE4 and the ratio of urinary LTE4 to fractionated exhaled nitric oxide to predict differential responses favouring initiation or step-up therapy with leukotriene receptor antagonists.

In the present study, there were some limitations. We did not estimate the basal levels of urinary LTE4 in stable asthma subjects before exacerbation. Though we had 60 patients in the study group, we could get only 24 controls as we were very stringent in selecting the controls. Study constituted of 50% asthmatic subjects who were on controllers and most of them were on controllers at least for a period of 4 months

Conclusions

In acute exacerbation of asthma, urinary LTE4 and total serum IgE levels were significantly elevated and there was a significant positive correlation between the urinary LTE4 levels and total serum IgE levels. Therefore urinary LTE4 can be used as an effective, non-invasive marker in monitoring the severity of acute exacerbations of asthma in children.

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