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Brief communication (Original)

Urinary leukotriene E4 in children with wheezing associated respiratory illness from influenza infection

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Background: Influenza virus can cause bronchiolitis and recurrent wheezing in young children. Although the pathophysiology is unclear, leukotriene is thought to play an important role.
Objectives: We conducted a prospective case-control study to measure urinary leukotriene E4 (uLTE4) levels in children with wheezing associated respiratory illness (WARI) from influenza infection, compared to children without respiratory tract infection and to identify the association between clinical features and uLTE4 levels in influenza-infected children.
Methods: Nasopharygeal secretions from patients less than 6-years-old hospitalized due to WARI were tested for influenza virus by RT-PCR. The uLTE4 levels were measured in the patients with influenza infection and compared with normal controls. The correlation between clinical features and uLTE4 levels was also studied.
Results: The study included 10 patients with influenza infection (median age 23.5 months) and 10 children as control group (median age 23 months). The uLTE4 levels were not significantly different between influenza and control group; 191.63 ng/mM creatinine (IQR 162.57-244.25) vs. 132.32 ng/mM creatinine (IQR 93.79-215.65), respectively (p = 0.096). There was no significant correlation between uLTE4 levels and clinical features including pulmonary index, eosinophil count, and length of hospital stay.
Conclusion: The uLTE4 level does not increase in pediatric patients with wheezing associated influenza infection and does not correlate with clinical features during infection. Although there is a trend towards increased uLTE4 in influenza patients and a correlation to the pulmonary index, the small sample size may have altered the significance of these results.

Keywords: Inflammatory mediator, influenza virus, leukotriene, lower airway obstruction, viral respiratory tract infection

Influenza virus is a common cause of respiratory tract infection among children with pneumonia, bronchiolitis and may cause recurrent wheezing [1-6]. Many studies have tried to discover the pathophysiology and mechanisms that cause lower airway obstruction in children with influenza virus infection. Some of these have detected many cytokines during the infection, including leukotrienes [7-12], which play an important role in children with wheezing associated respiratory illness (WARI) from respiratory syncytial virus (RSV) infection. However, previous studies have been in animals [7, 8], in vitro tests [9, 10] or in adults [11, 12]. This study tries to learn whether leukotriene plays an important role in children with WARI from influenza infection.

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Materials and methods

A prospective case-control study in children admitted to King Chulalongkorn Memorial Hospital with lower respiratory tract infection was conducted between February 2006 and January 2007. All children were less than six years of age and presented with lower airway obstruction during admission. Patients who had previously been diagnosed as having asthma or reactive airway diseases, who had taken antileukotrienes within one week of admission, or patients with nephrologic/urologic diseases, were excluded. All eligible patients underwent nasopharyngeal aspiration in the first two days of

admission. The secretions were then tested for influenza A and influenza B virus by reverse transcriptase polymerase chain reaction, tested for respiratory syncytial virus (RSV), and metapneumovirus by polymerase chain reaction. Patients with a positive test for influenza virus (A or B), negative test for RSV, and metapneumovirus were enrolled.

Within the first two days of admission, urine was collected from each eligible patient and stored at-80°C. Only the urine from influenza patients was tested for leukotirene E4 (LTE4) level by enzyme-linked immunosorbent assay (ELISA) with a commercial kit (ACE™ LTE4 ELISA kit; Cayman Chemicals, Ann Arbor, Michigan, USA) and for creatinine level, then the uLTE4 levels were determined as nanograms of uLTE4 per millimole of creatinine (ng/mM.Cr).

Data collected during admission, including age, sex, body weight, birth weight, gestational age at birth, breastfeeding period, physician-diagnosed cow's milk protein allergy, allergic rhinitis, atopic dermatitis, family history of atopic diseases, passive smoking, furred pet, severity of lower airway obstruction assessed by pulmonary index (**Table 1**), length of hospital stay, and eosinophil count.

Children visiting the well-baby clinic of King Chulalongkorn Memorial Hospital during the study period were selected as control group. Eligible children had to be age and sex-matched to each influenza patients, with no underlying respiratory or nephrologic/urologic diseases and were not to have received antileukotriene within 25 days before the sampling date. The urine was collected, stored, and tested in the same way as in the influenza group.

Data analysis

Continuous data are presented as median and analyzed with Mann-Whitney U test. Non-continuous data presented as percentage and analyzed with X^2 test. The data were collected and analyzed using SPSS version 13, p value <0.05 considered significant.

Results

During the study period, nasopharyngeal secretions for influenza RT-PCR were collected from 142 eligible patients, ten were positive for influenza A virus. No influenza B virus was detected, and the ten cases with positive influenza A result were recruited into the study group.

Patient characteristics

The patient characteristics of the influenza and control groups are presented in **Table 2**. Age, male:female ratio, birth weight, duration of breastfeeding between the two groups was comparable. The atopic history (cow's milk protein allergy, atopic dermatitis, family history of atopy) and the environment data (passive smoking, furred pets) were not significantly different between the two groups. The male:female ratio was 4:1 in both groups and the ratio of passive smoking was approximately 30 to 40%.

Clinical features at presentation: influenza group

The average pulmonary index at presentation was 6.5 (interquartile range 4.5 to 10.0; the maximum score is 15, representing the highest severity). The length of hospital stay ranged from 4 to 13 days, with a median of 6 days. None had respiratory failure. The eosinophil count ranged from 0 to 141 cell/cu.mm. Three of the ten patients had an eosinophil count of 0. The median eosinophil count was 23 cell/cu.mm, which is considered normal.

Effect of wheezing associated respiratory illness from influenza infection on uLTE4

The median uLTE4 level in the influenza group was higher than in the control group but without significant difference (191.63 vs. 132.32 ng/mM.Cr respectively; $p = 0.096$) as shown in **Figure 1**.

Table 1. Pulmonary index [13]

Parameters / Scores	0	1	2	3
Respiratory rate	<30	31-45	46-60	>60
Oxygen saturation (%)	99-100	96-98	93-95	<93
Inspiration:expiration	2:1	1:1	1:2	1:3
Accessory muscle use	None	Mild	Moderate	Severe
Wheezing	None	End expiration	Entire expiration	Inspiration and expiration or minimal air entry

Table 2. Baseline characteristics of influenza group and control group

Baseline characteristics	Influenza group (n=10)	Control group (n=10)	*p* value
Age (months)	23.5	23.0	0.820
(IQR)	*(13.3-37.8)*	*(12.0-45.5)*	
Sex (M/F)	8/2	8/2	1.000
Birth weight (grams)	3,020	3,200	0.177
(IQR)	*(2,885-3,425)*	*(3,000-3,962)*	
Duration of breastfeeding (months)	2.5	4.0	0.118
(IQR)	*(0.0-3.8)*	*(2.0-7.5)*	
Cow's milk protein allergy (%)	20	0	0.136
Atopic dermatitis (%)	10	0	0.305
Family history of atopy (%)	20	20	1.000
Passive smoking (%)	40	30	0.639
Furred pet (%)	10	40	0.121

Table 3. Clinical characteristics at presentation: influenza group

Clinical characteristics	Median *(IQR)*
Pulmonary index at presentation	6.5 *(4.5-10.0)*
Eosinophil count (cell/cu.mm.)	23.0 *(0.0-62.5)*
Length of hospital stay (days)	6.0 *(4.8-7.0)*

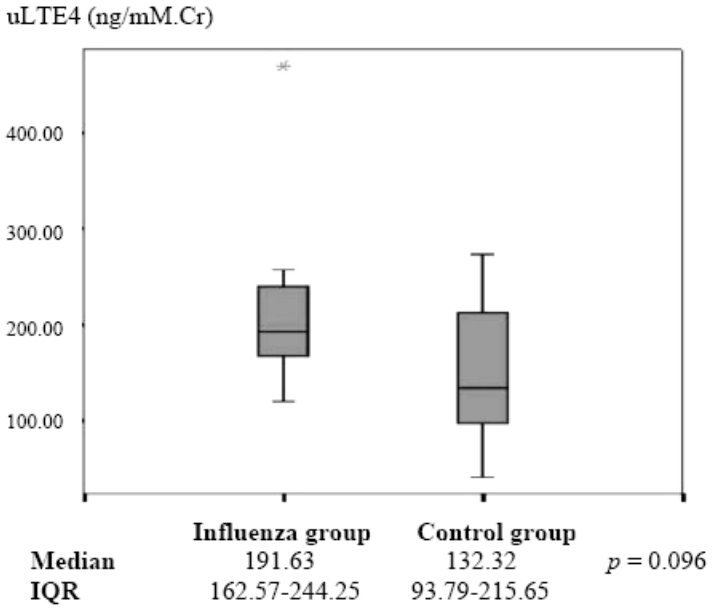

Figure 1. uLTE4 level between influenza and control group

uLTE4 level and correlation with clinical features

The pulmonary index at presentation correlated positively with the uLTE4 level but without statistical significance, $p = 0.063$ (**Table 4** and **Figure 2**).

The eosinophil count and length of hospital stay did not correlate with uLTE4 as can be seen in **Table 4**.

Table 4. Correlations between clinical features and uLTE4 level in influenza group

Clinical features	Pearson's correlation coefficient	*p* value
Pulmonary index at presentation	0.606	0.063
Eosinophil count	-0.473	0.168
Length of hospital stay	-0.055	0.880

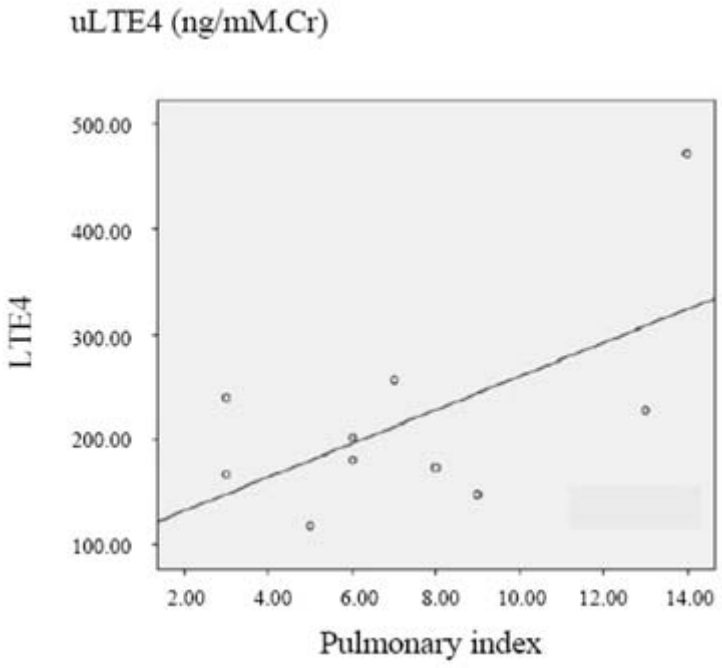

Figure 2. Correlation between pulmonary index at presentation and uLTE4 level in the influenza group uLTE4 (ng/mM.Cr)

Discussion

There have been several studies describing the relationship between respiratory tract infection from influenza virus and the level of leukotrienes. However, they have been done in animals [7, 8], in vitro [9, 10], in adults with experimental infection [11] or in asthmatic adults [12]. This is a study of children with wheezing associated respiratory illness from naturally-infected influenza virus. The study showed that influenza virus infection could cause lower airway obstruction. Previous studies showed that 25 to 38% of children with influenza virus infection had wheezing [4, 5].

LTE4 is the final cysteinyl leukotriene in the 5-Lipoxygenase pathway and is the most stable but least active form of the cysteinyl leukotrienes. The precursors of LTE4, leukotriene C4 and leukotriene D4 are potent bronchoconstrictors. They can stimulate mucus release into the airway and increase microvascular permeability. Because total leukotrienes in the lung are about 20-fold of the LTE4 level in urine, the concentration of uLTE4 is used as an estimation of total leukotriene production [14, 15].

In previous studies, among children with WARI from influenza virus, the male:female ratio was around 2-3:1 [16, 17]. In this study, the ratio is 4:1. The reason why boys are more susceptible is still unclear.

Few patients presented with severe respiratory manifestation. The average pulmonary index score is 6.5, which correlates to the overall clinical outcome. Only one patient was admitted for more than seven days and no patient stayed for more than 14 days. There was no respiratory failure or PICU admission in any patient and this is comparable to previous studies [5, 6, 18] where most respiratory illnesses from influenza virus in small children were not severe or in other words, less severe than RSV.

We found that the uLTE4 level in the influenza group was higher (191.63 ng/nM.Cr) than the control group (132.32 ng/mM.Cr) but without significant

difference. However, considering the small sample size (10 cases per group) with the *p* value 0.096, the uLTE4 level may be indeed significantly higher in the influenza-infected WARI. Oommen A et al. [16], and Oymar K et al. [19], found an increase in uLTE4 in children with WARI, whatever the pathogen (RSV or non-RSV). The study from Yasuda et al. [12] showed that in adults with asthmatic attacks from viral respiratory tract infection, of which influenza was the largest causative agent, the uLTE4 was significantly increased in comparison with the same asthmatic adults without attack. However, the previous study from Balfour-Lynn IM et al. [20], in 31 infants with WARI failed to show an increase in uLTE4 during the attack, compared to the recovery period.

The average eosinophil count in the influenza group was within normal limit (median 23cell/cu.mm.) comparable to Oh JW et al. [17] who found an average eosinophil count 36.1 cell/cu.mm in the influenza group. Furthermore, the uLTE4 did not correlate with the eosinophil count, which is similar to previous studies [19, 21, 22]. These results may imply that eosinophil does not play an important role in lower airway obstruction during influenza infection in children.

FEV1 is a good parameter to evaluate the severity of lower airway obstruction. However, all of our study patients are younger than 4-years-old and not suitable for FEV1 evaluation. Therefore, pulmonary index was used instead of FEV1 and it was found that the pulmonary index had a trend to correlate with the uLTE4 level, *p* value 0.063. According to previous studies in older children that evaluated FEV1 and uLTE4, two studies showed positive correlation [23, 24] but another did not [21]. Kott KS et al. [25] found a positive correlation between the uLTE4 level and length of hospital stay. However, this correlation does not exist in our study.

Conclusion

This study shows that WARI from influenza infection, similar to RSV, can increase uLTE4 levels. In addition, the uLTE4 level seems to correlate with the clinical severity of the lower airway obstruction. Although these findings are not significant, this may be due to sample size. A further study should be conducted in a larger population to confirm the hypothesis that leukotriene plays an important role in the pathophysiology of WARI from influenza infection, as it does in RSV. This may suggest use of antileukotriene in this population.

The authors have no conflicts of interest to declare.

References

1. American Academy of Pediatrics. Influenza. In: Pickering LK, ed. Red Book: 2003 report of the committee on infectious diseases. 26th ed. Elk Grove Village, IL. American Academy of Pediatrics; 2003: 382-91.
2. Glezen WP. Viral pneumonia. In: Chernick V, Boat TF, eds. Kendig's disorders of the respiratory tract in children. Philadelphia: WB Saunders, 1998:518-25.
3. Eriksson M, Bennet R, Nilsson A. Wheezing following respiratory tract infections with respiratory syncytial virus and influenza A in infancy. Pediatr Allergy Immunol. 2000;11:193-7.
4. Calvo Rey C, Garcia Garcia ML, Casas Flecha I, Martin del Valle F, Centeno Jimenez M, Perez-Brena P. Influenza virus infections in infants aged less than two years old. An Pediatr (Barc). 2005; 63:22-8.
5. Wolf DG, Greenberg D, Kalkstein D. Comparison of human metapneumovirus, respiratory syncytial virus and influenza A virus lower respiratory tract infections in hospitalized young children. Pediatr Infect Dis J. 2006; 25:320-4.
6. Vega-Briceno LE, Potin M, Bertrand P, Sanchez I. Clinical features of respiratory infections due to influenza virus in hospitalized children. Rev Med Chil. 2005; 133: 911-8.
7. Hennet T, Ziltener HJ, Frei K. A kinetic study of immune mediators in the lungs of mice infected with influenza A virus. J Immunol. 1992; 149:932-9.
8. Wong SS, Hyde J, Sun NN, Lantz RC, Witten ML. Inflammatory responses in mice sequentially exposed to JP-8 jet fuel and influenza virus. Toxicology. 2004; 197:139-47.
9. Clementsen P, Bisgaard H, Pedersen M. Staphylococcus aureus and influenza A virus stimulate human bronchoalveolar cells to release histamine and leukotrienes. Agents Actions. 1989; 27:107-9.
10. Huftel MA, Swensen CA, Borcherding WR. The effect of T-cell depletion on enhanced basophil histamine release after in vitro incubation with life influenza A virus. Am J Respir Cell Mol Biol. 1992; 7:434-40.
11. Gentile DA, Fireman P, Skoner DP. Elevations of local leukotriene C4 levels during viral upper respiratory tract infections. Ann Allergy Asthma Immunol. 2003; 91: 270-4.
12. Yasuda H, Suzuki T, Zayasu K, et al. Inflammatory and bronchospastic factors in asthma exacerbations caused by upper respiratory tract infctions. Tohoku J Exp Med. 2005; 207:109-18.

13. Becker AB, Nelson NA, Simons ER. The pulmonary index: assessment of a clinical score for asthma. Am J Dis Child. 1984; 138:574-6.
14. van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC II, Welliver RC. Increased production of IFN-gamma and cysteinyl leukotrienes in virus-induced wheezing. J Allergy Clin Immunol. 1999; 103: 630-6.
15. Barnes RMR. Principles and interpretation of laboratory tests for allergy. In: Kay AB, ed. Allergy and allergic diseases. Oxford: Blackwell Science, 1997:997-1006.
16. Oommen A, Grigg J. Urinary leukotriene E4 in preschool children with acute clinical viral wheeze. Eur Respir J. 2003; 21:149-54.
17. Oh JW, Lee HB, Park IK, Kang JO. Interleukin-6, interleukin-8, interleukin-11, and interferon-gamma levels in nasopharyngeal aspirates from wheezing children with respiratory syncytial virus or influenza A virus infection. Pediatr Allergy Immunol. 2002; 13: 350-6.
18. Gern JE, Martin MS, Anklam KA. Relationships among specific viral pathogens, virus-induced interleukin-8, and respiratory symptoms in infancy. Pediatr Allergy Immunol. 2002; 13:386-93.
19. Oymar K, Halvorsen T, Aksnes L. Mast cell activation and leukotrine secretion in wheezing infants. Relation to respiratory syncytial virus and outcome. Pediatr Allergy Immunol. 2006; 17:37-42.
20. Balfour-Lynn IM, Valman HB, Wellings R, Webster AD, Taylor GW, Silverman M. Tumour necrosis factor-alpha and leukotriene E4 production in wheezy infants. Clin Exp Allergy. 1994; 24:121-6.
21. Christie PE, Yntema JL, Tagari P, Ysselstijn H, Ford-Hutchinson AW, Lee TH. Effect of altitude on urinary leukotriene E4 excretion and airway responsiveness to histamine in children with atopic asthma. Eur Respir J. 1995; 8:357-63.
22. Takahashi Y, Ichikawa M, Nawate M, Kamoshida H, Shikano T. Clinical evaluation of urinary leukotriene E4 levels in children with respiratory syncytial virus infection. Arerugi. 2003; 52:1132-7.
23. Rabinovitch N, Zhang L, Gelfand EW. Urine leukotriene E4 levels are associated with decreased pulmonary function in children with persistent airway obstruction. J Allergy Clin Immunology. 2006;118:635-40.
24. Severien C, Artlich A, Jonas S, Becher G. Urinary excretion of leukotriene E4 and eosinophil protein X in children with atopic asthma. Eur Respir J. 2000; 16: 588-92.
25. Kott KS, Salt BH, McDonald RJ, Jhawar S, Bric JM, Joad JP. Effect of secondhand cigarette smoke, RSV bronchiolitis and parental asthma on urinary cysteinyl LTE4. Pediatr Pulmonolo. 2008; 43:760-6.