Urinary Leukotriene E4 Level in Non-Allergic Thai Young Children

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Background: *Urinary leukotriene E4 (uLTE4) concentration represents body leukotriene synthesis. The level increases especially in respiratory allergic diseases. Researches of leukotriene production role in children with respiratory allergic diseases required the normal levels in non-allergic children as references.*
Objective: *The study was to assess the reference values of uLTE4 in non-allergic Thai children measured by competitive enzyme-linked immunosorbent assay (ELISA) technique.*
Material and Method: *Children who were admitted for elective surgery, aged 6 months to 5 years, were included in the study. Subjects who had acute illness, respiratory allergy (allergic rhinitis and asthma), and chronic diseases were excluded. Morning urine (5 ml) was collected for uLTE4 analysis by competitive ELISA technique.*
Results: *There were 36 urinary samples from 36 subjects aged 6-60 months with 26 boys and 10 girls. The mean of uLTE4 concentration was 619.73±701.32 pg/ml and the mean of uLTE4 concentration adjusted for urinary creatinine concentrations was 1,328±788.54 pg/mg creatinine. The mean of uLTE4 concentration in boys and girls were 1,349±817.10 pg/mg creatinine and 1,275±747.79 pg/mg creatinine respectively. The mean of uLTE4 concentration adjusted for urinary creatinine concentrations were 1,363±886.65, 1,384±771.81 and 1,223±773.16 pg/mg creatinine for children with the age of 6-18, 19-36 and 37-60 months respectively.*
Conclusion: *The concentrations of uLTE4 and uLTE4 per creatinine were presented by age group in Thai very young children with non-respiratory disease. The concentrations were elevated in younger age group.*

Keywords: *Urine leukotriene E4, Creatinine, Reference, Children, Non-allergic*

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Leukotriene has been synthesized by cells including mast cells, eosinophils, basophils, macrophages, monocytes and neutrophils[1]. Leukotriene is raised from the split of the membrane, which is a phospholipid. It has been activated by the enzyme phospholipase A_2, and then released arachidonic acid (polyunsaturated 20 carbon fatty acid). Once arachidonic acid (AA) is released from the membrane, it is digested by several enzymes. If AA is digested by arachidonate 5-lipoxygenase (5-LO) enzyme, it's yield would be Leukotriene[2]. Properties of the following metabolite groups such as Leukotriene A4 (LTA4, unstable epoxide) and Leukotriene B4 (LTB4, chemo-attractant for neutrophils acting) consist of a combination of white blood cells. The other groups such as Leukotriene C4 (LTC4), Leukotriene D4 (LTD4) and Leukotriene E4 (LTE4) are correctively referred to as Cysteinyl leukotriene (Cys-LT) which is the most effective way of leukotriene vascular contraction, increase vascular permeability and induce bronchospasm conditions[3]. Leukotrienes (LTs) are inflammatory mediator; they can control the response of the immune system. LTs regulate lipid signaling through both autocrine signaling and paracrine

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signaling. It follows that LTs are able to predict the severity of the disease, especially diseases related to the respiratory system such as asthma and allergic rhinitis[4].

Pathway of LTs is starting from 5-LO converts AA into LTA4 with 5-Hydroperoxyeicosatetraenoic acid (5(S)-HPETE) as an intermediate mediator. The conjugation of glutathione to LTA4 results in the formation of LTC4; which is rapidly metabolized to LTD4 and LTE4. They are found to retain biological activity: LTD4 triggers construction in smooth muscle lining the bronchioles. LTD4 is detected in the fluid of the respiratory tract illness, asthma and rhinitis, but cannot be detected in urine. In the reverse direction, LTE4 is detected in the urine, it is relatively stable and accumulated in breath condensation, in plasma and in urine, making it's dominate. Therefore, measurement of LTE4, especially in the urine, is commonly monitored in clinical research studies[5].

Dal Negro, Visconti et al reported, atypical value for urine LTE4 in 2011. They processed by an immuno-enzymatic method, in four subject different class of age (0-14, 15-40, 40-60, >60 years)[6]. The study reported that uLTE4 levels were significantly different in each classes of age being higher in younger subjects (67.1$\pm$33.4, 69.8$\pm$27.5, 57.1$\pm$25.4 and 45.1$\pm$24.9 pg/ml). Moreover, Barry Kay and Allen Kapan reported that the uLTE4 levels in the Aspirin sensitive asthma (ASA) were 101 pg/mg creatinine, compared to 43 pg/mg creatinine in non-ASA sensitive and 34 pg/mg creatinine in normal control[7].

In 2003, Rubinsztajn R et al reported that, concentration of uLTE4 was 416.6$\pm$374.4 pg per ml in aspirin asthma patient group and 181.6$\pm$55.75 pg per ml in normal group[8]. In 2012, Brockmann PE et al also reported the effect of exercise on the uLTE4 profile. Urinary LTE4 concentration was measured by enzyme immunoassay and adjusted by urinary creatinine concentrations. Median concentration of LTE4 of the pretest was 17.82 (7.58-90.23) pg/ml in allergic group and 17.24 (4.64-64.02) pg/ml in non-allergic group (controls)[9], and result of the posttest was not different. As mentioned above, urinary LTE4 may be a promising non-invasive option to be used as marker in children; it can represent a useful index of the events underlying the airway inflammatory response during allergen challenge. Since references for Thai very young children of uLTE4 concentration in non-allergic children were still not available, this study aims to assess the reference values for urine LTE4 in non-allergic Thai lower 5 years old children.

Material and Method

Subjects

Children admitted for elective surgery, aged 6 months to 5 years, were included in the study with written informed consent from the parents. Subjects who have acute illness, respiratory allergy (allergic rhinitis and asthma), and chronic diseases including congenital heart diseases, immunodeficiency disease, cerebral palsy, chronic kidney disease, chronic liver disease, bronchopulmonary dysplasia, lobar/bacterial pneumonia were excluded from the study.

Sample preparation and uLTE4 assay

Morning urine (5 ml) was collected and centrifuged at 4,000 rpm (Hettich; universal 320 R) for 15 minute at 4°C. The supernatant was collected and stored at -80°C until used. The concentration of uLTE4 was measured by commercially available enzyme-linked immunoassay kits (The Invitrogen Human Leukotriene E4 ELISA Kit, Invitrogen) according to the manufacturer's instruction. Briefly, samples (100 µl) or standards were added to wells in duplicate. LTE4 alkaline phosphatase tracer (50 µl) was added to each well except the blank wells and total activity (TA) wells. LTE4 antiserum (50 µl) was added to each well except the blank wells, TA wells and non-specific binding (NSB) wells. Cover each plate with plate cover for 2 hours (incubate) at room temperature on an orbital shaker. Wells were then washed five times with wash buffer and para-Nitrophenyl phosphate (pNPP) solution (200 µl) added to each well including blank and TA wells. Add LTE4 alkaline phosphatase tracer (5 µl) to TA wells. Cover the plate and allowed developing in the dark on an orbital shaker. A stable yellow-colored product was produced that was proportional to the amount of enzyme present. The absorbance (412 nm) was read after 90 minutes and the absorbance of the sample were compared with those of standards by a computer program using a 4-parameter logistic or a log-log it curve fit for the calculation of sample LTE4. The uLTE4 concentrations were reported as pg/ml. Urine creatinine concentration was measured for adjusting the uLTE4 levels.

Data analysis

Children were categorized into 3 age groups including 6-18, 19-36 and 37-60 months. The means of uLTE4 concentration in pg/ml and standard deviations were reported, for all children and children in each age group. The adjusted means of uLTE4 concentration for urine creatinine in pg/mg creatinine and standard

deviations were also reported.

Results

There were 36 non- respiratory allergy children, 26 boys and 10 girls, in this study. Their mean age was 29.81±14.97 months. The mean uLTE4 concentration was 619.73±701.32 pg/ml. According to 3 age groups, the mean uLTE4 concentrations were 362.32±315.35, 497.77±423.30 and 1,032.36±1,052.66 pg/ml for children aged 6-18, 19-36, 37-60 months respectively (Table 1).

The mean uLTE4 concentrations adjusted by urinary creatinine concentrations were 1,328±788.54 pg/mg creatinine (1,349±817.10 pg/mg creatinine for boys and 1,275±747.79 pg/mg creatinine for girls). The adjusted (by urinary creatinine) mean uLTE4 concentrations were 1,363±886.65, 1,384±771.81 and 1,223±773.16 pg/mg creatinine for children aged 6-18, 19-36 and 37-60 months respectively. The concentrations of uLTE4 per creatinine were particularly elevated in younger children (Table 2).

Discussion

Cysteinyl leukotrienes have been mainly studied for their role in allergic respiratory disease and uLTE4 concentrations is a good, simple non-invasive marker for monitoring and follow-up of the inflammatory process in children. But it uses the high cost of this test so it is still limited in clinical practice.

LTE4, an extracellular metabolite, has been proved to be stable and detectable in urine analysis. Urinary LTE4 excretion is a good marker of the rate of total body production of LTs[10]. It can represent as a useful index of the events underlying the airway inflammatory responses such as asthma and allergic rhinitis. The normal values of LTE4 concentration in Thai very young children are still unknown. But the prevalence of asthma, allergic Rhinitis and Eczema in young children in Thailand (Khon Kaen) is increasing every year[11]. The reference study is essential since it has not yet been reported in Thai children. We found that the mean uLTE4 concentration is 1,328±788.54 pg/mg creatinine and when classified a group, they were decreased by increasing age, which is consistent with the previous reports[6].

Our results are similar to the study by Shahid Sheikh et al[12] that the mean of LTE4 is higher than the other reported. Because the samples were collected from the very young children compared to the other studies reported. Shahid Sheikh et al reported the mean uLTE4 was 2,800±2,080 pg/mg creatinine in infants without brochopulmonary dysplasia (BPD). It is possible that very young children when measuring uLTE4 is increased similar as Dal Negro et al reported, that the mean uLTE4 concentrations tend to a slight decrease with the increase of the subject' age[6]. But the report of Dal Negro RW el al was based on 4 age groups (0-14, 15-40, 41-60 and more than 60 years) and found the mean of uLTE4 levels was 67.1±33.4 pg/ml in the youngest group (n = 12) when to compared with our study (n = 36) the mean of uLTE4 concentrations was 619.73±701.32 pg/ml. This may be due to LT incomplete metabolism, or the genetic of non-allergic subjects in Asia. However, this study showed that the mean urine creatinine was 44.83±36.63 mg/dl. The uLTE4 concentration is accurate only in children patients with normal kidney function. Therefore, this study has

Table 1. Urinary leukotriene E4 concentration in non-allergic Thai very young children with different age groups

Age group (month)	Number	Mean uLTE4 ± SD (pg/ml)
6-18	11	362.32±315.35
19-36	14	497.77±423.30
37-60	11	1,032.36±1,052.66
Total	36	619.73±701.32

uLTE4 = urinary leukotriene E4

Table 2. Urinary leukotriene E4 concentration adjusted for urinary creatinine concentrations in non-allergic Thai very young children with different age groups

Age groups (month)	Urine creatinine mean concentration (mg/dl)	Mean uLTE4/creatinine (pg/mg creatinine)	
		Mean ± SD	Range
6-18	25.08±16.80	1,363±886.65	252-2,839
19-36	38.86±32.46	1,384±771.81	402-2,790
37-60	72.19±41.73	1,223±773.16	206-2,336
Total	44.83±36.63	1,328±788.54	206-2,839

adjusted the concentration of uLTE4 by urine creatinine to get more accurate measures regardless of kidney impairment. However of this study is weaknesses small sample size and lack of follow-up to compare between uLTE4 at different time such as week by week in each sample. For the further research, we plan to investigate uLTE4 concentration adjusted by urine creatinine in asthmatics (wheezing) Thai very young children and obstructive sleep apnea Thai children. The future and present studied will use to take more opportunity; such as monitor disease, control and aid in the prognosis of the respiratory disease in the very young children.

What is already known on this topic?

Urinary Leukotriene E4 (uLTE4) could be used as a non-invasive marker in children with airway inflammatory diseases, especially for allergic respiratory diseases. Urinary LTE4 levels of normal European individuals has been reported.

What is this study add?

Urinary LTE4 levels of Thai young children without allergic diseases is reported. The concentrations were elevated in younger age group.

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ระดับ Leukotriene E4 ในปัสสาวะของเด็กเล็กที่ไม่มีโรคภูมิแพ้

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ภูมิหลัง: ระดับ Leukotriene E4 ในปัสสาวะสามารถใช้เป็นตัวแทนแสดงถึงการสร้าง Leukotriene ทั้งหมดในร่างกายได้ ซึ่งค่านี้จะมีระดับความเข้มข้นสูงขึ้นเมื่อร่างกายมีการอักเสบของระบบทางเดินหายใจ งานวิจัยที่ต้องการศึกษาถึงการสร้าง Leukotriene ในผู้ป่วยเด็กที่เป็นโรคภูมิแพ้ในระบบทางเดินหายใจจึงจำเป็นต้องเปรียบเทียบกับค่าอ้างอิงในเด็กปกติ

วัตถุประสงค์: เพื่อศึกษาหาค่าอ้างอิงปกติของ Leukotriene E4 จากในปัสสาวะของเด็กไทยที่ไม่มีภาวะภูมิแพ้โดยวิธี competitive enzyme-linked immunoassay (ELISA)

วัสดุและวิธีการ: การศึกษานี้ทำในเด็กไทยอายุ 6 เดือนถึง 5 ปีที่ไม่มีประวัติโรคหืด โรคจมูกอักเสบจากภูมิแพ้และโรคเรื้อรังต่างๆ โดยเก็บปัสสาวะในช่วงเช้าปริมาตร 5 มิลลิลิตรนำไปปั่นตกตะกอนที่ความเร็วรอบ 4,000 rpm (Hettich;universal 320 R) เป็นเวลา 15 นาทีที่ 4 องศาเซลเซียส แล้วจึงนำไปเก็บที่ -80 องศาเซลเซียส จากนั้นนำตัวอย่างไปตรวจหาค่าความเข้มข้นของ Leukotriene E4 โดยการใช้วิธี ELISA

ผลการศึกษา: จากการศึกษาในเด็กเล็กจำนวน 36 รายที่มีอายุ 6-60 เดือนเป็นเด็กชาย 26 รายและเด็กหญิง 10 ราย พบว่าค่าเฉลี่ยของระดับ uLTE4 มีค่าเท่ากับ 619.73±701.32 pg/ml และในประชากรทั้งหมดที่ศึกษามีค่าเฉลี่ยของ uLTE4 ต่อ creatinine ในปัสสาวะมีค่าเท่ากับ 1,328±788.54 pg/mg creatinine โดยพบในเด็กชายมีค่าเท่ากับ 1,349±817.10 และเด็กหญิงมีค่าเท่ากับ 1,275±747.79 pg/mg creatinine เมื่อแบ่งเด็กตามกลุ่มอายุ 6-18 เดือน, 19-36 เดือนและ 37-60 เดือนพบว่าจะมีค่าดังนี้คือ 1,363±886.65, 1,384±771.81 และ 1,223±773.16 pg/mg creatinine ตามลำดับ

สรุป: เป็นการรายงานระดับความเข้มข้นของ uLTE4 ต่อ creatinine ในปัสสาวะของเด็กไทยที่ไม่มีประวัติโรคภูมิแพ้ของระบบทางเดินหายใจ ซึ่งแบ่งตามกลุ่มอายุและพบว่าระดับความเข้มข้นของ uLTE4 ต่อ creatinine ในปัสสาวะจะมีค่าสูงขึ้นในกลุ่มเด็กที่อายุน้อย