Plasma LTE₄/PGF₂α Ratio and Blood Eosinophil Count Are Increased in Elderly Asthmatics With Previous Asthma Exacerbation

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The tools for asthma control assessment recommended by the current guideline are cognitive function- and effort-dependent, which is substantially impaired in the elderly. The aim of this study is to investigate objective assessment tools of asthma control status and previous asthma exacerbation (AE) in elderly subjects. Asthmatics aged >60 years who were treated with step 2 or 3 by the Global Initiative for Asthma (GINA) guideline were enrolled. During the 12-week study period, the subjects used either 400 μg of budesonide plus 10 mg of montelukast or 800 μg of inhaled budesonide. The occurrence of AE during the 4-week run-in and 12-week treatment period was monitored. After 12-week of treatment, sputum eosinophil count, peripheral eosinophil count, the plasma leukotriene E₄ (LTE₄), and prostaglandin F₂α (PGF₂α) metabolite levels were measured using the UHPLC/Q-ToF MS system. The study subjects were divided into group 1 (asthmatics who experienced AE during the study period) and group 2 (those who did not). A total of 101 patients aged 60-85 years were enrolled. Twenty-three patients (22.8%) had experienced AE. The plasma LTE₄ level, LTE₄/PGF₂α ratio, and peripheral eosinophil count were significantly higher in group 1 than in group 2 ($P=0.023$, $P=0.010$, $P=0.033$, respectively). The plasma LTE₄/PGF₂α ratio and peripheral eosinophil count at week 12 were significantly associated with previous AE (odds ratio [OR]=1.748, $P=0.013$; OR=1.256, $P=0.027$). Receiver operating characteristic (ROC) curves to discriminate the subjects with previous AE, including these 2 parameters, showed that the area under the curve was 0.700 ($P=0.004$), with 73.9% sensitivity and 47.9% specificity. In conclusion, a combination of plasma LTE₄/PGF₂α ratio and peripheral eosinophil count can be an objective assessment tool which is significantly associated with asthma control status in elderly asthmatics.

Key Words: Elderly asthma; exacerbation; leukotriene

INTRODUCTION

The geriatric population is increasing worldwide and the burden of asthma is more significant in elderly asthmatics compared to younger ones. The number of elderly people is projected to reach 20% and 36% of total populations of the United States and China, respectively.[1,2] The prevalence of asthma is increasing at all ages, and it is reported to be 5% to 10% in elderly people, similar to younger ones.[1,3] A main burden of elderly asthma is attributed to asthma severity, hospitalization, near-fatal asthma related-events, and medical costs.[2,4,5] To reduce the severity, mortality, and burden of asthma in elderly asthmatics, assessment of asthma control is mandatory.

Asthma control is assessed in terms of symptom control and future risk for asthma exacerbation (AE).[6] Global Initiative for

Asthma (GINA) symptom control tool, Asthma Control Questionnaire, and Asthma Control Test (ACT) are validated methods for assessing symptom control. To perform these tools for symptom control, patients have to recall their symptoms, including day/night time symptoms, reliever use, and activity limitation during the previous 1 to 4 weeks. One of the most important risk factors for AE is the history of ≥1 exacerbation in

the previous year; therefore, patients should recall their AE history to evaluate the future risk for AE.[6] Spirometry which is recommended in assessing the future risk for AE requires proper coordination, and cognitive function and effort.[1] The tools for asthma control recommended by the current guideline[6] are dependent on cognitive function and effort which is substantially impaired in elderly. In addition, assessment of asthma symptoms, especially dyspnea, may be inappropriate for elderly because of multiple comorbidities that can cause dyspnea in elderly. Therefore, objective assessment tool which is significantly associated with asthma control status and AE in elderly subjects are needed.

MATERIALS AND METHODS

Asthmatics aged >60 years who were diagnosed with asthma for more than 6 months and treated with step 2 or 3 by the GINA guidelines were enrolled. Subjects were recruited between August 2011 and February 2012 from 4 university hospitals in Korea.[7] This study was approved by Ajou University Institutional Review Board (IRB No. GEN-CT4-10-095), and the other 4 hospitals approved the study. Informed consent was obtained from each patient. During the 12-week study period, the subjects used either 400 μg of inhaled budesonide plus 10 mg of montelukast or 800 μg of inhaled budesonide. The occurrence of AE during the 4-week run-in (maintaining with inhaled budesonide at 400 μg per a day) and 12-week treatment period was monthly monitored, which was defined when one of the following criteria was satisfied: use of systemic oral corticosteroids, asthma-related unscheduled visit/emergency department visit/hospitalization, and use of reliever medication. For the overall study period, all study subjects wrote a diary and the completeness was calculated as a compliance. After 12

weeks of antiasthmatic medication (maintaining same dose), the plasma levels of the metabolites leukotriene E_4 (LTE_4) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were measured. ACT score calculation, asthma quality of life (AQoL) assessment, sputum eosinophil counts, peripheral eosinophil counts, and pulmonary function tests were performed at week 12. The study subjects were divided into group 1 (asthmatics who experienced AE during the study period) and group 2 (those who did not). The LTE_4 and $PGF_{2\alpha}$ levels were quantified as previously reported.[8] In brief, Mass Hunter Quantitative Analysis B.07.00 (Agilent Technologies, Santa Clara, CA, USA) was used to quantify the LTE_4 and PGF_2 levels in plasma samples. The following deuterated internal standards were used: LTE_4-d5 for LTE_4 and 8-iso $PGF_{2\alpha}$-d4 for $PGF_{2\alpha}$ (Cayman Chemical Company, Ann Arbor, MI, USA). The concentration of each metabolite was determined from calibration curves using linear regression analysis. Correlation coefficients were >0.99 for all metabolites. All the chemicals were of high-performance liquid chromatograph (HPLC) grade. General linear regression analysis was performed to adjust for confounding factors in order to compare the metabolite levels between group 1 and 2 subjects. Receiver operating characteristic (ROC) curve analysis was used to discriminate the groups of patients who experienced AE.

RESULTS

A total of 101 patients aged 60-85 years were enrolled. Twenty-three patients (22.8%) had experienced AE over the study period. Among the group 1 patients, 5 (21.74%), 1 (4.35%), 14 (60.87%), and 3 (13.04%) had experienced the last AE in the first, second, third, and fourth months, respectively. Baseline clinical characteristics are summarized in Table 1. Group 1 subjects had a significantly higher plasma LTE_4 level and a LTE_4/$PGF_{2\alpha}$ ratio at

Table 1. Clinical characteristics of the study subjects

Characteristics	Total study subjects (n=101)	Group 1 (n=23)	Group 2 (n=78)	P value
Age (year)	67.82±5.46	67.48±5.15	67.92±5.57	0.820
Gender (M:F)	46:55	9:14	37:41	0.482
FEV1 (% predicted)	97.93±24.55	96.43±25.46	98.38±24.42	0.702
Sputum eosinophil count (%)	3.65±13.14	5.10±19.67	3.23±10.70	0.575
Peripheral eosinophil count (%)	2.89±2.37	3.74±3.21	2.63±2.01	0.033
ACT score	19.02±4.50	17.48±3.75	19.48±4.62	0.064
AQoL	27.87±23.04	32.48±24.18	26.49±22.68	0.293
Type of anti-asthmatic medication				0.551
Low dose ICS+montelukast	45	9	36	
High dose ICS	56	14	42	

General linear regression analysis adjusted by age, gender was performed. Continuous data are presented as mean±SD. Group 1: subjects that experienced AE during the study period, Group 2: subjects that did not experienced AE during the study period.
FEV1, forced expiratory volume in 1 second; ACT, asthma control test; AE, asthma exacerbation; AQoL, asthma quality of life; ICS, inhaled corticosteroid; SD, standard deviation.

week 12 than group 2 subjects (LTE$_4$ level: 20.83 ± 23.37 vs 13.49 ± 11.04 pg/mL, $P=0.023$; LTE$_4$/PGF$_{2\alpha}$ ratio: 1.43 ± 1.76 vs 0.79 ± 0.82, $P=0.010$) (Figure). The plasma PGF$_{2\alpha}$ level showed no significant difference between group 1 and group 2 subjects (17.17 ± 6.40 vs 23.24 ± 19.13, $P=0.132$). The compliance was not significantly different between group 1 and 2 subjects. The plasma LTE$_4$ level and LTE$_4$/PGF$_{2\alpha}$ ratio were not significantly different according to the time that subjects experienced the last AE. The plasma LTE$_4$ level and LTE$_4$/PGF$_{2\alpha}$ ratio showed no significant difference between the patients who had used 400 μg of budesonide plus 10 mg of montelukast and those who had used 800 μg of inhaled budesonide after adjusting for age and gender (LTE$_4$ level: 15.92 ± 12.04 vs 14.55 ± 17.02 pg/mL, $P=0.436$; LTE$_4$/PGF$_{2\alpha}$ ratio: 1.00 ± 0.96 vs 0.89 ± 1.26, $P=0.476$).

By a binary logistic regression model, the plasma LTE$_4$/PGF$_{2\alpha}$ ratio and peripheral eosinophil count (%) at week 12 were significantly associated with AE during the study period (odds ratio [OR]=1.748, $P=0.013$; OR=1.256, $P=0.027$, respectively) (Table 2). ROC curves were generated to discriminate the asthmatics with previous AE from those without previous AE using a logistic regression model for age, gender, plasma LTE$_4$/PGF$_{2\alpha}$ ratio, and peripheral eosinophil count (%) at week 12. The area

under the curve was 0.700 ($P=0.004$), with 73.9% sensitivity and 47.9% specificity. When ROC curves are generated from the LTE$_4$ level, LTE$_4$/PGF$_{2\alpha}$ ratio or peripheral eosinophil count (%) at week 12, the area under the curve was 0.608, 0.622, and 0.619, respectively.

DISCUSSION

This is the first study to suggest objective assessment tools which is significantly associated with previous AE in elderly asthmatics. A combination of plasma LTE$_4$/PGF$_{2\alpha}$ ratio and peripheral eosinophil count is reliable for assessing previous AE.

Biomarkers including fractional exhaled nitric oxide (FeNO), sputum eosinophilia, and urinary LTE$_4$, related with future AE have been widely studied.[9] However, objective assessment tools related with previous AE have been rarely studied. The current guideline recommends to treat asthmatics according to asthma control status.[6] The validated tools for assessing asthma control status are symptom control status by GINA, ACT score, asthma control questionnaire, spirometry, and presence of previous AE. Questionnaire items of GINA symptom control, ACT, and asthma control questionnaire all includes reliever usage for
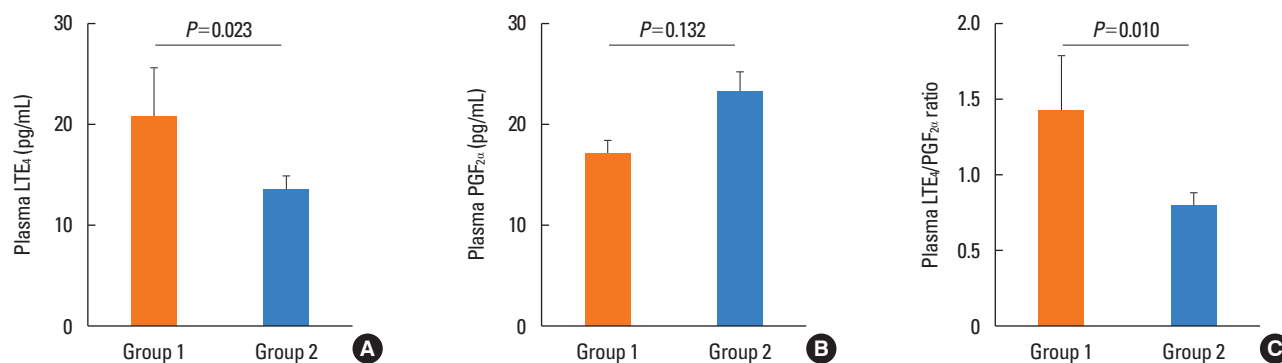
Figure. Comparison of the plasma levels of LTE$_4$ (A) and PGF$_{2\alpha}$, (B) and LTE$_4$/PGF$_{2\alpha}$ ratio according to the presence of previous AE (C). The plasma LTE$_4$ level and LTE$_4$/PGF$_{2\alpha}$ ratio were significantly higher in group 1 than in group 2 ($P=0.023$ and $P=0.010$, respectively). Data were analyzed by general linear regression analysis adjusted for age, gender, and type of anti-asthmatics treatment. Group 1: patients who experienced AE for previous 12 weeks, Group 2: patients who that did not experience AE for previous 12 weeks. AE, asthma exacerbation; LTE$_4$, leukotriene E$_4$; PGF$_{2\alpha}$, prostaglandin F$_{2\alpha}$.

Table 2. Factors associated with AE during the previous 16-week period

Characteristics	Group 1	Group 2	OR	95% CI		P value
				Lower	Upper	
Age (year)	67.48 ± 5.15	67.92 ± 5.57	1.029	0.930	1.138	0.578
Gender (M:F)	9:14	37:41	0.385	0.123	1.201	0.100
Plasma levels of LTE$_4$/PGF$_{2\alpha}$ ratio	1.43 ± 1.76	0.78 ± 0.82	1.748	1.009	1.082	0.013
Peripheral eosinophil count (%)	3.74 ± 3.21	2.63 ± 2.01	1.256	1.027	1.536	0.027
FEV1 (% predicted)	96.43 ± 25.46	98.38 ± 24.42	0.996	0.975	1.017	0.707
Type of medication (Low dose ICS+montelukast)	9	36	0.863	0.308	2.422	0.780

Data were analyzed by binary logistic regression analysis. Group 1: subjects that experienced AE during the study period, Group 2: subjects that did not experienced AE during the study period. Type of medication: Patients using either 400 μg of inhaled budesonide plus 10 mg of montelukast or 800 μg of inhaled budesonide. AE, asthma exacerbation; LTE$_4$, leukotriene E$_4$; PGF$_{2\alpha}$, prostaglandin F$_{2\alpha}$; FEV1, forced expiratory volume in 1 second; OR, odds ratio; CI, confidence interval.

previous 1 to 4 weeks. Therefore, we included reliever use in the definition of AE in the present study which is more relevant to assess asthma control status on the basis of real life in clinical practice. These tools are recommended to be applied to elderly asthmatics as in younger asthmatics, though they are substantially cognitive function- and effort-dependent. Taking into account the characteristics of elderly,[1] objective tools for assessing asthma control status, such as, previous AE are needed. In the present study, although the ACT score and AQoL were not significantly different whether the patients experienced AE or not, the plasma $LTE_4/PGF_{2\alpha}$ ratio and peripheral eosinophil count at week 12 were significantly associated with AE using a binary logistic regression model. With the ROC curve to discriminate asthmatics with previous AE, a combination of 2 parameters had 73.9% sensitivity and 47.9% specificity. Taken together, the plasma $LTE_4/PGF_{2\alpha}$ ratio and peripheral eosinophil count may be potential tools for assessing asthma control status along with GINA symptom control, ACT, and asthma control questionnaire, especially for elderly asthmatics.

LTE_4 mediates inflammatory cell infiltration, mucus secretion, vascular permeability, and smooth muscle contraction, and plays an important role in asthma pathogenesis.[10] Previous studies demonstrated that the increase in the LTE_4 level during acute AE were reversed after the resolution of AE.[11,12] In a previous study,[11] urinary LTE_4 was measured in asthmatic children during an acute exacerbation and 1 month later and the levels were compared. The authors demonstrated that the production of urinary LTE_4 was significantly higher than normal controls not only during AE, but also 1 month later after AE. In the present study, increased plasma LTE_4 level and $LTE_4/PGF_{2\alpha}$ ratio were noted in patients with previous AE during the previous 16-week period. Similar to the previous study,[11] our study showed no significant difference in the plasma LTE_4 level according to the time point of the last AE, suggesting that plasma LTE_4 level may represent an ongoing inflammatory process rather than acute inflammatory response and can be applied to assess asthma control status. Considering that plasma collection is convenient and that metabolite measurement is an objective method, plasma $LTE_4/PGF_{2\alpha}$ ratio representing dysregulation of arachidonic acid metabolism can be useful for assessing an episode of previous AE.

The plasma LTE_4 level and $LTE_4/PGF_{2\alpha}$ ratio showed no significant difference between the patients who used 400 μg of budesonide plus 10 mg of montelukast and those who used 800 μg of inhaled budesonide. Cysteinyl LTs are known to be not completely suppressed by treatment with corticosteroids alone. However, previous studies[13,14] demonstrated that not only systemic steroids, but also inhaled corticosteroids (ICSs) decrease cysteinyl LTs concentration. Therefore, it is difficult to directly compare the plasma LTE_4 level and $LTE_4/PGF_{2\alpha}$ ratio between patients treated with LT receptor antagonists and double dose of ICSs and to evaluate the effect of LT receptor antagonists on the LTE_4 level.

In conclusion, a combination of plasma $LTE_4/PGF_{2\alpha}$ ratio and peripheral eosinophil count can be an objective assessment tool which is significantly associated with asthma control status in elderly asthmatics. Further studies in a larger number of patients are needed to validate our results.

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