Original Article

Evaluation of correlation between airway and serum inflammatory markers in asthmatic patients

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ABSTRACT

Context: Asthma is one of the most common chronic diseases all over the world, resulting from a state of persistent sub-acute inflammation of the airways. Beside local inflammation, systemic inflammation is also present, which can be shown by increased levels of C-reactive protein (CRP). One of the most important cells in this disease is eosinophil, and sputum eosinophilia is used for its diagnosis. **Aims:** The purpose of the present study was to compare and evaluate the correlation between CRP level and sputum eosinophilia in asthmatic and control subjects. **Materials and Methods:** A total of 61 patients suffering from mild-to-moderate asthma participated in this study. They were divided into two groups based on whether they used inhaled steroid or not. Sputum was induced by ultrasonic nebulizer, and then samples of peripheral venous blood were collected to measure peripheral cell count and CRP by Enzyme-linked immunosorbent assay (ELISA). Thirty-seven healthy subjects were selected and their blood samples were taken. Thirty-seven healthy subjects were selected and their blood samples were taken. **Results:** Thirty asthmatic patients in user group (14 females/16 males) with the mean age of 39.4±9.37 years, 31 asthmatic patients in non-user group (13 females/18 males) with the mean age of 35.5±8.87 years, and 37 healthy controls (17 females/20 males) were included in our study. The mean serum concentration of CRP was 2.6 μg/mL, 3.32 μg/mL, and 1.16 μg/mL in user, non-user, and control groups, respectively. Compared to healthy controls, serum concentrations of high sensitivity-CRP (hs-CRP) significantly increased in the non-user group (P=0.0001), and user group as well. (P=0.016). The number of sputum eosinophils and peripheral blood eosinophils significantly increased in the non-users compared to the healthy controls (P=0.0001, P=0.003, respectively). In the non-user group, serum hs-CRP levels correlated negatively with FEV_1 and positively with numbers of sputum eosinophils, which was not statistically significant. Atopy status, age, and sex did not affect hs-CRP levels in both asthmatic groups. **Conclusions:** It was found that serum concentrations of hs-CRP significantly increased in asthmatic patients, and in the steroid-native group it partly correlated with FEV_1 and numbers of sputum eosinophils. It suggests that serum hs-CRP can indirectly indicate the degree of airway inflammation.

KEY WORDS: Asthma, C-reactive protein, eosinophilia, forced expiratory volume in one second, sputum

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INTRODUCTION

Asthma, one of the most common chronic diseases all over the world, currently affects 300 million people.[1] It is defined as a chronic inflammation of airways characterized by increased responsiveness of trachea and bronchial tree to a variety of stimuli.[2] Asthma results from a state of persistent sub-acute inflammation of the airways. The main pathogenesis of asthma is infiltration of such inflammatory cells as eosinophils, basophils, and CD4 + lymphocytes.[3,4] C-reactive protein (CRP) is a well-known inflammatory marker synthesized by hepatocytes. This marker increases in systemic inflammations such as diabetes, cardiovascular diseases, collagen vascular diseases, malignancies, and also obesity.[5-7] CRP is a marker of inflammation that predicts incidence of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death among healthy individuals without history of cardiovascular disease.[8] Recent population-based studies

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showed a correlation between increased levels of serum high sensitivity-CRP (hs-CRP) with a high frequency of airway hyper-responsiveness and low forced expiratory volume in one second (FEV_1) among subjects without heart disease.[9] In the present study, the serum levels of hs hs-CRP of asthmatic patients with and without inhaled corticosteroid (ICS) treatment were compared to those of healthy controls, and the correlation between serum CRP levels (systemic inflammatory marker) and sputum eosinophils (airway inflammatory marker) was evaluated as well.

MATERIALS AND METHODS

A comparative-descriptive cross-sectional study was conducted on 61 asthmatic patients (diagnosed according to American Thoracic Society criteria)[10] who visited the outpatient clinic. All patients suffered from mild-to-moderate persistent stage, according to GINA (the Global Initiative For Asthma) criteria.[2] Patients were divided into two groups: 30 patients receiving inhaled fluticasone (500 μg/day), named as the user group, and 31 patients who did not receive ICS, named as the non-user group. All of them were non-smokers without any history of respiratory tract infection or exacerbation of asthma during the previous 6 weeks. Subjects suffering from heart disease, diabetes mellitus, cancer, obesity (body mass index [BMI] ≥ 30kg/m^2) or systemic inflammatory disorders (such as collagen vascular diseases) were excluded from the study. Demographic data, duration of asthma, and history of asthma were recorded in questionnaires. Sputum was induced by ultrasonic nebulizer, so subjects were premedicated with inhaled salbutamol (two puffs), and inhaled hypertonic (5%) saline solution was administered to them for 10–20 min by an ultrasonic nebulizer (Shining World Health Co., Ltd. made by Taipei Taiwan), then they were asked to try to cough sputum into a plastic Petri dish. Then, smear of sputum was prepared and colored with Giemsa and cellular counts were performed. Thereafter, a group of healthy subjects (N=37) without history of smoking, ischemic heart disease, or other diseases, with normal pulmonary function tests, were selected as control group. These subjects were matched with asthmatic patients in terms of age and sex. Five cc of blood was taken from each person, poured into a clot tube, and then coagulated. Serum sample was separated by centrifugation and stored at −80°C. After collecting samples, ELISA test (DRG International, Inc.1167 US Highway 22 East, Mountainside, NJ 07092 USA) was performed. This kit showed CRP level as a quantitative measure and its sensitivity was 10 ng/mL. Data were analyzed by descriptive statistics (percentage, mean, and standard deviation) and comprehensive statistics (analysis of variance (ANOVA) and t test, Kruskal-Wallis test, and Mann-Whitney test) using SPSS (ver. 13.5).

RESULTS

This study was conducted on 30 patients in user group (14 females/16 males) and 31 patients in non-user group (13 females/18 males). Their mean age was 39.4±9.37 and 35.5±8.87 years, respectively. Thirty-seven healthy controls (17 females/20 males) and the subjects in case groups were matched in terms of age, sex, and BMI. The mean serum CRP concentration was 2.6 μg/mL, 3.32 μg/mL, and 1.16 μg/mL in user, non-user, and control groups, respectively. The difference was statistically significant between user and control groups (P=0.016) and non-user and control groups (P=0.0001), but was not significant between user and non-user groups (P=0.369). The number of sputum eosinophils and peripheral blood eosinophils significantly increased in the non-user group compared to users and healthy controls. In the non-user group, serum hs-CRP levels correlated negatively with FEV_1 and positively with numbers of sputum eosinophils, but this correlation was not statistically significant (P=0.072, P=0.437, respectively) [Table 1, Figure 1]. Atopy, age, and sex indices did not affect hs-CRP levels in both asthmatic groups.

DISCUSSION

According to our findings, serum CRP level was higher in asthmatic patients (users and non-users) than in controls. However, mean blood and sputum eosinophil count was higher in non-users. CRP level had a positive correlation with eosinophil count of sputum and blood and negative correlation with FEV_1, which was not significant statistically. On the other hand, there was no significant correlation between CRP and sex or history of atopy.

Besides chronic local inflammation of airways, systemic inflammation is also present in asthma, but there aren't enough studies about it.[11] Jousilaht reported higher level of amyloid A and plasma fibrinogen in asthmatic cases than in controls.[12] Buyukozturk *et al*. noted that although CRP level was higher in allergic rhinitis and asthma, mean CRP and fibrinogen level was not significantly higher than in healthy population. However, serum amyloid A level was significantly higher than in controls.[13] Takemura *et al*. showed that serum CRP level of non-user asthmatic patients was significantly higher than in controls. Although

Table 1: Characteristics and outcomes of asthmatic and control subjects

	ICS user	ICS non-user	Control	*P* value
Number	31	30	37	
Age (year)	39.4±9.37	35.5±8.87	38.6±9.34	0.22
Sex (male/female)	16/14	18/13	20/17	0.921
BMI (kg/m^2)	24.72±3.96	23.89±2.88	24.17±2.44	0.575
FEV_1% pred	66.36±19.35	76.74±19.85	91.29±5.33	0.0001
CRP (μg/ml)	2.6±2.09	3.32±2.91	1.16±0.85	0.0001
Blood eosinophil (number/μL)	402	517	211	0.003
Sputum eosinophil (percent)	6.36±5.77	9.96±5.5		0.015

ICS: Inhaled corticosteroid; FEV_1: Forced expiratory volume in one second; BMI: Body mass index; hs-CRP: High-sensitivity C-reactive protein; % pred: Percentage of the predicted value

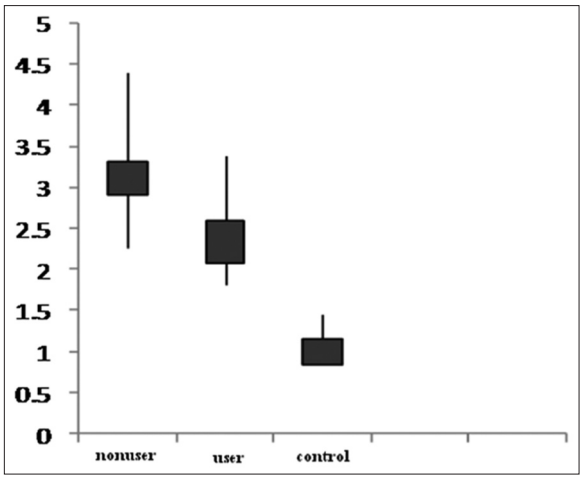

Figure 1: Serum high-sensitivity C-reactive protein (hs-CRP) levels (μg/mL) in a healthy control group (*n*=37) and two asthmatic groups (inhaled corticosteroid [ICS] + (*n*=31); ICS- (*n*=30)

serum CRP level among user patients was more than in control group, this was not statistically significant.[11]

Previous studies have shown that FEV_1 decline is associated with increased serum CRP level. Mean FEV_1 among subjects of user group of this study was lower than in patients in the Takemura study, so discrepancy between two studies may be due to difference in disease stage.

Our findings showed positive correlation between serum CRP of non-users and blood and sputum eosinophil and negative correlation with FEV_1, both of which were statistically insignificant. There was no such correlation among subjects of user group. Takemura findings confirmed this as well.[11]

Dahl *et al*. reported a negative correlation between plasma fibrinogen and FEV_1.[14] Engstrom also showed a negative correlation between FVC and plasma inflammatory proteins such as fibrinogen, α1 AT, haptoglobulin, and ceruloplasmin in males.[15] Kauffman showed a negative correlation between FEV_1 and haptoglobulin level in men.[16] This relationship indicates that with increasing severity of disease and further reduction of FEV_1, the inflammatory process and its serum markers will increase.

There was no statistically significant relationship between CRP and history of atopy. An epidemiologic study performed by Olafsdottir *et al*. showed a higher CRP level in non-allergic asthmatics than in healthy population but in allergic asthmatic patients, serum CRP was similar to that in healthy population.[17] Buyokoztu reported a higher level of CRP in allergic rhinitis and asthma groups than in the control group, which was statistically insignificant.[13] Takemura also didn't find any relationship between serum CRP level and atopy.[11] It seems that the correlation between atopy and CRP is not yet well understood and more research is needed. Our findings showed a lower CRP level in steroid users than in non-users, which was statistically insignificant either. Hashino and Nakamura reported that Beclomethasone Dipropionate suppressed cellular infiltration in airways and improved hyper-responsiveness in non-atopic asthma.[18] Takemura showed although serum CRP level of steroid users was higher than in control group, this difference was statistically insignificant.[11] Sin *et al*. studied the effect of steroid inhaler on systemic inflammatory markers in mild-to-moderate chronic obstructive pulmonary disease cases. They found that following discontinuation of inhaler fluticasone, serum CRP level increased and vice versa.[19] It seems that steroid inhalation decreases systemic inflammatory markers in asthma too, which should be studied more in cohort investigations. It should be noted that according to our findings, mean levels of sputum eosinophils in asthma group were significantly higher than upper limit of normal population in both user and non-user groups. Based on the European respiratory society (ERS) International Guidelines and other studies, a normal upper value of eosinophils of <3% or <2.5% has been reported.[20-22] Mean levels of sputum eosinophils were 6.3% and 9.96% in steroid user and non-user groups, respectively, which was more than upper limit of normal population. On the other hand, mean levels of sputum eosinophils were higher in non-users than user group, and this difference was statistically significant as well. In a similar study by Pin *et al*., sputum eosinophils and macrophages were significantly higher in asthmatics than controls, but no significant difference was seen between controlled and uncontrolled asthma groups.[23] Beasley and Kirby studies showed similar findings too.[24,25] Takemura reported that sputum eosinophils of steroid users was significantly higher than controls and non-users,[11] which was against our findings. It seems that according to the topical anti-inflammatory effects of inhaled steroids, levels of sputum eosinophils in user group should be less than non-user group.

Our study showed that levels of eosinophils in peripheral blood smear (PBS) were higher than in normal population. In asthmatic cases on steroid inhalers, mean levels of eosinophils in PBS were lower than non-users. Gibson *et al*. studied 18 allergic asthmatic cases being exposed to inhaled allergens and found a rise in the level of eosinophils and basophils during 24 h after exposure in 13 of them.[26] Gauvreau *et al*. performed a study on normal and atopic asthma groups to show the rise of PBS eosinophils following exposure to an allergen in asthmatic patients.[27] Bousquet also reported a rise in PBS eosinophils and eosinophils in bronchoalveolar lavage (BAL) in chronic asthma, which correlated with severity of the disease.[28] Evans *et al*. also showed that steroid inhalation decreased PBS eosinophils in 10 mild asthmatic individuals.[29] These findings showed a rise in PBS eosinophils in asthma, which can be decreased by steroid inhalers. To prove this hypothesis, more studies are needed.

It is concluded that in asthma, besides airway inflammation, systemic inflammatory markers are increased as well, and

steroid inhalers can decrease inflammation of airways and also systemic inflammation.

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