

GERIATRIC

The Differences of Eosinophil- and Neutrophil-Related Inflammation in Elderly Allergic and Non-Allergic Chronic Obstructive Pulmonary Disease

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Background. Chronic obstructive pulmonary disease (COPD) is a common disease in the elderly population and is characterized by airway inflammation. Whether it is a progressive condition resulting from allergic inflammation or a distinct condition involving a pathogen-induced reaction remains unclear. **Objectives.** To determine the role of allergic inflammation in the pathogenesis of elderly COPD. **Methods.** A total of 63 elderly adults (21 mite-allergic COPD patients, 29 non-allergic COPD patients, and 13 normal controls) were recruited in this study. The serum-specific IgE for mites, level of interleukin-5 (IL-5), IL-8, leptin, adiponectin, regulated upon activation normal T cell expressed and secreted (RANTES), growth-related oncogene- α (GRO- α), vitamin E, and glutathione (GSH) were determined. **Results.** The serum levels of GRO- α in patients with COPD were higher in comparison to normal controls (105.8 ± 32.7 vs. 7.5 ± 7.5 pg/mL, $p = .021$). Compared to patients with non-allergic COPD, patients with mite allergies had a higher serum level of IL-8 (63.2 ± 12.6 vs. 35.0 ± 8.2 pg/mL, $p = .022$). Although both IL-5 and RANTES levels were increased in COPD patients, there were no significant differences between allergic and non-allergic COPD. There were also no differences in serum levels of leptin, adiponectin, vitamin E, and GSH between COPD patients and normal controls. **Conclusions.** The increased serum levels of GRO- α indicate that it may have potential as a candidate biomarker for elderly COPD patients. There was no difference of eosinophils-related chemokines in allergic and non-allergic COPD. These results indicated that both adipokines and eosinophil-related chemokines only play trivial roles in the pathogenesis of COPD.

Keywords allergen-specific IgE, elderly COPD, growth-related oncogene- α , interleukin-8, storage mite, *Tyrophagus putrescentiae*

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) and asthma are common pulmonary diseases in the elderly population. Although different inflammatory cell profiles are observed in the lungs, both diseases are characterized physiologically by airflow limitations. COPD tends to be irreversible whereas asthma is largely reversible, either spontaneously or after bronchodilator administration (1). Studies using bronchoalveolar lavage, bronchial biopsy or, more recently, induced sputum have shown that the main cellular markers of intraluminal airway inflammation are eosinophils in asthma (2) and neutrophils in COPD (3). Moreover, good correlation has been found between eosinophils and airway hyper-responsiveness in asthma (4), as well as between neutrophils and airway obstruction in COPD (3). Because of poor public awareness of this disease and the overlap of symptoms with other comorbidities, many patients are not diagnosed until the disease has made a significant impact on their quality of life (5, 6).

The diagnosis and outcome of COPD are based on symptoms and/or measures of healthcare utilization. Various reports have demonstrated that systemic inflammation is upregulated during COPD exacerbations. Concentrations of many biochemical markers, including acute phase protein, inflammatory mediators, and systemic cytokines, have been shown to be higher in plasma or serum during COPD exacerbation (7–10). Elevated sputum levels of the neutrophil chemoattractants interleukin (IL)-8 and growth-related oncogene- α (GRO- α) have also been reported (11, 12). Leptin, a pro-inflammatory cytokine, is involved in the pathogenesis of physiological and pathological functions, including acute exacerbation of COPD (13, 14). Adiponectin is an adipose tissue-derived specific protein with anti-inflammatory effects (15, 16). Vitamin E and glutathione (GSH) are commonly used as food supplements to prevent diseases associated with oxidative damage in disease status in COPD (17).

It has been suggested that the discovery of new markers of disease severity would not only enhance our understanding of COPD's natural history and pathogenesis but also offer new endpoints for therapeutic monitoring. *Tyrophagus putrescentiae* (Tp) is one of the most dominant species of storage mites. The survey of Tp

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infestation in Taiwan was investigated, the results showed there are approximately 15% samples contaminated with Tp in the storage stuff. Among them, the pet feed, mushroom, and farina appeared to be most easily identified. A recent study found that prevalence of storage mite sensitization in the general adult population was high, and sensitization to the storage mite Tp appeared to be higher than sensitization to the house dust mite *Dermatophagoides pteronyssinus* (Dp) in subjects aged over 60 years (18). A similar sensitization pattern was observed in asthmatic patients aged over 60 years in Barbados: sensitization to the major allergen, the storage mite, was higher than to the house dust mite (19). Although there is one study of patients older than 65 years with severe persistent asthma, 75% of patients were positive to at least one allergen, as determined by skin test. The most prevalent antigens identified were typical indoor allergens, such as dust mites and animal dander (20). Because older adults often move into new institutional environments, exacerbation of late-onset asthma or COPD may occur because of allergen exposure. A history of allergy may predict the exacerbation of late-onset of asthma or COPD in older adults. However, there is no data regarding Tp- and Dp-sensitization in elderly COPD patients. Therefore, the aim of this study was to identify a biochemical marker for elderly COPD patients and to investigate the underlying allergic mechanism of COPD by comparing the pro-inflammatory and anti-inflammatory markers associated with mite-specific IgE in the sera.

MATERIALS AND METHODS

Patient Selection

A total of 63 elderly adults (21 mite-allergic COPD patients, 29 non-allergic COPD patients, and 13 normal controls) were recruited from the outpatient clinic of Veterans General Hospital and a veteran's nursing home in Yun-Lin County for this study. Most of the elderly subjects were veterans who had been in the army for more than 10 years, then retired and resided in the public veterans nursing home. The mean age of subjects was 83.9 ± 1.3 years for COPD patients and 85.1 ± 1.1 years for normal controls. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease criteria. The definition criteria for asthma diagnosis were based on the patient's reversible symptoms of breath and airway spasm, medical history, a physical examination, and laboratory tests that measure pulmonary function. All patients were clinically stable at the time of evaluation. Only men were enrolled in this study because subjects were recruited from a veteran's hospital and a nursing home for veterans. This study included age-matched healthy male controls from the veteran's nursing home. The study was approved by the Institutional Research Board of Taichung Veterans General Hospital, and informed written consent was obtained from each participant. A total of 20 mL of peripheral blood was

collected from all participants. Serum was separated and stored at -70°C before assay.

ELISA for Specific IgE to Tp/Dp Allergens and Cytokine Measurement

Specific IgE to Tp/Dp mites was determined using the Pharmacia CAP system (Uppsala, Sweden). A titer of antigen-specific IgE higher than 0.35 kU/L was considered to be positive. The ELISA Development kit contains the basic components to measure human-specific chemokines (GRO- α , IL-8, IL-5, and RANTES) and adipokines (leptin and adiponectin). The commercially available enzyme-linked immunosorbent assays (ELISA) kits were obtained from R&D Systems (Minneapolis, MN, USA), except for IL-5 (Bender MedSystems, Vienna, Austria). The detectable ranges of chemokines were described as following: GRO- α (30–1000 pg/mL), IL-8 (30–2000 pg/mL), IL-5 (10–500 pg/mL), and RANTES (30–2000 pg/mL). All ELISA assays were performed according to the manufacturer's instructions. The results were run in duplicate with at least three separate experiments being examined. Vitamin E and GSH were measured using high-performance liquid chromatography based on the method described in previous reports (21).

Statistical Analysis

Results are expressed as an arithmetic mean $\pm$ SEM of each group. The differences between groups were analyzed with a non-paired *t*-test. *P*-values less than .05 were considered statistically significant.

RESULTS

General Characteristics of Subjects

The baseline characteristics of the COPD patients group ($N = 50$) and the normal control group ($N = 13$) were shown in Table 1. Sensitization to Tp and Dp among the 63 elderly adults was determined by measuring IgE antibodies against Tp and Dp in the sera. The results showed that 42% of the COPD patients (21 out of 50 subjects) were sensitized to either Tp or Dp. In addition, the prevalence of mite allergy, especially Tp-sensitive (19 subjects), was more common than Dp-sensitive (7 subjects) allergy in elderly COPD patients. Five out of seven Dp-sensitive subjects were also sensitized to Tp. Fifty-eight percent of COPD patients were non-sensitized to either Tp or Dp. Among the COPD patients, there were 76% subjects (38 out of 50) smoking. Among the 38 smoking subjects, allergic COPD versus non-allergic COPD patients were 27% (14/38) versus 63% (24/38). There was a higher percentage of smoking in the population of non-allergic COPD patients than allergic COPD patients.

Differences in Levels of Chemokines between Elderly COPD Patients and Normal Controls

The serum levels of chemokines in elderly COPD patients and normal healthy controls were compared.

TABLE 1.—General characteristics (Mean $\pm$ SEM) of the study subjects.

	COPD (N = 50)	Normal (N = 13)
Age (years)	83.9 $\pm$ 1.3	85.1 $\pm$ 1.1
Tp + allergic	19 (38%)	0 (0%)
Dp + allergic	7 (14%)	0 (0%)
Smoking (%)	38 (76%)	3 (23%)
Asthma (%)	18 (36%)	0 (0%)
Eczema (%)	0 (0%)	2 (15%)

Note. Data are expressed as mean $\pm$ SEM.

The results showed that GRO- α (53.6 $\pm$ 13.8 vs. 7.5 $\pm$ 7.5 pg/mL, p = .038), IL-8 (48.9 $\pm$ 7.6 vs. 37.9 $\pm$ 7.4 pg/mL), and IL-5 (0.7 $\pm$ 0.4 pg/mL vs. non-detectable) levels all increased in COPD patients, however, only GRO- α levels increased significantly (Figure 1A). Furthermore, levels of IL-8 in mite-allergic COPD patients were found to be significantly higher than those in non-allergic COPD patients (63.2 $\pm$ 12.6 vs. 35.0 $\pm$ 8.2 pg/mL, p = .022, Figure 1B). Levels of GRO- α (75.5 $\pm$ 23.7 vs. 33.5 $\pm$ 14.3 pg/mL), IL-5 (1.3 $\pm$ 0.9 vs. 0.2 $\pm$ 0.1 pg/mL), and RANTES (1100.7 $\pm$ 37.6 vs. 1097.9 $\pm$ 19.4 pg/mL) were not significantly different between the two groups (Figure 1B).

Differences in Levels of Adipokines and Antioxidants between Elderly COPD Patients and Normal Controls

Serum levels of adipokines, leptin, and adiponectin were compared between elderly COPD patients and normal healthy controls. The results showed that there were no significant differences between the two groups. There were also no significant differences between mite-allergic

TABLE 2.—The differences in levels of adipokines and antioxidants between elderly COPD patients and normal controls.

	COPD (N = 50)	Normal (N = 13)
Leptin (pg/mL)	60.5 $\pm$ 11.1	74.9 $\pm$ 23.9
Adiponectin (ng/mL)	552.6 $\pm$ 76.1	570.9 $\pm$ 90.6
Vitamin E (μ M)	36.8 $\pm$ 3.8	39.7 $\pm$ 4.0
GSH (μ M)	1682.1 $\pm$ 82.2	1770.7 $\pm$ 120.8

Note. Data are expressed as mean $\pm$ SEM.

TABLE 3.—The differences in levels of adipokines and antioxidants between allergic and non-allergic COPD patients.

	Allergic COPD (N = 21)	Non-allergic COPD (N = 29)
Leptin (pg/mL)	66.8 $\pm$ 15.7	51.2 $\pm$ 15.3
Adiponectin (ng/mL)	558.6 $\pm$ 102.3	543.6 $\pm$ 121.0
Vitamin E (μ M)	34.1 $\pm$ 4.3	41.6 $\pm$ 7.5
GSH (μ M)	1624.8 $\pm$ 85.1	1780.5 $\pm$ 172.5

Note. Data are expressed as the mean $\pm$ SEM.

and non-allergic COPD patients (Table 2). Similar findings were observed for serum levels of vitamin E and GSH. There was no significant difference between the COPD and the control group, or between the mite-allergic and non-allergic COPD groups (Table 3).

DISCUSSION

In this study, the percentage of mite sensitization in our elderly COPD patients was higher for Tp than for Dp. This finding was consistent with that of a similar study which showed elderly subjects were more sensitized to Tp than to Dp, and that many subjects were sensitized to both Dp and Tp (22). Allergic COPD was associated with high serum levels of IL-8 indicating that IL-8-related neutrophilic airway inflammation in COPD might be upregulated by mite allergy. Neutrophil-dominant airway inflammation is considered to be a major feature in COPD. GRO- α and IL-8 are typically associated with the development of neutrophil infiltration and activation in the airway. Both chemokines have been detected in the sputum of stable COPD patients and are believed to be related to disease severity. Our study yielded a similar finding, namely, that GRO- α levels were elevated in COPD patients, however, IL-8 levels did not increase significantly. As the elevation of IL-8 has been reported in the acute exacerbation (10), the less increase of IL-8 level may have been because of low disease activity of COPD in our study. Another potential confounder may have been the low number of COPD cases with mite allergies in the current study.

The role of eosinophils in the airways of patients with COPD remains controversial. In this study, we found that both IL-5 and RANTES were present in COPD patients. In line with our findings, eosinophils have been identified in COPD patients during exacerbation (5). The lack of significant difference of IL-5 and RANTES concentrations between the mite-allergic and non-allergic COPD groups might be because of the similar nature of clinical

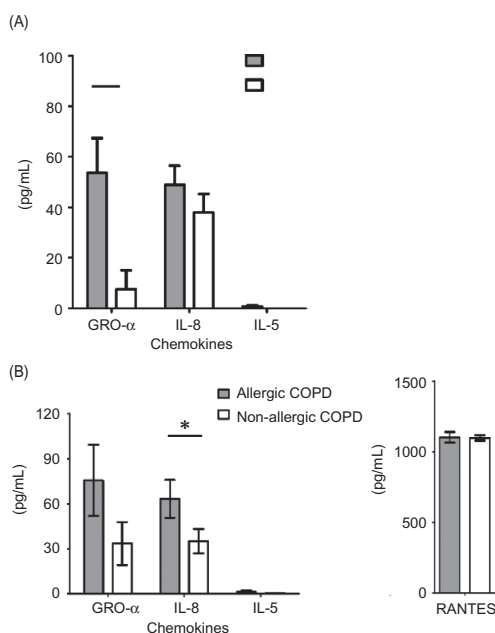


FIGURE 1.—The levels of chemokines and RANTES among these subjects. (A) The differences in levels of chemokines between elderly COPD patients (n = 50) and normal controls (n = 13); *: p = .038. (B) The differences in levels of GRO- α , IL-8, IL-5 and RANTES between mite-allergic (n = 21) and non-allergic (n = 29) COPD patients; *: p = .022.

conditions of our recruited patients. These results indicate that eosinophils may have a role in the pathogenesis of COPD.

Various studies have shown that systemic inflammatory markers are upregulated during COPD exacerbation. Both leptin and adiponectin have been linked to acute exacerbation and stable COPD (14). Serum levels of antioxidants have been found to be related to airway function and inflammation (23). In our study, we did not find any significant difference between COPD patients and normal controls with regard to levels of antioxidants. This apparent discrepancy might be because of the clinical conditions of patients selected for inclusion in the study. In this study, the study population comprised of patients with stable COPD. Serum leptin and adiponectin have been reported to be associated with gender and obesity, regardless of development of asthma (16). Our study also showed that both leptin and adiponectin were not significantly different in the allergic and non-allergic COPD groups. These results indicate that adipokines might be related to sample size and not be related to allergic inflammation in COPD. A large sample size should be recruited for further clarification.

There were some limitations in this study, including sample size and clinical conditions of patients. In order to minimize selection bias and enable researchers to identify the variables affecting the role of mite allergy in the pathogenesis of COPD, future studies should be conducted with a large sample size, a wide range of clinical conditions, including clinical severity and a greater number of subjects with mite-species allergy.

In conclusion, although the serum levels of GRO- α in COPD patients were higher than the normal healthy controls, and levels of IL-8 in mite-allergic COPD patients were higher than those in non-allergic COPD patients, there were no difference of adipokine and eosinophil-related chemokines between these two groups. These results indicated that both adipokines and eosinophil-related chemokines only play trivial roles in the pathogenesis of COPD.

ACKNOWLEDGMENTS

This study was supported by grants from Taichung Veterans General Hospital (TCVGH-977319E) and from Veterans General Hospital University System of Taiwan (TCVUST97-G2-2). The authors sincerely appreciate the assistance of the TCVGHAGE research group and Biostatistics Task Force of Taichung Veterans General Hospital, Taichung, Taiwan.

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