The Role of Eosinophil Cationic Protein in Patients with *Mycoplasma pneumoniae* Infection

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Background: To study the role played by eosinophil cationic protein (ECP) in patients with *Mycoplasma pneumonia* infection.

Methods: Pediatric patients aged 4 to 14 years old were divided into 3 groups, each consisting of 30 patients. Group 1 comprised patients with known *M. pneumoniae* infection. Group 2 comprised patients with asthma who were in a stable condition with no infection, acute asthma exacerbation or steroid use in the last 2 months. Group 3 consisted of healthy children and was designated the control group. The level of ECP in patients' serum was measured by an ECP radioimmunoassay kit.

Results: There were 90 children enrolled in this study; 59 (65.56%) were boys and 31 (34.44%) were girls. Mean serum ECP levels between males and females was not significantly different ($p = 0.544$). The variance of serum ECP levels decreased as patient age increased, but there was no relationship between serum ECP level and patient age ($\gamma = 0.118$, $p = 0.267$). Serum ECP levels were similar in both the *M. pneumoniae*-infected and asthma groups; serum ECP levels in the control group were less than the levels seen in the other 2 groups. The difference in serum ECP levels among the 3 groups was statistically significant ($p < 0.001$).

Conclusion: Both the children who had *M. pneumoniae* infection and the children with asthma had significantly increased serum ECP levels compared to normal healthy children. The elevated ECP levels found in the serum of patients with *M. pneumoniae* infection may be associated with damage to the respiratory epithelium and accelerated hypersensitivity in the respiratory system. Decreasing the serum level of ECP may potentially be a method of relieving symptoms in patients with *M. pneumoniae* infection. Additional studies are warranted to further validate this conclusion. [*J Chin Med Assoc* 2008;71(1):37–39]

Key Words: asthma, eosinophil cationic protein, *Mycoplasma pneumoniae*, pneumonia

Introduction

Previous studies have demonstrated that serum total immunoglobulin E (IgE) levels rise in respiratory tract infections caused by parainfluenza virus, respiratory syncytial virus (RSV) and rhinoviruses.[1] Both IgE and eosinophil cationic protein (ECP) are important markers for atopy and allergic inflammation; they are commonly used for the diagnosis of and for following the course of allergic diseases.

Mycoplasma pneumoniae is a common cause of lower respiratory infection in young children.[2–4] The major clinical symptom of most patients is chronic cough. Initially, a dry cough is accompanied by thick sputum; this may persist for several months without adequate treatment. This presentation is similar to that seen in patients with asthma. Some studies suggest that the pathogenic mechanism of action of *M. pneumoniae* is due to its specialized attachment tip, the P1 protein, causing liberation of hydrogen peroxide and inhibition of host cell catalase activity.[5,6] In 1985, Kitahara and colleagues found that 44% of children with *M. pneumoniae* infection had eosinophilia.[7] Yamashita and colleagues reported that the serum concentration of ECP in children with *M. pneumoniae* infection was higher than that seen in normal healthy children, and that the level of serum ECP in children with *M. pneumoniae* infection was as high as that seen in children who had

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asthma attacks.[8] ECP may play a role in inducing chronic cough in children with *M. pneumoniae* infection. These correlative studies are rarely, if ever, performed in Taiwan. The objective of this study was to clarify the role of ECP in children with *M. pneumoniae* infection.

Methods

Patients

Group 1 consisted of 30 pediatric patients with *M. pneumoniae* infection, without a history of asthma, and who ranged in age from 4 to 14 years. Blood samples for serum ECP levels were collected between days 5 and 20 of infection. The diagnosis of *M. pneumoniae* infection was determined by either a positive mycoplasma ELISA IgM test or by a 4-fold increase in the complement fixation test between the acute and convalescent stages.

Group 2 consisted of 30 pediatric patients with asthma who ranged in age from 7 to 14 years. These patients were in a stable condition with no infection, acute asthma exacerbation or steroid use within the past 2 months. Blood samples for ECP levels were collected in the outpatient department.

Group 3 consisted of 30 healthy children who ranged in age from 3 to 12 years; this was designated the control group.

Procedure

All blood samples were centrifuged for 10 minutes at 3000 rpm. The serum was collected and immediately stored at –20°C for further study. Serum ECP levels were measured using an ECP radioimmunoassay kit (Pharmacia, Uppsala, Sweden).

Statistical analysis

When the distribution of data sets was normal, the independent 2-sample *t* test was applied to compare the mean values between 2 groups, and 1-way ANOVA was performed to compare the mean values between more than 2 groups. When the data sets were not normally distributed, the Mann–Whitney U and Kruskal-Wallis tests were applied to compare the medians in different groups. If the means or medians in the groups were significantly different, then pair-wise multiple comparisons were performed using the Bonferroni method.

For a categorical variable, we used the χ^2 test to investigate the homogeneity of the proportion of each category. In addition, Pearson's correlation coefficient was used to measure the strength of the linear correlation between 2 quantitative variables. All statistical assessments were 2-sided and evaluated at the 0.05 level of statistical significance. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

There were 90 children enrolled in this study. Of these, 59 (65.56%) were boys and 31 (34.44%) were girls. The mean serum ECP levels were not significantly different between males and females ($p=0.544$). The variance of serum ECP levels decreased as patient age increased. No obvious trend, pattern or relationship was noted between serum ECP levels and the children's age ($r=0.118$, $p=0.267$).

We compared the ages, serum ECP levels, and gender in all 3 groups. The children in the asthma group tended to be older than the children in the other 2 groups. The ages of the children in the *M. pneumoniae* and control groups were similar. We performed 1-way ANOVA to compare the mean ages among the 3 groups and found a significant difference ($p=0.001$). When multiple comparisons were performed with the Bonferroni method, a significant difference in mean age between the asthma and control groups was found (7.11 years *vs.* 4.53 years).

Figure 1 shows that the serum ECP levels in the *M. pneumoniae* and asthma groups were similar, and that the serum ECP levels in the control group were lower than those found in the other 2 groups. A statistically significant difference in serum ECP levels

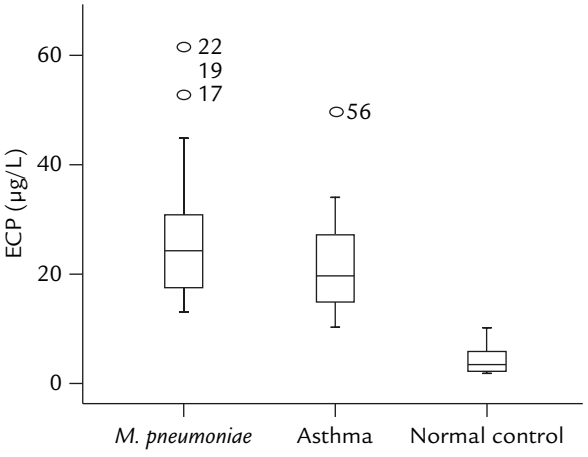

Figure 1. Serum eosinophil cationic protein (ECP) levels in patients with *Mycoplasma pneumoniae*, patients with asthma, and normal controls.

was found among the 3 groups ($p<0.001$). Both the asthma and control groups as well as the *M. pneumoniae* and control groups had different mean serum ECP levels. The mean serum ECP levels in the *M. pneumoniae*, asthma and control groups were 28.88 μg/mL, 23.01 μg/mL and 4.48 μg/mL, respectively. Since gender was a categorical variable, we compared the proportions of males in the 3 groups using the χ^2 test to evaluate homogeneity. We did not have enough evidence to conclude that the proportion of males and females was significantly different among the 3 groups ($p=0.162$).

Discussion

Eosinophilic airway inflammation is a characteristic feature of asthma.[9] ECP is a potent cytotoxic secretory protein with both bactericidal and antiviral properties; it reflects the degree of activation of the circulating eosinophilic pool in the body. Elevated ECP levels may be found in the serum as well as the bronchoalveolar and nasopharyngeal secretions in many diseases, including asthma, bronchiolitis, atopic dermatitis and rheumatoid arthritis.

ECP levels may also increase during infection. ECP levels in the nasopharyngeal secretions of infants with RSV bronchiolitis were significantly higher than those found in infants with non-RSV bronchiolitis.[10] In 1985, Kitahara et al reported that 44% of children with asthma-like *M. pneumonia* infection had eosinophilia.[7] The characteristic symptoms of *M. pneumoniae* infection are prolonged cough with or without sputum, which is similar to the symptoms seen with asthma. In our study, the serum ECP levels in the *M. pneumoniae* and asthma groups were similar; the serum ECP levels in the control group were lower than those in the other 2 groups. The activities of ECP as mentioned previously include toxic effects on human respiratory epithelium seen *in vitro* and stimulating effects on human mast cell histamine release.[11,12] Kitahara et al suggested that elevated concentrations of ECP in the serum of patients with *M. pneumonia* may be associated with damaged respiratory epithelium and accelerated hypersensitivity in the respiratory system.[7] This may act as a factor in causing persistent cough similar to asthma in children with *M. pneumonia*.[8] Serum ECP levels can decrease after treatment with corticosteroids, as it does also in asthma. Patients with *M. pneumoniae* infection have elevated serum levels of ECP and have clinical symptoms similar to an asthma attack. For this reason, decreasing the serum level of ECP might play a role in relieving the symptoms of patients with *M. pneumoniae* infection.

In conclusion, both children with *M. pneumoniae* infection and children with asthma have significantly elevated serum ECP levels compared to normal healthy children. The elevated serum concentrations of ECP in patients with *M. pneumonia* may be associated with damaged respiratory epithelium and accelerated hypersensitivity in the respiratory system, which may cause chronic persistent cough for several months. Decreasing the serum level of ECP might play a role in relieving the symptoms of patients with *M. pneumonia*. Additional studies are warranted to further validate this conclusion.

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