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Eosinophil cationic protein mRNA expression in children with bronchial asthma

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ABSTRACT. Studies have shown that eosinophils are closely related to pathogenesis of bronchial asthma. Eosinophils release eosinophil cationic protein (ECP), which plays an important role in infection and allergic reactions. Serum ECP mRNA expression in children with bronchial asthma has not been adequately investigated. We analyzed serum ECP mRNA expression in 63 children with bronchial asthma and 21 healthy children by using reverse-transcriptase polymerase chain reaction to understand the role of ECP in children with bronchial asthma. The children with bronchial asthma were segregated into acute-phase and stable-phase groups, based on the severity of the illness. Serum ECP mRNA expression in children with bronchial asthma (0.375 ± 0.04) was significantly higher than that in healthy controls (0.20 ± 0.02; $P < 0.05$). Additionally, children in the acute-phase group showed

higher ECP mRNA expression level (0.44 ± 0.06) than those in the stable-phase (0.31 ± 0.03) and healthy control groups (0.20 ± 0.02; $P < 0.05$), while the level in the stable-phase (0.31 ± 0.03) was markedly higher than that in the healthy control group (0.20 ± 0.02; $P < 0.05$). Detection of serum ECP mRNA expression level has possible applications in the diagnosis and treatment of children with bronchial asthma.

Key words: Bronchial asthma; Children; Eosinophils; ECP; mRNA

INTRODUCTION

Bronchial asthma occurs mostly in children and adolescents and is mainly characterized by high airway reactivity, causing reversible airflow limitation. Symptoms include cough, dyspnea, and an expiratory wheezing sound, which can be alleviated with spasmolytics, antiasthmatics, or rest. However, some patients show serious symptoms, which can be life-threatening (Agodokpessi et al., 2014; Folmsbee et al., 2015). Recently, the incidence of bronchial asthma and resulting deaths has increased due to the impact of air pollution as well as environmental and lifestyle changes (Araújo et al., 2014). The pathogenesis of bronchial asthma is not clear. Current literature suggests that bronchial asthma is a type of chronic inflammatory respiratory system disease involving smooth muscle cells, neutrophils, airway epithelial cells, and eosinophils working together, while its pathogenesis is closely related to eosinophils (Zhang et al., 2015). Eosinophils secrete eosinophil cationic protein (ECP), which plays an important role in the incidence of infection and allergic response (Gao et al., 2014; Glover et al., 2014; Norback et al., 2014). Serum ECP mRNA expression in children with bronchial asthma has not been adequately investigated. In this study, we determined serum ECP mRNA expression levels in children with bronchial asthma.

MATERIAL AND METHODS

Reagents

RNA was extracted and reverse-transcriptase polymerase chain reaction (RT-PCR) was performed using kits by Sunbio company (Beijing, China). PCR primer was designed and synthesized by Sunbio company ScienBio (Beijing, China).

Subjects

Children with bronchial asthma

Serum specimens were collected from 63 children with bronchial asthma, consisting of 35 males and 28 females, with mean age of 10 ± 2 years, who visited the Department of Pediatrics at Qilu Hospital of Shandong University between April 2014 and October 2014. Diagnosis was performed as per standards published by the Global Initiative for Asthma (Bateman et al., 2008). This group included 44 cases in acute phase and 19 cases in stable phase of the disease. The clinical data in both acute versus stable phase groups were comparable.

Control group

As the control group, 21 healthy children, 10 males and 11 females, with mean age of 11 ± 3 years, were enrolled from the physical examination center in the same period. The clinical data were comparable.

The study protocol was approved by the Research Ethics Committee at the hospital, and all patients provided informed consent before study commencement.

Methods

RT-PCR was used to determine serum ECP mRNA levels. Blood samples were collected in the morning and centrifuged at 3000 rev/s and stored at -80°C. RNA extraction was performed as per manual instructions. cDNA was synthesized using Invitrogen reverse-transcription kit Sunbio company (Beijing, China). The cycling conditions for RT-PCR consisted of an initial, single cycle at 95°C for 2 min, followed by 32 cycles of 60 s at 95°C, 60 s at 55°C, and 60 s at 72°C, after which 10 μL PCR amplification product was used for electrophoresis and analyzed by statistical software. The following primers were used: ECP sense: 5'-GAAAGATGGGGCTGTTGAGT-3'; anti-sense: 5'-CTTCTCACCACGAGGTAGCG-3'; β-actin sense: 5'-ATTCCTATGTGGGCGAOGAG-3'; anti-sense: 5'-AGAGGC‑GTACAGGGATAGCA-3'

Statistical analysis

All statistical analyses were performed using the SPSS18.0 software (Chicago, IL, USA). Differences between means were analyzed using the *t*-test, where $P < 0.05$ was considered as statistically significant result.

RESULTS

Comparison of serum ECP mRNA levels in children with bronchial asthma *vs* healthy controls

Serum ECP mRNA expression levels in children with bronchial asthma (0.375 ± 0.04) were significantly higher than those in healthy controls (0.20 ± 0.02) ($P < 0.05$; Table 1).

Table 1. Comparison of serum ECP mRNA levels in children with bronchial asthma *vs* healthy controls.

Group	Cases	ECP mRNA level
Bronchial asthma group	63	0.375 ± 0.04
Healthy control group	21	0.20 ± 0.02

$P < 0.05$.

Comparison of serum ECP mRNA level in children with acute phase bronchial asthma *vs* those with stable phase bronchial asthma

The children with bronchial asthma were further divided into acute-phase and stable-phase groups. Children with acute-phase bronchial asthma showed visibly higher ECP mRNA expression levels (0.44 ± 0.06) than did those with stable-phase (0.31 ± 0.03; $P < 0.05$; Table 2).

Table 2. Comparison of serum ECP mRNA levels in children with bronchial asthma in acute phase *vs* those with bronchial asthma in stable phase.

Group	Cases	ECP mRNA level
Bronchial asthma group	44	0.44 ± 0.06
Healthy controls	19	0.31 ± 0.03

$P < 0.05$.

Comparison of serum ECP mRNA levels in children with stable bronchial asthma *vs* healthy controls

Serum ECP mRNA levels in children with stable-phase bronchial asthma (0.31 ± 0.03) were markedly higher than those in healthy controls (0.20 ± 0.02; $P < 0.05$; Table 3).

Table 3. Comparison of serum ECP mRNA levels in children with stable bronchial asthma *vs* healthy controls

Group	Cases	ECP mRNA level
Bronchial asthma group	21	0.31 ± 0.03
Healthy controls	19	0.20 ± 0.02

$P < 0.05$.

Agarose gel electrophoresis of RT-PCR products

Serum samples from children in acute-phase group exhibited stronger bands than did those from children in the stable-phase group, which in turn showed stronger bands than did those from the healthy controls (Figure 1).

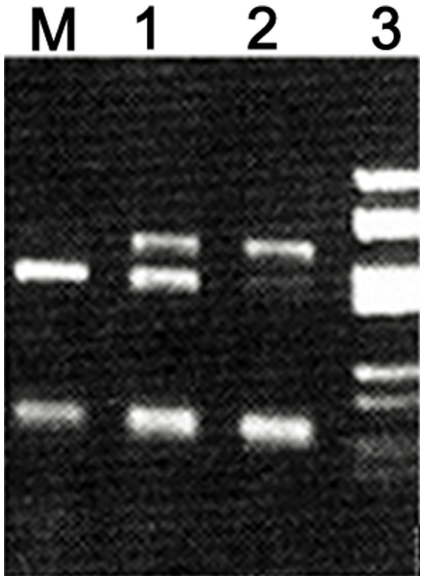

Figure 1. ECP mRNA electrophoresis. *Lane M*, marker; *lane 1*, control; *lane 2*, bronchial asthma in acute phase; *lane 3*, bronchial asthma in stable phase.

DISCUSSION

The etiology of bronchial asthma is still not fully elucidated. Previous studies have revealed that, in addition to infections caused by bacteria, viruses, and mycoplasma, environmental and genetic factors also play an important role in pathogenesis (Masid-de-Brito et al., 2014; Namjou et al., 2014; Martineau et al., 2015). Airway obstruction caused by bronchial asthma is reversible, while airway hyper-responsiveness and stimulation caused by inflammatory mediators are characteristic of bronchial asthma (Schoepf and Heun, 2014; Lozano et al., 2014; Gadhe, 2015; López-Expósito et al., 2015; Turner et al., 2015).

Several studies have indicated that eosinophils are widely distributed in the respiratory mucosa, mainly functioning to clear and engulf bacteria. ECP is a specific marker of eosinophils (Ursaciuc et al., 2010; Shah et al., 2012; Beigelman, 2014; Smith et al., 2015), which is mainly activated and released by eosinophils and causes neurotoxicity and cytotoxicity. In addition, ECP induces release of histamine by mast cells, causing airway hyperresponsiveness, resulting in bronchial asthma attack (Reed et al., 2007; Choi et al., 2010; Chua et al., 2010; Ursaciuc et al., 2010; Wei et al., 2010; Braga et al., 2011; Olive et al., 2011).

A previous study reported a comparison of serum ECP levels in 60 children with capillary bronchitis and 50 healthy controls, where children in acute phase exhibited visibly higher serum ECP mRNA expression levels than did those in stable phase as well as the healthy controls (Brightling et al., 2008). This indicated that ECP mRNA expression increases with increasing severity of the condition. Lee et al. (2003) reported that ECP mRNA was detected in induced sputum samples from 80 children with bronchial asthma and 62 normal controls by using chemiluminescence enzyme-linked immune assay. They found that ECP mRNA level in the sputum was significantly higher in children with acute-phase bronchial asthma than that in children with stable phase or in normal controls (Lee et al., 2003), suggesting that ECP mRNA in the sputum can reflect the severity of asthma.

Our results showed that children with acute-phase bronchial asthma showed obviously higher ECP mRNA expression levels than did those in the stable phase and healthy controls. Serum ECP mRNA expression levels in children with stable-phase bronchial asthma were markedly higher than those in healthy controls. This is in accordance with previous studies.

In summary, serum ECP mRNA expression levels can reflect the severity of bronchial asthma and thus, function as an effective indicator to monitor bronchial asthma. This suggests potential applications in the diagnosis and treatment of children with bronchial asthma.

Conflicts of interest

The authors declare no conflict of interest.

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