Is Serum or Sputum Eosinophil Cationic Protein Level Adequate for Diagnosis of Mild Asthma?

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ABSTRACT

Spirometry has been used as a common diagnostic test in asthma. Most of the patients with a mild asthma have a FEV1 within normal range. Hence, other diagnostic methods are usually used. The aim of this study was to evaluate whether eosinophil Cationic Protein (ECP) could be an accurate diagnostic marker of mild asthma.

In this study diagnosis of asthma was made according to internationally accepted criteria. Asthma severity was evaluated according to frequency of symptoms and FEV1. Adequate sputum samples were obtained in 50 untreated subjects. A control group of 12 normal subjects that showed PC20 more than 8 mg/dl was also examined. Sputum was induced by inhalation of hypertonic saline. Inflammatory cells in sputum smears were assessed semi-quantitatively. ECP and IgE concentrations, eosinophil (EO) percentage and ECP/EO ratio in serum and sputum were also determined.

The results revealed that Cough and dyspnea were the most frequent clinical findings. Dyspnea and wheezing were the symptoms that correlated with staging of asthma. FEV1 was within normal range (more than 80% of predicted) in 22 (44%) subjects. Asthmatic patients showed significantly higher numbers of blood eosinophils (4.5± 3.1% vs. 1.2±0.2%, P=0.009), and higher levels of serum ECP than control group (3.1± 2.6 % and 22.6± 15.8 ng/ml, respectively). Sputum ECP level in asthmatics was significantly higher than non-asthmatics (55.3±29.8ng/mL vs. 25.0±24.7ng/mL, P=0.045). Regression analysis showed no significant correlation between spirometric parameters and biomarkers, the only exception was significant correlation between FEF25-75 and serum ECP (r= 0.28, P 0.041). Regarding clinical symptoms, wheezing was significantly correlated with elevation of most of biomarkers. Since, serum and sputum ECP levels are elevated in untreated asthmatics, the ECP level could be used for accurate diagnosis of mild form of asthma in which spirometry is unremarkable.

Key words: Asthma; Eosinophil cationic protein; Spirometry

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INTRODUCTION

Eosinophils are potent effectors cell in asthma and allergic diseases. Upon activation, eosinophils release their granular proteins and numerous mediators such as eosinophil cationic protein (ECP), major basic protein, eosinophil peroxidase and eosinophil derived neurotoxin in the airway mucosa. The content of these granules are accompanied with other cytotoxic proteins such as arachidonic acid metabolites, and oxygen-derived radicals, in damage to the airway epithelium. Serum levels of ECP show the magnitude of activated circulating eosinophils. Hence, serum level and more specifically sputum ECP level might reflect ongoing eosinophilic airway inflammation. Spirometry is an accurate, noninvasive and easy to use as diagnostic tool for diagnosis of asthma, but this test is normal in mild asthma. In this condition methacholine challenge test should be used. Objective of this study was to evaluate whether eosinophilic cationic protein (ECP) could be considered as a diagnosis marker of asthma and to assess this biomarker to be replaced with other diagnostic tests in mild asthma when spirometry is in normal range. In addition, we tried to evaluate the accuracy of ECP/Eosinophil count ratio (ECP/EO) as a new marker for diagnosis and severity of asthma.

PATIENTS AND METHODS

Subjects

Fifty subjects suffering from asthma (31 females and 19 males) were enrolled in this cross sectional prospective study. Case group were asthmatic subjects that possessed following criteria: 1- history of cough, dyspnea, wheeze and air way hyper responsiveness, 2- increasing the symptoms during nights and some seasons, 3- A spirometry value that showed obstructive pattern with more than 12% increase with bronchodilator or PC20 less than 8mg/l. All of the patients were new cases or subjects that withheld their drugs for a long time.

Subjects with asthma who had a positive history of atopy and/or a total serum immunoglobulin E (IgE) level > 200 IU/ml were defined as having allergic asthma. Asthma was classified as mild intermittent (step 1), mild persistent (step 2), moderate persistent (step 3) and severe asthma (step 4) according to Global Initiative for Asthma classification (GINA).

The control group consisted of twelve normal subjects, with no history of asthma and other allergic disorders. To rule out hyper responsiveness and asthma, all control subjects performed methacholine challenge test. Subjects with PC20 more than 8 mg/ml were accepted. Subjects who had evidence of peripheral blood eosinophilia, abnormal chest X ray, history of smoking, corticosteroid usage (systemic or inhaled) and recent infection were excluded from study.

The experiments were approved by the Ethical Committee of Islamic Azad University of Mashhad and each subject gave his informed consent.

Spirometry

Standard spirometry was performed on all subjects before collecting sample (Superspiro, Microedical Inc. England) according to American Thoracic Society standards. Main variables which were measured consisted of Forced vital capacity (FVC), Forced expiratory volume in one second (FEV1), Forced expiratory flow in 25 to 75% of vital capacity (FEF_{25-75}) and FEV1/FVC. Severities of obstructive lung disease were classified according to Global initiative for Asthma (GINA). Criteria which consisted of mild intermittent and persistent (FEV1 more than 80% predicted), moderate persistent (FEV1 60-79% predicted) and severe persistent (FEV1 less than 60% predicted) disease.

Sputum Specimen Collection

Before sample collection, 400µg salbutamol was delivered to asthmatic subjects. Sputum induction, collection and processing were performed by nebulizing hypertonic saline (nebulizer used was Omron CX3) according to European respiratory society guidelines.

Analysis

Total IgE levels were measured in sputum supernatants as well as in serum samples using a commercially available ELISA Kit according to the manufacturer's instructions (Radim, Pomezia Terme, Italy). Total ECP content was determined in eosinophil lysates, using an ELISA kit (MBL; Naka-ka Nagoya, Japan.). The lower detection limit was 2µg/l.

Peripheral blood eosinophil count was also determined by using automated cell counter (Sysmex; Tokyo, Japan).
Statistical Analysis

Descriptive analysis such as frequency, mean and standard deviation (SD) were applied to qualitative and quantitative variables respectively. Variables such as amount of serum and sputum ECP, IgE and eosinophile were compared within two groups using unpaired two tailed t-test, Mann-Whitney U test and ANOVA analysis. Person's and Spearman's non parametric tests were used to assess correlation between variables. The significance level was set at P<0.05.

RESULTS

General Data

Fifty asthmatic subjects (31 females and 19 males) with average age and SD 41.3 ± 14.6 years (range 9-76 years) were enrolled in this study. The controls were 12 subjects (6 females and 6 males) with average age and SD 37 ± 16.5 years, who showed no significant differences with case group (t= -1.38, P=0.17).

Clinical Findings

The most frequent symptoms in asthmatic subjects were cough in 41 (85%), followed by dyspnea in 35 (72%). Comparison of symptoms in different stages of asthma was shown that frequencies of dyspnea and wheeze were increased as the severity of asthma elevated (rho spearman=0.383, P=0.005 and rho spearman=0.393, P=0.007 respectively). Family history of asthma was present in 21 (43%) of asthmatic subjects and other allergic symptoms were seen in 29 (60%). History of gastroesophageal reflux was mentioned in 17 (35%) of asthmatic subjects that was not significantly higher than control group (X²= 0.48, P=0.5).

Spirometry Results

Mean FEV1 and FEV1/FVC were decreased from 103±14.9 and 101.0±18.6 respectively in mild intermittent asthma to 45.5±24.6 and 76.5±15.6 in severe persistent asthma. All parameters except FEV1/FVC showed close correlation with asthma stages. In 22 (44%) of asthmatic subjects FEV1 as main parameters for diagnosis of asthma was in normal range (more than 80% of predicted).

Laboratory Analysis

Asthmatic patients, had significantly higher numbers of blood eosinophils, and higher levels of serum ECP than control group (Table 1), but total serum IgE levels and ECP/EO ratio in asthmatics were not significantly more than control group.

<table>
<thead>
<tr>
<th>Variables in Blood &amp; Sputum</th>
<th>Asthmatics</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild intermittent n=6</td>
<td>Mild persistent n=16</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>2±1.4 (0-4)</td>
<td>3.6 ± 3.8* (0-12)</td>
</tr>
<tr>
<td>Total IgE (IU/ml)</td>
<td>68.2±44.2 (40.5-157.1)</td>
<td>170±168 (4.9-500)</td>
</tr>
<tr>
<td>ECP (ng/ml)</td>
<td>15.1±14.3 (26-84)</td>
<td>30.7±15.9* (3-86)</td>
</tr>
<tr>
<td>ECP/EO ratio</td>
<td>633±329 (1554±1443)</td>
<td>706±533 (70±10)</td>
</tr>
<tr>
<td>Sputum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>13.1±8.6 (8-25)</td>
<td>13.1±12 (3-34)</td>
</tr>
<tr>
<td>Total IgE (IU/ml)</td>
<td>0.3±0.26 (0.1-0.2)</td>
<td>0.6±0.49 (0.1-1.5)</td>
</tr>
<tr>
<td>ECP (ng/ml)</td>
<td>52.5±19.2* (2.8-40.8)</td>
<td>51.4±29.3 (4.5-48.1)</td>
</tr>
<tr>
<td>ECP/EO ratio</td>
<td>6.4±6.2 (4.5-19.2)</td>
<td>9.19±10 (4.5-48.1)</td>
</tr>
</tbody>
</table>

*= Significant elevation of biomarkers in subjects presenting this symptoms
Sputum eosinophils in asthmatic patients (15.1±11.3% vs. 13.0 ±7.6%, P=0.366) and total sputum IgE levels were not significantly different from control subjects (0.67±0.44 IU/mL vs. 0.27±0.20 IU/mL, P=0.061). However sputum ECP levels in asthmatics were significantly higher than control group (55.3±29.8ng/mL vs. 25.0±24.7ng/mL, P=0.045) (Table 1).

**Correlation of Biomarkers with Spirometry Parameters**

Regression analysis showed no significant correlation between spirometric parameters (including FEV1, FEV1/FVC and FEF25-75) and serum and sputum ECP or ECP/EO indices. The only exception was significant correlation between FEF25-75 and serum ECP (r= 0.28, P 0.041).

Blood and sputum ECP, and also blood eosinophils were significantly more than control group in most of asthma stages (Table 1). Regression analysis showed that elevation of ECP (serum and sputum) were not significantly different in four asthma stages.

**Correlation of Serum and Sputum ECP and IgE with Clinical Symptoms**

Table 2 shows the mean and SD of important biomarkers in subjects with presenting specific clinical findings of asthma. Wheezing was significantly correlated with elevation of most of biomarkers, but night symptoms were correlated only with serum ECP and dyspnea with sputum ECP/EO ratio.

Comparison of biomarkers and spirometry parameters between asthmatic subjects that suffered from other allergic symptoms and the remaining asthmatic subjects showed that serum ECP and blood eosinophils were significantly higher in subjects with other allergic symptoms (27± 16 VS 15±13 ng/ml of ECP and 4.5± 3.1 VS 2.3± 1.7% of eosinophil). Sputum biomarkers and spirometry results were not significantly different in two groups.

**DISCUSSION**

In the present study we evaluated serum and sputum ECP in asthmatic subjects in order to compare their differences. In addition to ECP, the ECP to eosinophil ratios in serum and sputum were mentioned to above measurements.9 We have also made an attempt to discuss whether ECP and ECP/EO ratio have any potential to become an accepted tests for the diagnosis of severity of asthma and to predict clinical response.

Our principal findings indicated that the blood eosinophil percentage and ECP (and total IgE with borderline result) in serum are significantly higher in asthmatic patients than in control subjects. However, sputum ECP was the only biomarker in sputum that revealed significant difference with control group (Table 1). Therefore ECP in serum and sputum are a reliable test for diagnosis of asthma. Spirometry is also a valuable test for diagnosis of asthma.10 This diagnostic test is noninvasive, widely available and reproducible.11 In this study spirometry was able to precisely diagnose asthma in 56% of subjects and showed close correlation with asthma stages. On the contrary, spirometry was normal in 44% of asthmatic subjects were classified as mild (intermittent and persistent) asthma. For resolving this weak point of spirometry, some advanced diagnostic methods were
introduced such as bronchial challenge test\textsuperscript{16}, exhaled nitric oxide\textsuperscript{12} and ECP.\textsuperscript{13} In this study sputum ECP was able to show significant change in all stages of asthma from mild intermittent to severe asthma. In a large series, Bartoli et al\textsuperscript{14} revealed that sputum ECP in different stages of untreated asthma was uniformly elevated. According to this study and ours, sputum ECP is elevated in all cases in uncontrolled asthma in any stages in a similar manner, therefore ECP can be used as firm diagnosis of asthma.

Several studies have shown the association between stages of asthma and ECP concentration. Fernandez et al suggested that the severity of bronchial obstruction in asthmatic patients might be estimated by ECP concentration in sputum.\textsuperscript{15} However Noguchi et al reported that serum ECP levels were not elevated in some patients with asthma, even when they were symptomatic.\textsuperscript{16} In these studies low ECP levels may be related to presence of non-eosinophilic asthma (with definition of sputum eosinophil less than 2\%).\textsuperscript{17} Fortunately in our patients minimum sputum eosinophil count was 13\%, so we did not have non-eosinophilic asthma in our patients.

ECP was evaluated for detecting the presence of subjective clinical findings. Jang and Choi correlated dyspnea as assessed by Borg scale with ECP. They showed that significant relationship was presented between the baseline perception score and FEV\textsubscript{1}/FVC, sputum eosinophils and sputum ECP.\textsuperscript{18} In our study dyspnea was correlated only with sputum ECP/EO ratio. But wheezing was correlated with serum and sputum ECP, sputum ECP/EO and sputum IgE. Therefore in subjects with respiratory symptoms and normal spirometry, we recommend to consider wheezing as the best predictor and the most adjacent symptom to asthma.

Elevation of ECP is a common laboratory finding in subjects with prolonged cough. In a study conducted by Rytila et al.\textsuperscript{19} most of subjects showed increase sputum ECP but they could not confirm specific diagnosis in some of the subjects. We believe that these subjects were mild asthmatic subjects that were not able to be confirmed by conventional diagnostic tests. In this situation ECP seems to be more successful for diagnosis of asthma than other tests with exception of MCT.

Relationship of ECP concentrations and spirometry changes has been reported by a number of other studies. Papadopouli et al have reported the correlation of serum ECP with a decrease in percent predicted FEV\textsubscript{1}(r = 0.6).\textsuperscript{20} In another report Weyer et al have shown a significant negative correlation of serum ECP with lung function tests. In their study strongest correlation was with FEV\textsubscript{1}/FVC ratio (r = - 0.61; P<0.001).\textsuperscript{21} In contrast, our study similar to other studies,\textsuperscript{22,23} did not confirm any significant correlation between ECP and lung function tests. Therefore if eosinophilic inflammation is active during asthma pathology then ECP elevation is anticipated; and this finding is not affected by degree of pulmonary function tests derangement or asthma stages. Also in mild stage of asthma spirometric finding is unremarkable; ECP measurement provides good evidence for diagnosis of asthma.

Authors of this study believe that for diagnosis of asthma spirometry is the first choice. But this test is diagnostic in moderate to severe asthma where FEV\textsubscript{1} is decreased. In mild asthma that FEV\textsubscript{1} is in normal range, induced sputum and evaluating ECP are a good diagnostic method.

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REFERENCES


