Original Article

Role of serum eosinophil cationic protein as a biological marker to assess the severity of bronchial asthma

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ABSTRACT

Objective: The study was carried out to evaluate the role of serum eosinophil cationic protein (ECP) as a biological marker for the diagnosis and to assess the severity of bronchial asthma.

Methodology: This observational cross-sectional study was conducted among 70 bronchial asthma patients and 45 disease controls (tuberculosis-15, chronic obstructive pulmonary disease-15, interstitial lung disease-15) enrolled from patients attending the outpatient department of the National Institute of Disease of the Chest and Hospital (NIDCH), Dhaka, Bangladesh during July 2010 to June 2011. Global Initiative of Asthma Management and Prevention (GINA) criteria were followed for selection of both atopic and non-atopic patients with intermittent or persistent (mild, moderate & severe) asthma. Serum level of eosinophil cationic protein (ECP), IgE, forced expiratory volume in 1 second (FEV_1% predicted) and circulatory eosinophil (CE) count were estimated.

Results: Mean serum ECP level (28.8 ± 42.9 vs. 6.82 ± 3.5 ng/mL; *P*<0.001), IgE level (383.59 ± 225.3 vs. 135 ± 131.8 IU/mL; *P*<0.001) and percent circulatory eosinophil count (9.95 ± 3.7 vs. 5.95 ± 1.4; *P*<0.024) were all found significantly raised among asthma patients than disease controls but %FEV_1 was equivocal. All grades of persistent asthma patients had significantly (*P*<0.025 & *P*<0.002) higher mean ECP level than intermittent cases but serum IgE level and CE count did not differ significantly. FEV_1 % predicted correlated well among moderate and severe persistent asthma but was equivocal for intermittent and mild persistent cases.

Conclusion: This study has reinforced that serum eosinophil cationic protein is a dependable biological marker with more discriminatory power over other indicators for bronchial asthma and to assess its severity.

KEY WORDS: Bronchial asthma, Eosinophil cationic protein, Circulating eosinophil count, Serum IgE level, Biological marker.

Pak J Med Sci July - September 2012 Vol. 28 No. 4 597-601

How to cite this article:

Begum A, Sattar H, Miah MRA, Saleh AA, Hassan MR, Salam MA. Role of serum eosinophil cationic protein as a biological marker to assess the severity of bronchial asthma. Pak J Med Sci 2012;28(4):597-601

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* Received for Publication: May 22, 2012
* 1st Revision Received: May 29, 2012
* 2nd Revision Received: June 12, 2012
* Final Revision Accepted: June 15, 2012

INTRODUCTION

Bronchial asthma is one of the important causes of morbidity as well as mortality in severe cases.[1] More than 300 million asthma cases with 180,000 deaths occur every year throughout the world.[2] According to second National Asthma Prevalence Study (NAPS) during 2010 in Bangladesh, it was estimated that out of 150 million people about 10.5 million (7%) have been suffering from bronchial asthma.[3] The disease can be classified based on the severity of clinical features into four categories viz.

intermittent, mild persistent, moderate persistent and severe persistent asthma, according to Global Initiative of Asthma Management and Prevention (GINA) criteria.[4]

Bronchial asthma is a chronic inflammatory airway disease mediated mainly by eosinophils with its degranulation causing epithelial damage, desquamation and increased airway hypersensitivity.[5,6] To date, the assessment of asthma has been based mainly on surrogate measures of airway inflammation such as airflow limitation or reversibility using spirometry and related techniques. Direct measurement of airway inflammation using biological markers could potentially refine asthma management. In recent years, clinical research has suggested an emerging clinical usefulness of activated eosinophil granule proteins in particular eosinophil cationic protein (ECP), one of the four major cationic granule proteins as dependable biological marker for the management of asthma.[7,8]

Serum ECP level remains significantly elevated in asthmatic subject compared to that of healthy control, which indicates the role of eosinophilic inflammation in the pathogenesis of asthma.[9,10] Although, circulatory eosinophil (CE) count has long been accepted as a good adjunct in the clinical diagnosis of bronchial asthma but patient with symptomatic asthma may not have significantly higher initial CE count compared those with asymptomatic asthma. Higher serum ECP level can help to diagnose asthma in such cases. Moreover serum ECP level correlates well with other indicators of clinical asthma such as peak expiratory flow rate (PEFR), airway hyperresponsiveness, number of inhaler puff needed, symptom onset, seasonal asthma attack, disease activity throughout the year etc.[11] Further, serum ECP increases significantly with increase severity of asthma and is considered to be a good predictor for the assessment of severity as well as risk factor for asthma exacerbation.[12,13] The present study was undertaken to determine the role of serum ECP as a biological marker for bronchial asthma and to assess its severity.

METHODOLOGY

Subjects: This observational cross-sectional study was approved by the Ethical review committee of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. After obtaining voluntary informed consent, 70 clinically suspected bronchial asthma patients (both atopic and non-atopic) of different age and sex who fulfilled the internationally accepted Global Initiative for Asthma Management and Prevention (GINA) criteria[4] were included from the patients attending the outpatient department of NIDCH (National Institute of Disease of the Chest and Hospital), Dhaka, Bangladesh from July 2010 to June 2011. We also included 45 patients with chronic pulmonary diseases (Tuberculosis-15, Chronic obstructive pulmonary disease-15 and Interstitial lung disease-15) who served as disease control for comparison of diagnostic parameters.

Inclusion criteria for asthmatic subjects were:

* A physician's diagnosis of asthma (individuals with a history of oral corticosteroid use within previous 4 weeks of the visit were excluded).
* A positive skin test (Creative Diagnostic Medicare Pvt. Ltd. Mumbai, India) to one of the common aeroallergens to distinguish atopic and non-atopic asthma patients.
* Bronchoprovocation test (BPT) was done for those asthma patients who had normal lung function test ($FEV_1 \geq 80\%$ predicted) but symptoms were suggestive of asthma.
* Positive family history of atopy or allergy.

Laboratory procedures: All study population underwent lung function test (spirometry) and blood sampling (5mL for adult and 3mL for child) for circulatory eosinophil count, serum ECP and IgE levels.

Lung function test: Spirometry (Micro, UK) was performed for measurement of force expiratory volume in one second (FEV_1 % predicted) of the study population at their enrollment after proper explanation of the test procedure.

Circulating Eosinophil count (CE count): Circulating eosinophil count was done from the collected blood for all patients and disease controls by automated cell analyzer and expressed as percentage.

Determination of serum ECP: Quantitative assay of serum ECP was measured for bronchial asthma patients and disease controls by using Mesacup ECP kit (MBL, Naka-Ku Nagoya, Japan). This kit measures human ECP by sandwich ELISA with a minimum detection limit of 0.125 ng/mL. Briefly, the samples and standards were incubated into the microtiter wells coated with anti-human ECP monoclonal antibody. After washing, a peroxidase conjugated anti-human ECP polyclonal antibody was added into the microtiter wells and incubated again. After another washing, the peroxidase substrate was mixed with the chromogen and allowed to incubate for an additional period. An acid solution was then added to each well to terminate

Table-I: FEV1, serum ECP level, CE count and serum IgE level among bronchial asthma patient and disease control.

Group	*FEV1 (% predicted) Mean ± SD*	*Serum ECP level (ng/mL) Mean ± SD*	*C. E. count (%) Mean ± SD*	*Serum IgE level (IU/mL) Mean ± SD*
Bronchial asthma (n=70)	68.50 ± 17.5 (18-92)	28.84 ± 42.9 (0.8-190.5)	9.95 ± 3.7 (7-14)	383.59 ± 225.3 (34.5-712.5)
Disease control (n=45)	64.33 ± 19.2 (22-85)	6.82 ± 3.5 (0.8-13.16)	5.95 ± 1.4 (1-13)	135 ± 131.8 (16.7-353.9)
P value		<0.001	<0.024	<0.001

the enzyme reaction and to stabilize the developed color. The optical density of each well was then measured at 450 nm using a microtiter plate reader. The concentration of ECP was calibrated from a standard curve based on reference standards. Test was considered positive with ECP > 15.6 ng/mL and negative with ECP <15.6 ng/mL.

Determination of serum IgE: Serum total IgE level from all asthma cases and disease controls were measured by DRG IgE quantitative test (IgE EIA kit**,** DRG, USA**).** It is a solid phase sandwich ELISA with an assay range from 5 IU/mL to 800 IU/mL. Manufacturer's instructions were strictly followed for the assay.

Statistical Analysis: The values were reported as mean ± SD at 95% confidence interval. For statistical analysis between group, paired *t* test was used. The levels of each marker were compared between study group and disease control by using SPSS version 16.0. *P* value of <0.05 was considered as significant.

RESULTS

A total of 70 bronchial asthma patients including 30 atopic and 40 non-atopic were enrolled in the study with age ranging from 5 to 70 years. Of patients, 54 were adults (20-male & 34-female) and 16 were children (14-boys, 2-girls).

FEV_1, serum ECP level, CE count and serum IgE level among asthma patients and disease control are shown in Table-I. Mean serum ECP level (28.8 ± 42.9 vs. 6.82 ± 3.5 ng/mL); *P*<0.001), IgE level (383.59 ± 225.3 vs. 135 ± 131.8 IU/mL; *P*<0.001) and circulatory eosinophil count (9.95% ± 3.7 vs. 5.95% ± 1.4; *P*<0.024) were all found significantly raised among bronchial asthma patients than those of disease controls. On the contrary, no significant difference of FEV_1 % predicted was noted between asthma patients (68.50 ± 17.5%) and disease controls (64.33 ± 19.2%).

Patients with bronchial asthma were divided into two broad categories depending on their presenting symptoms viz. intermittent (n= 30) and persistent asthma (n=40). Again persistent asthma patients were subdivided into 3 grades according to their severity of symptoms viz. mild (n=4), moderate (n=24) and severe (n=12). All four laboratory parameters were evaluated among different categories and grades of asthma patients (Table-II). Percent FEV_1 showed significantly higher (*P*<0.001) value among mild (82.5 ± 1.4%) than moderate (67.83 ± 3.9%) persistent asthma. It also showed significantly higher (*P*<0.001) value among moderate (67.83 ± 3.9%) than severe (52.54 ± 12.6%) persistent asthma. However, FEV_1 % predicted did not differ significantly between intermittent and mild persistent asthma patients. Serum ECP

Table-II: FEV1, serum ECP level, CE count and serum IgE level among different categories of bronchial asthma patients (n= 70).

Type of patients	*No. of patients (n)*	*FEV1 (% predicted) Mean ± SD*	*Serum ECP level (ng/mL) Mean ± SD*	*C. E. count(%) Mean ± SD*	*Serum IgE level (IU/mL) Mean ± SD*
Intermittent	30	86.80 ± 4.91 (80-92)	4.48 ± 1.39 (0.8-10.38)	10.06 ± 1.86 (7-13)	219.90 ± 231.4 (34.5-590.5)
Mild Persistent	04	82.5 ± 1.4 (80-85) (P<0.001)	16.85 ± 6.8 (14.20-21.60) (P<0.025)	10.75 ± 4.0 (8-12)	398.15 ± 261.3 (215-612)
Moderate persistent	24	67.83 ± 3.9 (60-79) (P<0.001)	28.51 ± 14.8 (16.79-68.5) (P<0.025)	12.23 ± 3.6 (7-13)	500.16 ± 244.8 (268-674)
Severe persistent	12	52.54 ± 12.6 (18-55) (P<0.001)	92.20 ±70.05 (34.6-190.5) (P<0.002)	13.46 ± 8.41 (7-14)	606.28 ± 167.2 (574.3-712.5)

level showed gradual increasing value among different grades of persistent asthma and all grades had significant (P<0.025 & P<0.002) values than intermittent cases but circulating eosinophil count and serum IgE level did not differ significantly among different categories and grades of asthma patients (Table-II).

DISCUSSION

Conventional diagnosis of bronchial asthma depends upon clinical history along with some objective tests like FEV_1, bronchodilator reversibility test, PEFR, bronchoprovocation test and some routine investigations like CBC, sputum AFB, chest and sinus X-rays to exclude asthma.[2] Recently, serum ECP is being widely studied and investigated to include it as a valuable marker for its diagnostic and prognostic value in bronchial asthma.[14,15]

We found significant higher level of serum ECP (P<0.001) among bronchial asthma patients (28.84 ± 42.9 ng/mL) than disease control (6.82 ± 3.5 ng/mL) (Table-I). In a study done by Samarai *et al.*, (2010), serum ECP level was found significantly higher (p<0.00001) in asthmatic patients (36.12 ± 17.7 ng/mL) than healthy control (7.68 ± 5.63 ng/mL).[1] Similarly, Koller *et al.*, (1995) reported that mean serum ECP level was 34.3 ng/mL in bronchial asthma cases and 9.8 ng/mL in healthy control.[16] Our findings are in good concordance with these two studies. Interestingly serum ECP level observed among disease control in our study was just like that of healthy controls of those similar studies, which has reinforced the good prediction role of ECP in bronchial asthma. Although serum ECP was found to be a useful diagnostic marker for bronchial asthma in several studies, a systematic review of ECP and its usefulness in asthma, Koh *et al.,* (2007) have nullified the diagnostic role of ECP because of its lack of specificity as it was raised in other atopic diseases (e.g. allergic rhinitis, recurrent wheezing) and infections (e.g. rhinovirus infections and bacterial sinusitis).[11]

ECP has also been correlated well with different grades of bronchial asthma in our study. Serum ECP level differentiated (P<0.001) significantly among different categories and grades of persistent asthma (Table-II). We found increasingly higher level of ECP among moderate and severe persistent asthma patients than mild persistent or intermittent cases. These findings are well consistent with Badar *et al.*, (2004) and Samarai *et al.*, (2010)[1,12], which reflects lesser extent airway inflammation in patients with intermittent asthma than in patients with persistent asthma. Thus estimation of serum ECP may be a dependable marker for the assessment of extent of pulmonary inflammation in bronchial asthma.

Considering the FEV_1 as a frequently practiced screening test for bronchial asthma, our findings showed that there was no significant difference of FEV_1 between intermittent and mild persistent cases. On the contrary, serum ECP level was found to be more discriminatory marker for accurate diagnosis of such asthma cases. This finding corroborates with reports by Badr *et al.,* (1999) that serum ECP has a significant negative correlation with FEV_1.[15] Circulating eosinophil count and serum IgE level as predictors of severity of bronchial asthma have been ruled out in our study because number of CE and IgE level did not vary significantly among different grades of persistent asthma. Whereas serum ECP performed better as a discriminatory marker of asthma severity over these two conventional markers and is supported by other investigators.[17,18] In fact, serum ECP level appears to correlate better with the severity of asthma than does eosinophil count as only activated eosinophils release this granular protein.[19]

There were a number of limitations in our study like discrepancy in number of cases and controls, inconsistency of number among different grades of persistent asthma, absence of inclusion of healthy controls, lack of monitoring of cases for ECP and other predictors etc. Had these factors been ruled out, precise role of ECP in asthma could have been better predicted. We conclude from our endeavour that serum ECP level may reasonably help to differentiate asthmatic patients from non-asthmatics, is a dependable biomarker for assessing the severity of asthma and perform better than other predictors. Although substantial knowledge has been gained about ECP but still there are many characteristics yet to explore to validate ECP as inflammatory biomarker in asthma management and to translate our understanding of ECP from bench to bedside.

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Authors' contribution:

All authors have made substantial contributions in the drafting, editing, writing and revising of this article. AB and MAS have coordinated the write-up of the manuscript and are responsible for final edits of the article. MRH has contributed in clinical case selection while HS, MRAM and AAS have supervised the laboratory works and data analysis. All authors have read and approved the final version of the manuscript.

Conflict of interest: Authors disclose no conflict of competing interest.

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