Study of serum C-reactive protein level and sputum eosinophils in patients with bronchial asthma

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Background Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils, and lymphocytes. It is a major chronic airway disorder that poses a serious public health problem worldwide. C-reactive protein (CRP) is used mainly as a marker of inflammation.

Aim of the work This study aims to clarify the relationship between serum CRP, sputum eosinophils, and the degree of airway inflammation in asthmatic patients (stable or in exacerbation) for use as a prognostic marker in detecting the severity of the disease.

Participants and methods The study was carried out on 60 patients who were admitted to the chest department, Benha University Hospital. They were divided into two groups: 40 patients with bronchial asthma (20 patients with controlled asthma and 20 patients with exacerbated asthma) and 20 apparently healthy individuals. Patients and controls were subjected to a full assessment of history and clinical examination. Spirometry, serum CRP level, and sputum eosinophil count were measured in asthmatic patients and in healthy control individuals.

Results Serum CRP was significantly increased in 85% of patients with acute exacerbation, whereas only 30% of patients with controlled asthma showed increased serum CRP. Its level was markedly increased during exacerbation. The sputum eosinophil count was highly increased in the exacerbated asthma group and 25% of patients in the controlled asthma group. There was a negative correlation between CRP, forced expiratory volume in the first second (FEV_1), FVC, and FEV_1/FVC and a highly significant positive correlation with sputum eosinophils.

Conclusion There is an association between airway inflammation in bronchial asthma and elevated level of CRP and sputum eosinophils. *Egypt J Broncho* 2015 9:43–47

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Keywords: bronchial asthma, C-reactive protein, forced expiratory volume in the first second, sputum eosinophils

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Introduction

Asthma is an inflammatory disorder of the airways that involves several inflammatory cells and multiple mediators that result in characteristic pathophysiological changes. The airway inflammation in asthma is persistent even though symptoms are episodic [1]. Two main mechanisms have been identified that underlie airway obstruction in experimental asthma. The first, type I hypersensitivity, is principally an antibody-mediated reaction. The second mechanism that contributes toward airway obstruction, type IV hypersensitivity, also crucially involves Th2 cells [2].

Eosinophils are present in increased numbers in the airways, and release basic proteins that may damage airways epithelial cells. They may also play a role in the release of growth factors and airway remodeling [3,4]. Two-thirds of patients with mild to moderate asthma are reported to have increased sputum eosinophils [5]. Blood eosinophilia is known to be an indirect marker of airway inflammation in asthma [6].

C-reactive protein (CRP) is one of the acute-phase reactants whose levels increase in response to inflammation; thus, it is a marker of airway inflammation. Its synthesis by the liver is regulated to a large extent by the proinflammatory cytokine interleukin-6 [7,8]. Increased CRP levels have been associated with many conditions such as cardiovascular diseases, obesity, smoking, and diet/nutritional state [9]. It is a powerful predictor of adverse cardiovascular events. Respiratory impairment is also associated with cardiovascular events [8,10]. Al-Aarag *et al*. [11] reported elevated levels of CRP in chronic obstructive pulmonary disease patients without clinically relevant ischemic heart diseases (IHD) and independent of cigarette smoking. CRP is associated negatively with indices of pulmonary function and associated positively with sputum eosinophils in steroid-naive asthmatics, but not in those treated with steroids [11]. The association between asthma and CRP is by no means clear. A recent population-based study showed associations of increased levels of serum CRP with a high frequency of bronchial hyperresponsiveness (BHR) [12,13].

The aim of this study was to clarify the relationship between serum CRP and sputum eosinophils in asthmatic patients, either stable or in exacerbation, and also its relation to the respiratory impairment measured by pulmonary function tests (PFT) for use as a prognostic marker in detecting the severity of the disease.

Patients and methods

This study was carried out on 60 individuals, 20 men and 40 women. They were divided into 40 patients with bronchial asthma and 20 healthy individuals as a control group. The patients were admitted in the chest department of Benha University Hospital in the period between March 2011and March 2012. They were divided into three groups: group I included 20 patients with controlled bronchial asthma, two men and 18 women. Their ages ranged from 20 to 40 years, mean age 36.1 ± 7.50 years. Group II included 20 patients with bronchial asthma on exacerbation, six men and 14 women, ranging in age from 20 to 45 years, mean age 32.8 ± 8.89 years. Group III included 20 healthy individuals, 12 men and eight women, ranging in age from 25 to 48 years, mean age 36.1 ± 7.32 years.

According to GINA 2011 [14], controlled asthma was defined as the need for rescue medications twice or less a week (short-acting b2-agonists), no limitation of daily activity, no nocturnal symptoms, twice or less a week, daytime symptoms, and forced expiratory volume in the first second (FEV_1)% greater than 80% predicted. There were no exacerbations and no use of systemic steroids in the previous 12 months. Acute asthma exacerbation was defined as dyspnea and wheezing with or without increased coughing [15]. Patients were excluded if they were smokers, had respiratory infection within the month preceding the study, a rheumatologic illness, malignancy, diabetes, heart failure, a history of venous embolisms, coronary heart disease, and liver or kidney diseases (diseases that may result in elevation of CRP levels) [16].

The diagnosis and classification of asthma were performed according to GINA guidelines (2011) [14]. Informed consent was obtained from all the patients before enrollment. They all underwent a full clinical examination, pulmonary function tests (FEV_1 before and after salbutamol inhalation), sputum and blood sampling for sputum eosinophil count, and measurement of serum CRP. Normal volunteers were also enrolled in the study as healthy controls. None of them had any history of lung or allergic disease and were not using any medication. They had a normal lung function test (FEV_1 >80%). All participants were submitted to a full assessment of history, clinical examination, chest radiography (posteroanterior view), pulmonary function tests, assessment of serum CRP, sputum for eosinophils and others (echocardiography, complete blood count, liver and kidney function tests, and fasting blood sugar).

Pulmonary function tests were performed using Sensor-medics V max series, 2130 spirometer, V6200 Autobox, 6200DL. (Sensor Medics Corporation, 22705 Savi Ranch Parkway Yorba Linda, 92887-4645 California, USA). Short-acting bronchodilators were stopped at least 8 h before the test. Dynamic spirometry was performed by measurement of FEV_1% predicted according to the standards of the European Respiratory Society [17]. The highest value of FEV_1 of three forced expiratory maneuvers was used.

Blood sampling

Fresh serum samples (stable 7 days at 2–8°C or 3 months at −20°C) were centrifuged in the presence of fibrin before testing. The CRP-latex agglutination test was used for the qualitative and semiquantitative detection of the CRP in human serum. Latex particles coated with IgG anti-human CRP were agglutinated when mixed with samples containing CRP. The CRP-latex sensitivity was calibrated to the Reference material CRM 470/RPPHS.

Sputum induction and processing

Sputum was collected either spontaneously or induced with hypertonic saline nebulization from all participants. Before sputum induction, patients inhaled 200 μg of salbutamol to minimize bronchoconstriction during the induction procedure. Sputum was induced by inhalation of a 3% hypertonic saline solution for 5 min (DeVilbiss 65 ultrasonic nebulizer; DeVilbiss, Somerset, Pennsylvania, USA), and the participants were encouraged to cough and expectorate sputum into sterile containers between each dose of nebulized saline. This procedure continued until an adequate sample containing more than 0.5 ml visible mucoid material was obtained. If a satisfactory sputum sample was not obtained at the time the FEV_1 had decreased more than 20% compared with the baseline values occurred or if troublesome symptoms appeared, the procedure was stopped [17]. Nebulization with 4.5% saline was continued for 4-min periods once the FEV_1 had returned to within 10% of the baseline.

Sputum was selected from saliva and was treated by adding four volumes of 0.1% dithiothreitol (DTT-sputolysin 10%; Calbiochem Corp., La Jolla, California, USA) and mixed by rotating for 30 min at 37°C, followed by four volumes of PBS. The suspension was filtered through a 60 mm nylon gauze (Millipore,

North Ryde, New South Wales, Australia) and the total cell count of leukocytes and viability was determined. The cell suspension was centrifuged at 200*g* for 10 min and the supernatant was aspirated and stored at –70°C. The cell pellet was resuspended in PBS to obtain a concentration of 1×10^6 cells/ml and 70 ml was placed in cups of a Shandon III cytocentrifuge (Shandon Cytospin, Sewickey, Pennsylvania, USA) for slide preparation. An adequate sample is defined as less than 50% squamous cells. The eosinophil and neutrophil counts are then expressed as a percentage of the total cell count as it is more accurate than the absolute count [18].

Statistical analysis

The values were reported as mean ± SD. For statistical analysis between two groups, the χ^2-test was used. The levels of each marker were compared between the study groups and the control group using SPSS computer package. (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, USA). *P* values of less than 0.05 were considered significant (Rosner, 1988) [19].

Results

Patients' demographic and laboratory data are presented in Table 1.

CRP was significantly higher ($P < 0.001$) in asthmatic patients compared with the control group. Overall, 30% of the patients in the controlled asthma group and 85% of patients with exacerbated asthma had increased levels of CRP, whereas the healthy group had normal levels of CRP. There was a highly significant difference in CRP between the three groups Tables 2 and 3.

Sputum eosinophils were found in all cases of asthma during exacerbation, whereas only five of patients with stable controlled asthma had positive eosinophils in their sputum. None of the healthy control participants had eosinophils in their sputum as shown in Table 4.

Pulmonary function tests: it was found that FEV_1, FVC, and FEV_1/FVC were significantly decreased in all patients with asthma exacerbation in relation to the stable asthma group and the healthy group (Table 5).

There were highly significant positive correlations between CRP and leukocytosis, erythrocyte sedimentation rate (ESR), a significant positive correlation between CRP and both eosinophils% and peak expiratory flow (PEF), and a highly significant negative correlation between CRP and FEV_1, and FVC. No significant negative correlation was found between CRP and FEV_1/FVC (Table 6).

Table 1 Patients' demographic data

Groups	Age (years) (mean ± SD)	Sex (male/female)
Controlled asthma	36.1 ± 7.50	2/18
Exacerbated asthma	32.8 ± 8.89	4/16
Healthy participants	36.1 ± 7.32	12/8

Discussion

It is well known that CRP increases during infection and autoimmune disorders [19]. A positive relationship has been reported between elevated CRP levels and current asthma [20,21], respiratory impairment [22], and BHR [23]. In recent years, there have been some reports on the measurements of serum levels of Hs-CRP as a useful tool for the detection of systemic inflammation in asthma [24–26].

Eosinophil inflammation is a hallmark feature of asthma [27]. Eosinophils play a crucial role in the pathogenesis and course of asthma as most allergic and nonallergic asthmatic patients, including those of mild asthma, have a bronchial eosinophilia and there is a significant association between eosinophils and severity of asthma as well as BHR [28].

For this reason, several studies were carried out to determine the relationship between asthmatic patients, CRP, and sputum eosinophils [20–30].

In the present study, high serum levels of CRP were related strongly to asthma exacerbation, whereas lower levels of CRP were observed in stable asthma. The difference in CRP was very significantly higher during exacerbation than in stable asthma patients and control individuals. These results were in agreement with other

Table 2 Percentage of positive C-reactive protein in the groups studied

CRP	*N* (%)			*P*-value	Significance
	High CRP (>6 mg/l)	Normal CRP (0–6 mg/l)	Total		
Controlled asthma group (*N* = 20)	6 (30)	14 (70)	20 (100)	0.001	HS
Exacerbated asthma group (*N* = 20)	17 (85)	3 (15)	20 (100)		
Total asthma	23 (57.5)	17 (42.5)	40 (100)		
Healthy group (*N* = 20)	0 (0)	20 (100)	20 (100)		

CRP, C-reactive protein; HS, highly significant.

studies [8,26]. These results support the concept that exacerbated asthma is always associated with increased inflammatory response, which increases the level of CRP in these patients.

These results were also in agreement with Mojtaba *et al*. [29], who showed that CRP levels in asthma patients were significantly higher than those in normal individuals. These results were supported by other authors, such as Takemura *et al.*[24] and Fujita *et al.* [25], who reported that measurement of serum CRP was a useful tool for the detection of systemic inflammation in asthma. Razi *et al.* [30] reported that serum CRP levels measured by high-sensitivity assays increased in acute asthma and may be used as a diagnostic tool for the detection and monitoring of inflammation in these patients.

In the current study, there was a highly significant difference in sputum eosinophil% between the three groups and the eosinophilic count was correlated strongly to asthma exacerbation compared with the stable asthma group and the healthy control group. This result was in agreement with the results of other studies [31,32]. The observation of increased numbers of eosinophils was a feature of asthma for many decades. Eosinophils and their granule products, including Charcot Leyden crystals were a hallmark of spontaneously induced sputum and were plentiful in the airways in post-mortem specimens [32]. Subsequent studies have confirmed this association [33].

In the current study, spirometry was performed for patients during asthma exacerbation, patients with stable asthma, and in normal individuals; there were significant differences in spirometric data between the three groups. There was a highly significant negative correlation between asthma severity and PFT measured by FEV_1, FVC, and FEV_1/FVC. These results were in agreement with those of Mojtaba *et al.* [29], who showed that spirometry markers such as FEV_1, and FVC or FEV_1/FVC in asthma patients were significantly lower than those in normal individuals.

Also, there was a highly significant negative correlation between PFT (the same parameters) and serum CRP. These results were in agreement with those of Alobaidi *et al.* [8], who found that FEV_1 was significantly inversely correlated with serum CRP in all asthmatic patients whether in a stable or an exacerbation state. Many studies found that increasing serum CRP in asthmatic patients was associated with significantly impaired lung functions indicated by decrease in FEV_1, FVC, and FEV_1/FVC, which also support the results of the current study [24, 25, 30, 36–38].

Table 3 C-reactive protein (mg/l) in the groups studied

CRP	Range	Mean ± SD	*P* value	Significance
Controlled asthma group (*N* = 20)	0–22	15.33 ± 2.4	≤0.001	HS
Exacerbated asthma group (*N* = 20)	0–90	55.36 ± 12.36		
Healthy group (*N* = 20)	0–6	2.1 ± 0.7		

CRP, C-reactive protein; HS, highly significant.

Conclusion and recommendations

There is an association between airway inflammation in bronchial asthma and systemic inflammation. CRP is markedly increased in asthmatic patients, especially during exacerbation. Increases in CRP levels were associated with a steeper decrease in FEV_1 and impaired other pulmonary function parameters. CRP showed

Table 4 Sputum eosinophil percentage in the groups studied

Eosinophil (%)	Range	Mean ± SD	Number of cases	*F* test	*P* value	Significance
Controlled asthma group (*N* = 20)	0–16	12.1 ± .2.99	5	8.362	0.009	HS
Exacerbated asthma group (*N* = 20)	0–70	49.36 ± 10.3	20			
Group III (*n* = 20)	Negative	–	0			

HS, highly significant.

Table 5 PFT findings in all groups

PFT	FEV_1		FVC		FEV_1/FVC	
	Mean ± SD	Predicted (%)	Mean ± SD	Predicted (%)	Range	Mean ± SD
Controlled asthma group (*N* = 20)	2.63 ± 0.96	72.6 ± 8.62	3.52 ± 0.63	88.6 ± 9.74	72–97	76.9 ± 6.1
Exacerbated asthma group (*N* = 20)	1.12 ± 0.41	47.6 ± 12.1	2.41 ± 0.28	55.7 ± 8.96	40–68	53.2 ± 8.9
Healthy group (*N* = 20)	2.53 ± 0.63	86.3 ± 6.65	3.99 ± 0.74	91.3 ± 6.96	73–97	74.3 ± 6.7
F test	13.36		2.632		0.635	
P value	0.001		0.015		0.225	
Significance	HS		S		NS	

HS, highly significant; NS, nonsignificant; S, significant.

Table 6 Correlation coefficient (*r*) of CRP with age, sex, pulmonary functions, ESR, total leukocytic count, and eosinophil% in all the groups studied (*n* = 60)

Patients variables	CRP		
	Correlation coefficient (*r*)	*P* value	Significance
Age	−0.253	0.410	NS
Sex	−0.324	0.658	NS
Leukocytosis	−0.852	0.001	HS
ESR	−0.811	0.001	HS
FEV_1	0.563	0.001	HS
FVC	0.510	0.001	HS
FEV_1/FVC	0.017	0.201	NS
PEF	−0.314	0.004	S
Eosinophil%	0.320	0.002	S

CRP, C-reactive protein; HS, highly significant; NS, nonsignificant; S, significant.

a highly significant positive correlation with sputum eosinophils. The degree of sputum eosinophilia correlates with the disease severity in asthma. It is recommended to use serum CRP as a sensitive marker and a diagnostic tool for the detection and monitoring of airway inflammation in patients with bronchial asthma.

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Conflicts of interest

None declared.

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