Original Research Article

Relationship between pulmonary function tests, sputum eosinophilia and total serum IgE levels among asthmatic patients in Duhok, Iraq

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ABSTRACT

Background: Bronchial asthma is a disease characterized by reversible airway obstruction, airway inflammation; and hyper-responsiveness. The prevalence of asthma is high, and both its prevalence and burden have increased over the last several decades. The study of inflammatory markers has implications for the appropriate management of this disease. Inflammatory markers has implications for the appropriate management of this disease. Objective of the study is to determine the correlation between asthma severity using pulmonary function tests with sputum eosinophilia and total serum IgE levels.
Methods: This case-control study was conducted from March 2017 to September 2018 in the respiratory unit of Azadi general teaching hospital. It included 42 asthmatic patients and 18 healthy subjects. They underwent pulmonary function tests and measurement of total serum IgE levels. Induced sputum was done for asthmatic patients.
Results: The age of asthmatic patients ranged from 16-70 years (mean 42±19 years). The asthmatic patient's female: male ratio was 1.8. Mild asthma was the most common severity group (N=18, 43%) followed by moderate asthma (N=14, 33%) then severe asthma (N=10, 24%). Abnormal sputum eosinophilia (≥3%) was detected in 90% of severe asthma (N=9) compared to 36% in moderate asthma (N=5) and 5.6% in mild asthma (N=1). There was significant statistical association between asthma severity and sputum eosinophilia (p=0.00004). The association between asthma severity and total serum IgE levels was highly significant (p<0.0000) with levels of total serum IgE increasing as the severity of asthma increases.
Conclusions: Severe asthma is the least common severity group in this study. Both abnormal sputum eosinophilia and total serum IgE levels are associated with the severity of asthma.

Keywords: Asthma, Eosinophilia, IgE level, Sputum

INTRODUCTION

Asthma is a chronic inflammatory respiratory disorder affecting the airways characterized by respiratory symptoms, airway hyper-responsiveness, structural remodelling and variable reversible airflow obstruction.[1]

Asthma is a major health problem worldwide, with an estimated 300 million affected patients. The global prevalence of asthma appears to range from 1 to 16% of the population in different countries based on standardized methods for assessing asthma clinical features.[2] Trends suggest an increase in both the prevalence and morbidity of asthma.[3]

Annually, the World Health Organization has estimated that 15 million disability-adjusted life-years are lost and 250,000 asthma deaths are reported worldwide.[4]

Asthma has complex pathophysiology where inflammation and pathobiology derangements are present throughout the respiratory system. Airways can be affected by inflammation, hyper-reactivity, remodelling like smooth muscle hyperplasia, and changes of the surrounding tissue, all contributing to the clinical expression of asthma.[5] The inflammatory process in asthma is assumed to be a T helper cell type 2 (Th2) driven process that enhances eosinophil accumulation and immunoglobulin (IgE) product ion, leading to airway hyper-responsiveness.[6]

The asthma severity staging is usually assessed by subjective tools which include clinical assessment and quality of life questionnaires and objective measures including spirometry, peak expiratory flow rate, and Broncho-provocation testing. Current guidelines assess the level of asthma severity according to the severity of symptoms, use of rescue medications, asthma exacerbations, and severity of airflow obstruction.[1] The correlation between clinical, functional, and various biological markers of airway inflammation in asthma severity is not well established.[7] Over the last decade, various non-invasive markers for measurement of airways inflammation such as exhaled nitric oxide, sputum eosinophilia, and serum proteins such as eosinophilic cationic protein have been used in monitoring of asthma.[8]

Serum levels of IgE are strongly associated with the clinical grade of sensitization and disease severity in allergic asthmatic patients.[9] IgE has been shown to be a major contributing factor for bronchial hyper-responsiveness in asthmatics, and ongoing research focuses on the possibility and clinical feasibility of blocking IgE-mediated disorders by monoclonal anti-IgE-antibodies.[10] Determination of total serum IgE levels is used as a screening tool for atopy, although the sensitivity and specificity of serum IgE determination still remains controversial.[9]

Sputum analysis is increasingly used to assess airway inflammation in asthma. Sputum is induced using hypertonic saline. It has been shown to distinguish between asthma and normal subjects, to detect acute airway inflammation during allergen challenge, and to detect reduced airway inflammation after treatment.

The lack of enough local data regarding the pathophysiology of asthma in this area have urged us to conduct this study with a primary aim of examining different parameters related to lung function and inflammation in asthmatic patients.

METHODS

Study design and patients

This case control study was conducted from March 2017 to September 2018 in the respiratory unit of Azadi general teaching hospital. It included 42 asthmatic patients and 18 healthy subjects. The study design was approved by the Duhok General Directorate of Health ethical committee. The informed consent was taken from all patients and healthy controls.

Asthma was diagnosed according to the criteria recommended by the Global Initiative for Asthma. Asthma was described as a medical history of recurrent wheezes, cough, tightness of the chest or dyspnea, and reported reversible airflow limitation with FEV1 ≥20% improvement after albuterol (200μg) or being on treatment for bronchial asthma.[12] At least 1 month before the study, drugs remained unchanged, except for β2-agonists taken as needed. There was no history of upper respiratory infection in the previous four weeks. The healthy controls were non-smokers with no history of bronchial asthma or other respiratory symptoms and predicted FEV1 >80%.[11]

The patients and healthy controls were interviewed before the study data were collected from them using a special questionnaire form which included the demographic and clinical data. The demographic data were name, age and sex. The clinical data included the clinical presentation, past medical history, history of smoking. Then height and weight were measured.

Spirometry was conducted on all patients in accordance with American Thoracic Society/European Respiratory Society guidelines using Spirovit SP-1 spirometer (SCHILLER AG Co., Baar, Switzerland) to determine Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC), and FEV_1/FVC (FEV_1%). Subjects with asthma were instructed to withhold their bronchodilator medications for at least 8 hours before pulmonary function tests. The highest value from three technically satisfactory attempts was recorded. FEV_1 and FVC values were expressed as a percentage of the predicted value.[13] Severity of asthma was classified as follows:[14]

- Mild asthma: symptoms less than once a week, with brief exacerbations; nocturnal symptoms not more than twice a month; FEV_1 ≥80%.
- Moderate persistent asthma: symptoms daily; exacerbations may affect activity and sleep; FEV_1 between 60-80%.
- Severe persistent asthma: symptoms daily with frequent exacerbations; frequent nocturnal symptoms; limitations physical activities; FEV_1 ≤60%.

Laboratory testing

All the asthmatic patients were subjected to induced sputum using 3% hypertonic saline. The sputum was homogenized by adding phosphate-buffered saline (PBS), vortexed for 30s, and centrifuged for 10 min. Authors added 0.1% dithiothreitol to the cells in a ratio of 4:1,

which was agitated for 20 min to break up the disulphide bonds and disperse the cells. Cells were washed once more with PBS and re-suspended. The cell suspension was aspirated and filtered to remove any remaining debris. Supernatant was separated from cell pellet. Sputum sample was transferred to the slide and was distributed thinly and evenly over the slide. Staining was done by hematoxylin and eosin and analysed using microscopy to determine the count for eosinophils. Sputum eosinophil count ≥3% was considered abnormal.[1]

Under aseptic precaution, 5ml of blood was taken into vacutainers from each asthmatic patient and the healthy controls and measured for serum total IgE in Vin Laboratory in Duhok governorate by Roche Diagnostic Cobas e411 Immunoassay System utilizing electrochemiluminescence (ECL). The results were expressed in International Units (IU/ml).

Statistical analysis

Analysis of data was done using Statistical Package for the Social Sciences (SPSS) program version 21.0 for windows. Statistical analysis was conducted to calculate the p-value using χ^2 (or Fisher's exact test if an expected number in any cell was less than 5). Testing for differences among the means of groups was done using Analysis of Variance (ANOVA). For the associations or differences to be significant, the p-value should have been less than 0.05.

RESULTS

The age of asthmatic patients ranged from 16 to 70 years with a mean of 42 years (Standard deviation-SD=19 years) while that of the control ranged from 18 years to 64 years with a mean of 33 years (SD=10 years). Out of the 42 asthmatic patients, 27 were females and 15 were males with a ratio of 1.8 to 1 while out of the 18 control subjects, 9 were females and 9 were males with a ratio of 1:1. The mean body mass index of the asthmatic patients was 26.7 (SD=6.3)Kg/m² while that of the control

subjects was 23.4 (SD=4.1)Kg/m². There was no significant statistical difference between asthmatic patients and control subjects regarding age, sex and body mass index as shown in Table 1.

Table 1: Characteristics of Study Population.

	Asthmatic patients N=42	Control subjects N=18	p-value
Age (Mean±SD) years	42±19	33±10	0.06
Sex (Female/ Male)	27/15	9/9	0.15
Body mass index (Kg/m²) (Mean±SD)	26.7±6.3	23.4±4.1	0.11

The severity of asthma was classified into mild, moderate and severe. Mild asthma was the most common group (N=18, 43%) followed by moderate asthma (N=14, 33%) then severe asthma (N=10, 24%) as shown in Figure 1.
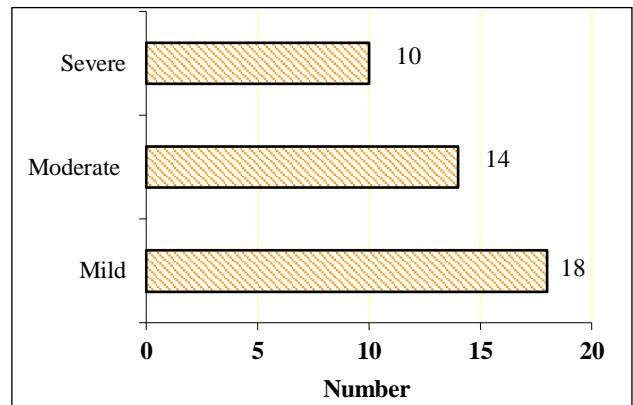

Figure 1: Severity of asthma (N=42).

The results of pulmonary function tests of the asthmatic patients are shown in Table 2. There are significant statistical differences among the asthma severity groups regarding FEV_1 (p=0.00002), FVC (p=0.02) and FEV_1/FVC (p=0.03), respectively.

Table 2: Pulmonary function tests in different asthma severity groups (N=42).

Parameters	Mild (N=18)	Moderate (N=14)	Severe (N=10)	p- value
FEV_1 % predicted	83.2±1.5	67.3±4.9	48.1±5.4	0.00002
FVC % predicted	89.1±4.8	78.7±9.4	62.2±10.1	0.02
FEV_1/FVC % predicted	91.6±5.2	80.5±7.5	69.3±10.9	0.03

Induced sputum was used for measuring sputum eosinophilia (abnormal if ≥3%) which was detected in 90% of severe asthma cases (N=9) compared to 36% of cases of moderate asthma (N=5) and 5.6% of case of mild asthma (N=1). There was a significant statistical

association between asthma severity and sputum eosinophilia (p=0.00004) as shown in Table 3.

Table 4 the total serum IgE levels in the control subjects and asthmatic patients. The association between asthma

severity and total serum IgE levels was highly significant (p<0.0000) with levels of total serum IgE increasing as the severity of asthma increases.

Table 3: Sputum eosinophilia in different asthma severity groups (N=42).

Asthma severity	Number of patients having abnormal sputum eosinophilia (≥3%)	p-value
Mild asthma	1/ 18 (5.6%)	
Moderate asthma	5/ 14 (36%)	0.00004
Severe asthma	9/ 10 (90%)	

Table 4: Total serum IgE levels in study population.

Study groups	Serum total IgE level (Mean±SD)	p-value
Control subjects	52.6±42.8	
Mild asthma	111.6±79	
Moderate asthma	390±94	<0.0000
Severe asthma	921±533	

DISCUSSION

Asthma is a leading chronic disease affecting patients of all age groups with its prevalence increasing steadily internationally since 1980s, prompting concern regarding the genetic and environmental factors, which may underlie the ''asthma epidemic''. Asthma accounts for significant work absence days among adults aging 18 years and older and its annual economic burden in the United States is estimated to be $12.7 billion.

Various factors related to asthma have been investigated for the last 60 years including genetic, immunologic, and environmental factors. Numerous studies have established the hereditary nature of asthma. Atopy, defined as the predisposition to develop IgE antibodies against environmental substances, is associated strongly with asthma. Environmental factors including infections, smoking and allergen exposure have been linked to asthma development.[16,17]

Matching of the age, sex and body mass index was done between the asthmatic patients and the control subject. The mean age of the asthmatic patients in this study was 42 years with 76% of asthmatic patients in this study having the onset of their disease before the age of 40 years. This is identical to the results of previous studies.[18,19]

In this study, the incidence of bronchial asthma is higher in females than males, which is also similar to previous studies.[19,20] Before the age of 10 years, asthma is more prevalent in boys than in girls which is probably related to narrower airways, increased airway tone, and possibly

higher IgE in boys but this difference disappears after the age of 10 years when airway diameter/length ratio becomes the same in both sexes, because of changes in thoracic size that occurs at puberty in males. Also, more females than males develop asthma during puberty, so the prevalence of adult asthma becomes higher in females than males.[20] The mean body mass index of asthmatic patients was $26.7 Kg/m^2$, and asthma incidence is positively related to both body mass index and changes in body mass index as shown in previous studies.[21]

In this study, the most common group of asthma severity was mild followed by moderate then severe. The tools for assessing asthma severity are not consistent among studies and different guidelines recommend using different methods to identify asthma severity e.g., the Global Initiative for Asthma (GINA) criteria classifies asthma as mild, moderate, and severe while National Institute of Health in USA (NIH) classifies it as intermittent and persistent.[22] Most patients with asthma have a mild or moderate disease and data on the prevalence of severe asthma is sparse but reports indicate between 5 and 10% of the asthmatic populations to have had severe disease.[23] In this study, the method used for classifying asthma severity incorporated patient's symptoms into pulmonary function test but the main parameter was the results of FEV_1 which made the results of pulmonary function tests statistically significantly different among the different asthma severity groups.[1,14,19]

The induced sputum identifies eosinophilic inflammation that is inversely associated with lung function parameters. Studies have shown convincing evidence for multiple roles that immune, inflammatory, and structural cell types can play in this complex disease.[24] Abnormal sputum eosinophilia (≥3%) was observed in 90% of case of severe asthma with significant statistical association between asthma severity and sputum eosinophilia (p=0.00004). This was observed in many studies with significant inverse correlation between sputum eosinophil count and predicted FEV_1.[1,25-27] although few studies have demonstrated conflicting results.[28,29] Eosinophilic asthma is a phenotype of severe asthma that is associated with elevated levels of eosinophils in tissues and sputum. Eosinophils play a role in airway inflammation, and increased concentrations of eosinophils are associated with increased frequency of exacerbations and poor disease control.[30] The importance of identifying sputum eosinophilia is related to steroid responsiveness. A treatment strategy that normalizes induced sputum eosinophil count reduces asthma exacerbations and admissions without the need for additional anti-inflammatory treatment.[31]

In this study, serum IgE level was statistically significantly higher in the asthma group compared with the control group with levels of total serum IgE increasing as the severity of asthma increases. This is in agreement with other studies.[32-34] This is attributed to that

there is a link between total IgE and asthma, which appears to be independent of allergen sensitization. Total IgE levels are related to the degree of exposure and severity of disease. Inability to control the environment causes higher exposure to allergens and more persistent asthma. Measuring total IgE levels may be used as a biomarker to determine whether efforts to control the environment of suspected allergens was enough or needs reevaluation. The total IgE levels decline with steroid therapy, a double-blind study concluded that using inhaled beclomethasone dipropionate at 800 mg per day for 3 months significantly reduces total IgE levels and improves asthma symptoms. Also, total IgE levels decreases 2 weeks after corticosteroid treatment.[35]

CONCLUSION

In conclusion, severe asthma is the least common severity group in this study. Both abnormal sputum eosinophilia and total serum IgE levels are associated with the severity of asthma.

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