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The study of fibulin-1 as a novel biomarker in bronchial asthma and its association with disease severity

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ABSTRACT

A key feature of asthmatic airways is remodeling which involves thickening of the airway wall, altered deposition of extracellular matrix (ECM) proteins and increased airway smooth muscle (ASM) mass. Fibulin-1 (FBLN-1) assists in stabilizing the ECM which maintains airway function and structure by providing mechanical support in addition to constituting a dynamic and complex network that influences cellular function. The present study aimed at investigation of possible association between the fibulin-1 levels in asthmatic patients and its relation to asthma severity.

Subjects: The study was carried out on forty five asthmatic patients and thirty control normal subjects age and sex matched.

Methods: All subjects included in the present study were subjected to: full history taking, complete clinical examination, laboratory investigation and chest X-ray. Pulmonary function test and reversibility test. Serum samples from all studied patients and controls were taken for estimation of level of fibulin-1 Enzyme-linked immune-sorbent assay (ELISA). All asthmatic patients will be examined with fibroptic bronchoscope and bronchoalveolar lavage were taken for estimation of level of fibulin-1.

Results: The mean level of serum fibulin-1 in asthmatic patients was 244.10 ± 98.28 pg/ml in mild group, 217.97 ± 121.16 pg/ml in moderate group, 172.20 ± 53.85 pg/ml in severe group compared to a mean level of 187.23 ± 67.97 pg/ml in control group. It was found that fibulin-1 increased in serum of asthmatic patients than in controls with statistical significant difference ($p < 0.05$) but there was no significant relation to asthma severity. Estimation of BAL fibulin-1 in the three asthmatic groups, the mean level of BAL fibulin-1 was 507.0 ± 152.27 pg/ml in mild group, 692.81 ± 207.14 pg/ml in moderate group, 702.0–127.67 pg/ml in severe group. It was found that fibulin-1 was increased in BAL of severe degree bronchial asthma than in mild and moderate degrees with statistical significant difference ($p < 0.05$).

Conclusion: This may highlights the potential role of fibulin-1 in airway wall remodeling.

Introduction

Asthma is a chronic inflammatory disorder of the airways characterized by variable and reversible airflow obstruction and airway hyper-responsiveness (AHR). A key feature of asthmatic airways is remodeling which involves thickening of the airway wall, altered deposition of extracellular matrix (ECM) proteins [1,2] and increased airway smooth muscle (ASM) mass. These structural changes may result from an aberrant repair process in the lung, which includes increased proliferation of the ASM cells [3,4]. Whilst current treatments control the symptoms of asthma, they are unable to fully prevent or reverse airway remodeling.

The ECM maintains airway function and structure by providing mechanical support in addition to constituting a dynamic and complex network that influences cellular function [5]. The ECM deposited by asthma derived ASM cells is altered such that increased amounts of collagen I and laminin [6–8], as well as

Abbreviations: FBLN-1, fibulin-1; ECM, extracellular matrix; AHR, airway hyper-responsiveness; ASM, airway smooth muscle; FN, fibronectin; TGF-β, transforming growth factor β; BAL, bronchoalveolar lavage; ELISA, Enzyme-linked immune-sorbent assay.

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fibronectin (FN) are produced which mediate a range of cellular interactions including migration, growth and differentiation.

Levels of the profibrotic cytokine transforming growth factor β (TGF-β) are elevated in the bronchoalveolar lavage (BAL) in asthma [9], and are increased in bronchial tissue [10]. TGF-βstimulated FN deposition is also enhanced in asthma derived bronchial epithelium and fibroblasts [11,12].

Fibulin-1 (FBLN-1), a secreted glycoprotein, assists in stabilizing the ECM. It associates with FN and a variety of other ECM proteins including laminin and fibrinogen [13]. FBLN-1 expression has been reported in human lung tissue using microarray technology, however, FBLN-1 was not verified by PCR or at the protein level, nor were functional studies carried out [14–16]. In other study reported reduction of FBLN-1D expression in asthma derived bronchial biopsies compared with those derived from non-asthmatics [14]. However, the function of FBLN-1 in the lungs and its role in asthma is unknown. The present study aimed at investigation of possible association between the fibulin-1 levels in asthmatic patients and its relation to asthma severity.

Subjects

The study was carried out on forty-five asthmatic patients were classified into three groups [17] group I: Fifteen asthmatic patients (mild stage ,(group II: Fifteen asthmatic patients (moderate stage (and group III: Fifteen asthmatic patients (severe stage) and thirty age and sex- matched normal control subjects with no history of asthma or other lung disease and normal spirometry recruited from the clinical and chemical pathology department and chest diseases department of the Alexandria Main University Hospital.

An informed consent was taken from all subjects prior to the onset of the study.

Inclusion criteria: asthmatic patients in different degrees of disease (mild, moderate and severe degrees).

Exclusion criteria: renal, hepatic, immunological diseases, connective tissue diseases and smoking.

Methods

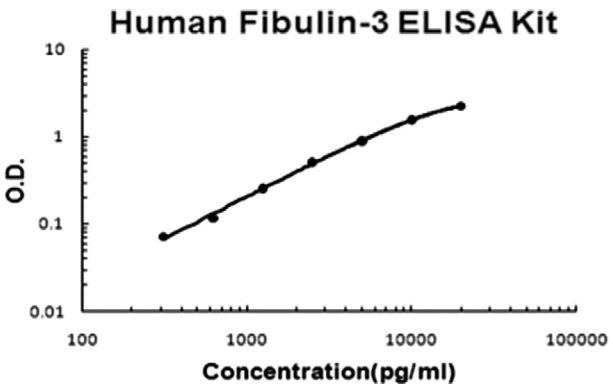
All subjects included in the present study were subjected to: full history taking, complete clinical examination, laboratory investigation and chest X-ray with Pulmonary function and reversibility tests. Serum samples from all studied patients and controls were taken for estimation of level of fibulin-1 by Enzyme-linked immune-sorbent assay (ELISA). All asthmatic patients were examined with fibroptic bronchoscope and bronchoalveolar lavage was taken for estimation of level of fibulin-1. Bronchoalvoelar lavage (BAL) [18] was obtained (from patients only)by flexible fibreoptic bronchoscopy. Specimens were collected via normal saline lavage of the segmental airways and alveolar spaces in sterile containers, centrifuged for 20 min at speed of 2000–3000 r.p.m. to remove mucus and cells. Supernatant was removed, aliquot and stored at −20 °C till later use. Samples were centrifuged again after thawing before they were processed.

Enzyme-linked immune-sorbent assay (ELISA) [19–22].

The assay measure Human FBLN1 level in the sample using Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human Fibulin-3/EFEMP1. The sensitivity is up to <10 pg/ml It use Purified Human FBLN1 antibody to coat microtiter plate wells, Combined FBLN1 antibody which with enzyme labeled, become antibody - antigen - enzyme-antibody complex. After washing completely, add substrate. Substrate becomes blue color at HRP enzyme-catalyzed. Reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of FBLN1 in the samples is then determined by comparing the optical density (O.D) of the samples to the standard curve.

Calculation: The standard curve was drawn on graph paper with the standard density as the horizontal, the OD value for the vertical. The corresponding density according to the sample OD value had been found out by the Sample curve, multiplied by the dilution multiple.

Assay range 20 varied from 20 pg/ml to 800 pg/ml.



Results

Table 1 shows comparison between the four studied groups according to demographic data. The control group included 3 males (10%) and 27females (90%) with a mean age of 40.66 ± 9.33 years (ranging from 1962 years). The asthmatic patients include 2 male (13.3%) and 13 females (86.78%) with a mean age of 46.20 ± 10.42 (ranging from 26–62 years) in mild stage, 1males (6.7%) and 14 females (93.3%) with a mean age of 45.53 ± 14.6 (ranging from 26–72 years) in moderate stage, 2 males (13.3%) and 13 females (86.7%) with a mean age of 42.33 ± 10.24 (ranging from 22–56 years) in severe stage.

Table 2 shows estimation of serum levels fibulin-1 in both asthmatic patients and control group. The mean level of Serum fibulin-1 in asthmatic patients was 244.10 ± 98.28 pg/ml (ranging from 170.50–577.0 pg/ml) in mild group, 217.97 ± 121.16 pg/ml (ranging from 45.50–454.50 pg/ml) in moderate group, 172.20 ± 53.85 pg/ml (ranging from 101.50–265 pg/ml) in severe group compared to a mean level of 187.23 ± 67.97 pg/ml (ranging from 49.50–426.50 pg/ml) in control group. It was found that fibulin-1 increased in serum of asthmatic patients but without relation to asthma severity.

Table 3 shows estimation of BAL fibulin-1 in the three asthmatic groups. The mean level of BAL fibulin-1 was 507.0 ± 152.27 pg/ml (ranging from 290.50–750.0 pg/ml) in mild group, 692.81 ± 207.14 pg/ml (ranging from 260.50–960.0 pg/ml) in moderate group, 702.0–127.67 pg/ml (ranging from 550.0–1050.0 pg/ml) in severe group.

It was found that fibulin-1 increased in BAL of severe degree bronchial asthma than in mild and moderate degrees with statistical significant difference ($p < 0.05$).

Discussion

Asthma is a chronic inflammatory disorder of the airways characterized by variable and reversible airflow obstruction and airway hyper-responsiveness (AHR). It is thought to be caused by a combination of genetic and environmental factors [23]. Its diagnosis is usually made based on the pattern of symptoms and/or response to therapy over time [24].

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