Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20161121065943

Original Research

Clinical significance of periostin in Egyptian asthmatic patients

Amr Yehia[1], Mohamed Badr[1], Fawzy El-Emery[2], Mohamed El-Zahabi[1]

[1]Department of Biochemistry, Faculty of Pharmacy, Alazhar University, Nasr City, Egypt, [2]Department of Chest, Faculty of Medicine, Tanta University, Tanta, Egypt

Address for correspondence: Amr Yehia, Department of Biochemistry, Faculty of Pharmacy, Alazhar University, Nasr City, Egypt. E-mail: dr.amr_yehia@yahoo.com

Received: October 24, 2016
Accepted: November 21, 2016
Published: February 15, 2017

ABSTRACT

Objective: Asthma is an inflammatory disease of the airways appropriate to pulmonary eosinophilia, airway hyperresponsiveness, and mucus overproduction. We now investigate the level of periostin serum concentration in Egyptian asthmatic patients and define any correlation between periostin and pulmonary function tests (PFTs). **Methods:** Totally, 80 subjects were enrolled in the study: 20 control (11 males and 9 females), 28 atopic asthmatic patients (19 males and 9 females), and 32 non-atopic asthmatic patients (14 males and 18 females). Blood samples were obtained from the subjects for laboratory investigations which divided into two aliquots, first aliquot for whole blood used for complete blood count to determine blood eosinophil count by using Sysmex XP-300 and second aliquot for serum used for estimation of periostin, transforming growth factor beta 1 (TGF-β1) and total immunoglobulin E (IgE) using enzyme-linked immunosorbent assays. **Results:** Periostin concentrations were significantly higher in atopic and non-atopic groups (mean ± standard error [SE]; 6889 ± 393.8 and 6212 ± 348.8 pg/ml respectively) compared to control group (3053 ± 446.5 pg/ml) at $P < 0.05$. TGF-β1 were significantly higher in atopic and non-atopic groups (mean ± SE; 75.83 ± 1.53 and 73.47 ± 1.09 pg/ml respectively) compared to control group (51.26 ± 2.65 pg/ml) at $P < 0.05$ and IgE were significantly higher in atopic group (mean ± SE; 151.3 ± 15.10 IU/ml) compared to control group and non-atopic groups (52.47 ± 3.045 and 48.51 ± 1.827 IU/ml respectively) at $P < 0.05$. Periostin correlated negatively with PFT which forced vital capacity, forced expiratory volume 1 (FEV1) and FEV1%. **Conclusions:** Serum periostin is increased in Egyptian asthmatic (atopic and non-atopic) patients compared to control and periostin correlated negatively with PFTs in asthmatic patients. Asthma is the most significant variable relates to high periostin serum concentration.

KEY WORDS: Asthma, atopic and non-atopic, immunoglobulin E, periostin, transforming growth factor beta 1

INTRODUCTION

Asthma is an inflammatory disease of the airways showing bronchial hyperresponsiveness, movable airflow limitation and pulmonary eosinophilia affecting about 300 million people in the world [1]. Asthma can be classified as allergic "atopic" or non-allergic "non-atopic" based on the presence (atopic) or absence (non-atopic) of specific immunoglobulin E (IgE) antibodies to natural environmental allergens. Atopic asthma is the most regular type of asthma. In allergen-sensitized patients including atopic asthma, re-exposure to an aeroallergen will induce an IgE-mediated inflammatory cascade in the airways [2].

Periostin, act as osteoblast-specific factor 2, was first described in 1993 and was named related to its expression in the periodontal ligament and periosteum of adult mice [3]. Periostin is a 90 kDa member of the fasciclin-involving protein family. Periostin is a matricellular protein that negotiates cell activation by binding to receptors present on the cell surface [4-6].

Epithelial cell-derived periostin in asthma alters collagen fibrillogenesis or cross-linking and bring about solidifying of the matrix and mutation of the biomechanical properties of the airway. Interleukin 13 induced periostin in bronchial epithelial cells in asthma obtain autocrine effects to up-regulate the expression of transforming growth factor beta 1 (TGF-β1) and Type 1 collagen through integrins and matrix metalloproteinase production. Meanwhile, the epithelial cell-derived periostin brings to bears paracrine effects to induce secretion of Type 1 collagen by airway fibroblasts in a TGF-β-confident manner [7].

TGF-β1 is relevant fibrogenic, and immunomodulatory factor advised to have pivotal roles in the pathogenesis of airway remodeling [8]. TGF-β1 promotes propagation of fibroblasts and degradation of collagen [9].

Periostin is expressed at higher levels in patients influenced by conditions that are held by elevated cell division, cell turnover, cell invasion, and angiogenesis [10].

Pulmonary function testing (PFT) justly documents these conclusions and is the standard diagnostic test for asthma. PFT also has been analytically shown to advance asthma diagnosis and treatment and rule out other diseases, thus, lack of

testing increases the incidental of misdiagnoses and associated morbidities in the population [11,12].

This work is, therefore, designed to investigate the influence of periostin serum concentrations in Egyptian asthmatic patients. We also surveyed the correlation between periostin serum concentration and PFTs in asthmatic patients.

Subjects

A total of 80 subjects aged 39-57 years were collected, 60 moderate attending patients were chosen from outpatient Chest Clinic, Chest Department, Tanta University Hospitals, and 20 healthy volunteers were collected as controls, from December 2014 till October 2015. The experimental protocol was approved by the Ethics Committee of Chest Department, Tanta Hospital University and Biochemistry Department, Faculty of Pharmacy Boys, Al-Azhar University, Cairo. In total, 60 asthmatic patients were enrolled in the study with forced expiratory volume 1% (FEV1%) <80% of the predicted based on Global Initiatives of Asthma Scale for Asthma Severity: 28 atopic asthmatic patients (19 males and 9 females), their ages ranged between 39 and 57 years they had shown positive skin prick test and high level of IgE in serum and 32 non-atopic asthmatic patients (14 males and 18 females), their ages ranged between 39 and 57 years they had shown negative skin prick test and normal level of IgE in serum. Exclusion criteria were the presence of specific respiratory diseases such as cystic fibrosis and tuberculosis, identified by a physician, and the presence of other seriously interfering diseases. None of the subjects used oral corticosteroids on a regular basis. Inhaled corticosteroids or cromoglycate were not available. All patients were in good physical and mental health. A detailed medical history and drug treatment were collected from all subjects. All patients have been signed an informed consent before the study. They were informed about the purpose and nature of the study.

METHODS

Venous blood samples were collected from all subjects for laboratory investigations which divided into two aliquots: The first aliquot for whole blood for complete blood count was done using Sysmex XP-300 to determine blood eosinophil count and the second aliquot was collected in gel separating tube, for serum used for estimation of Periostin, TGF-β1, and Total IgE. Blood samples of the second aliquot were allowed to clot at room temperature for 30 min followed by centrifugation at 4000 rpm for 10 min. and then samples were stored in three eppendorf tubes at - 20°C till the time of assay. The concentrations of serum Periostin, TGF-β1 and IgE were determined at the Central Lab of Biochemistry Department, Faculty of Pharmacy (Boys), Al-Azhar by enzyme-linked immunosorbent assay (ELISA) using commercially applicable kits: Human Periostin ELISA kit (Boster Biological Technology Co., Ltd, USA) [3], Human TGF-β1 ELISA kit (Boster Biological Technology Co., Ltd., USA) [13] and Human IgE ELISA kit (R-Biopharm, Germany) [14] respectively. All ELISA plates were measured by State fax 2100 according to the manufacturer's instructions.

PFTs

Forced vital capacity (FVC), FEV1 and FEV1% of all subjects were recorded using (Spiro lab II 125-00155, Roma, Italy).

Skin prick test and PFTs for all subjects were done at Chest Department, Tanta University Hospital.

Data Analysis

All analysis and graphics were performed using GraphPad prism 6 (windows version 7; GraphPad software 2010). All results were presented as mean values ± standard error unless indicated otherwise. Differences between groups were evaluated by the calculation of unpaired Student's *t*-test. Correlations between biochemical markers and other contiguous wavering were tested using the Spearman or the Pearson's correlation coefficients. A $P < 0.05$ was studied to be statistically important. All reported *P* values are based on two-sided tests and compared to a significance level of 5%. D'Agostino-Pearson omnibus test was used to identify whether the variables were normally distributed.

RESULTS

We found that FVC was significantly decreased in atopic and non-atopic groups (mean ± SE; 88.64 ± 0.382 and 88.81 ± 0.438 respectively) compared to control group (97.30 ± 0.241). FEV1 was significantly lower in atopic and non-atopic groups (mean ± SE; 67.50 ± 0.446 and 66.41 ± 0.580, respectively) compared to control group (87.20 ± 0.953) and FEV1% was significantly lower in atopic and non-atopic groups (mean ± SE; 75.64 ± 0.615 and 74.38 ± 0.651 respectively) compared to the control group (88.90 ± 1.02) [Table 1].

Serum periostin concentrations were significantly higher in atopic and non-atopic groups (mean ± SE; 6889 ± 393.8 and 6212 ± 348.8 pg/ml, respectively) compared to control group (3053 ± 446.5 pg/ml) at $P < 0.05$. TGF-β1 was significantly higher in atopic and non-atopic groups (mean ± SE; 75.83 ± 1.53 and 73.47 ± 1.09 pg/ml respectively) compared to control group (51.26 ± 2.65 pg/ml) at $P < 0.05$ and IgE was significantly higher in atopic group (mean ± SE; 151.3 ± 15.10 IU/ml) compared to control group and non-atopic groups (52.47 ± 3.045 and 48.51 ± 1.827 IU/ml respectively) at $P < 0.05$ [Table 2].

Table 1: Anthropometric parameters in all studied groups (mean±SEM)

Factor	Controls	Atopic	Non-atopic
N	20	28	32
Age (years)	48.50±0.844	48.21±1.085	47.84±1.027
Sex			
Male	11	19	14
Female	9	9	18
Eosinophil (%)	2.00±0.059	5.211±0.166[a]	5.00±0.153[a]
FVC	97.30±0.241	88.64±0.382[a]	88.81±0.438[a]
FEV1	87.20±0.953	67.50±0.446[a]	66.41±0.580[a]
FEV1%	88.90±1.02	75.64±0.615[a]	74.38±0.651[a]

[a]Significant from control group at $P<0.05$. SEM: Standard error of mean, FVC: Forced vital capacity, FEV1: Forced expiratory volume 1

Table 2: Levels of periostin, TGF-β1 and IgE (Mean±SEM) in all studied groups

Factor	Controls	Atopic	Non_atopic
Periostin	3053±446.5	6889±393.8[a]	6212±348.8[a]
TGF-β1	51.26±2.65	75.83±1.53[a]	73.47±1.09[a]
IgE	52.47±3.045	151.3±15.10[ab]	48.51±1.827

[a]Significant from control group at $P<0.05$, [b]Significant from non-atopic group at $P<0.05$. TGF-β1: Transforming growth factor beta 1, IgE: Immunoglobulin E, SEM: Standard error of mean

Periostin correlated negatively with PFTs (FVC, FEV1, and FEV1%). Figures 1-3 shows a statistical comparison of periostin, TGF-β1, and IgE in all studied groups, respectively.

Receiver Operating Curve (ROC) Analysis

Using ROC analysis in the comparison between asthmatic patients and control, we found that the critical serum level of periostin associated with the risk of asthma was 1421 pg/ml (61.6% sensitivity and 70% specificity).

Using ROC analysis in comparison between asthmatic patients and control, we found that the critical serum level of TGF-β1 associated with the risk of asthma was 0.37 pg/ml (60% sensitivity and 65% specificity).

Using ROC analysis in comparison between asthmatic patients and control, we found that the critical serum level of total IgE associated with the risk of asthma was 5330 IU/ml (60% sensitivity and 65% specificity).

Figures 4-6 show ROC curve of periostin, TGF-β1, and IgE, respectively.

DISCUSSION

Asthma is a highly prevalent chronic respiratory disease affecting 300 million people worldwide [15]. The disease is appropriate to airway inflammation, bronchial hyperresponsiveness, and recurrent episodes of reversible airway obstruction. Asthma can be divided to "atopic" or "non-atopic" based on the presence (atopic) or absence (non-atopic) of specific IgE antibodies to common environmental allergens [16]. Periostin secreted by airway epithelial cells is capable of activating TGF-β-mediated increases in Type I collagen production in fibroblasts [7].

Our study showed that serum periostin levels in asthmatic subjects were significantly higher than in control subjects, which was confirmed by Jia *et al.* reported that serum periostin levels were significantly higher in asthmatic patients [17]. Moreover, the present findings are similar to what Bentley *et al.* observed in a recent study that in murine models, periostin has been linked to more severe asthmatic airway inflammation responses and hyperresponsiveness [18].

There was a significantly higher level of periostin in asthmatic subjects as compared to control subjects, which was in agreement with Woodruff *et al.* reported that periostin expression is elevated in the bronchial epithelial cells of a subset of patients with asthma [19]. Furthermore, the concentrations of PFTs (FVC, FEV1, and FEV1%) were a significant statistical decrease in asthmatic subjects compared to the control subject. This data is supported by the report of Bremner *et al.* [20] that

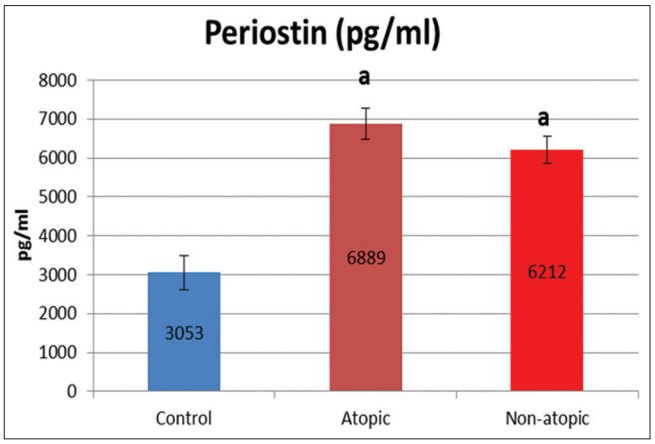

Figure 1: Periostin levels in all studied groups. [a]Significant from control group at $P < 0.05$

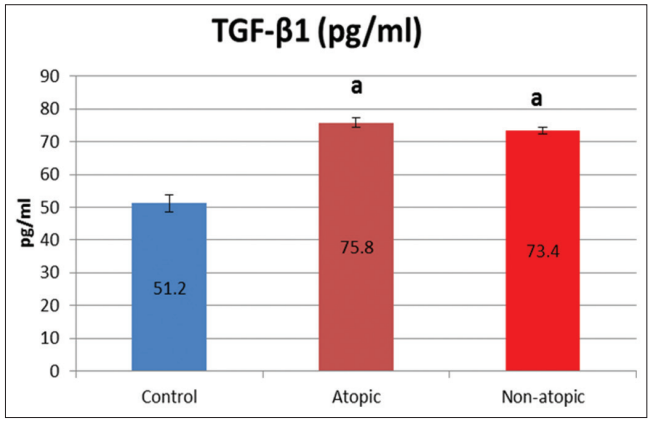

Figure 2: Transforming growth factor beta 1 levels in all studied groups. [a]Significant from control group at $P < 0.05$

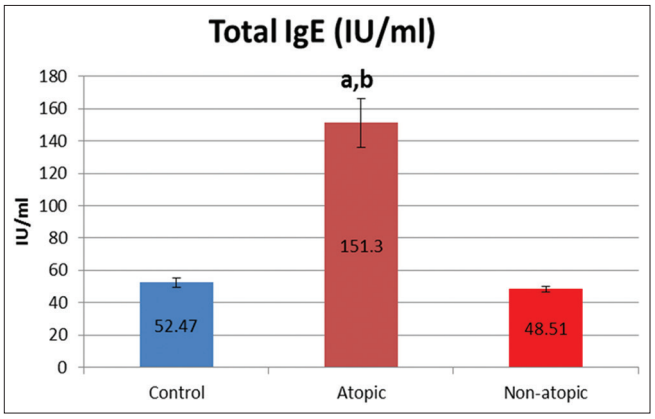

Figure 3: Immunoglobulin E levels in all studied groups. [a]Significant from control group at $P < 0.05$. [b]Significant from non-atopic group at $P < 0.05$

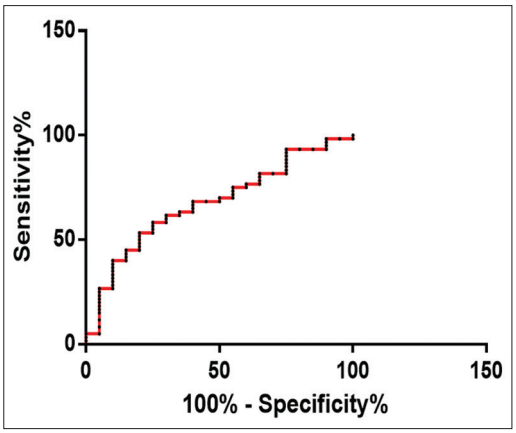

Figure 4: Illustrated receiver operating curve of periostin

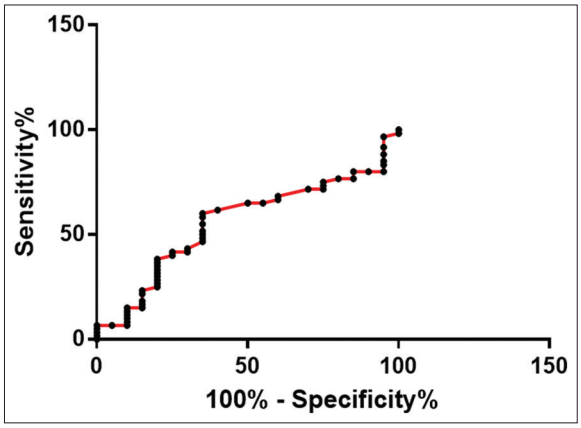

Figure 5: Illustrated receiver operating curve of transforming growth factor beta 1

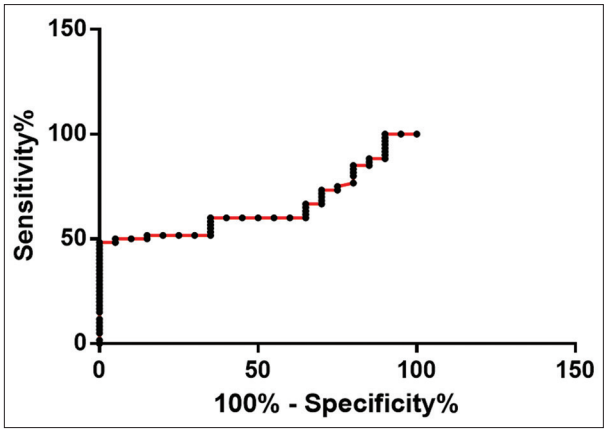

Figure 6: Illustrated receiver operating curve of total immunoglobulin E

increased bronchial responsiveness associated with lower levels of lung function.

In addition, Kanemitsu *et al.* published their findings and discovered that the serum periostin concentrations coordinate with an annual downturn in FEV1, independently of the severity of asthma [21].

Also, we can differentiate between atopic and non-atopic asthma by skin prick test and serum level of IgE. In atopic asthma, skin prick test is positive, and the amount of serum IgE is high, in contrast, non-atopic asthma negative skin prick test, and normal serum IgE. With the agreement of these results Stenius *et al.* reported that the weal size of the individual skin prick tests have been shown to coordinate well with the amount of specific serum IgE for that allergen [22].

CONCLUSION

Serum periostin is increased in Egyptian asthmatic patients compared to control subjects and periostin correlated negatively with PFTs in asthmatic patients. Periostin may be considered as a biomarker to distinguish asthmatics from non-asthmatics individuals.

ACKNOWLEDGMENT

The authors are dreadful to all staff members the Departments of Biochemistry, Faculty of Pharmacy, Al-Azhar University, the nursing and all staff members the Chest Department, Tanta University Hospitals.

REFERENCES

1. Wenzel SE. Asthma phenotypes: The evolution from clinical to molecular approaches. Nat Med 2012;18:716-25.
2. Busse WW, Lemanske RF Jr. Asthma. N Engl J Med 2001;344:350-62.
3. Takeshita S, Kikuno R, Tezuka K, Amann E. Osteoblast-specific factor 2: Cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. Biochem J 1993;294:271-8.
4. Ohta N, Ishida A, Kurakami K, Suzuki Y, Kakehata S, Ono J, *et al.* Expressions and roles of periostin in otolaryngological diseases. Allergol Int 2014;63:171-80.
5. Izuhara K, Arima K, Ohta S, Suzuki S, Inamitsu M, Yamamoto K. Periostin in allergic inflammation. Allergol Int 2014;63:143-51.
6. Conway SJ, Izuhara K, Kudo Y, Litvin J, Markwald R, Ouyang G, *et al.* The role of periostin in tissue remodeling across health and disease. Cell Mol Life Sci 2014;71:1279-88.
7. Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, *et al.* Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. Proc Natl Acad Sci U S A 2010;107:14170-5.
8. Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. Pulm Pharmacol Ther 2003;16:181-96.
9. Ignotz RA, Massagué J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 1986;261:4337-45.
10. Nair P, Kraft M. Serum periostin as a marker of T(H)2-dependent eosinophilic airway inflammation. J Allergy Clin Immunol 2012;130:655-6.
11. Cowie RL, Underwood MF, Field SK. Asthma symptoms do not predict spirometry. Can Respir J 2007;14:339-42.
12. Holt EW, Tan J, Hosgood HD. The impact of spirometry on pediatric asthma diagnosis and treatment. J Asthma 2006;43:489-93.
13. Sporn MB, Roberts AB, Wakefield LM, Assoian RK. Transforming growth factor-beta: Biological function and chemical structure. Science 1986;233:532-4.
14. Renz H, Becker WM, Bufe A. Positionspapier der deutschen Gesellschaft für allergologie und klinische immunologie (DGAI). Allergo J 2002;11:492-506.
15. Dougherty RH, Fahy JV. Acute exacerbations of asthma: Epidemiology, biology and the exacerbation-prone phenotype. Clin Exp Allergy 2009;39:193-202.
16. Masoli M, Fabian D, Holt S, Beasley R; Global Initiative for Asthma

(GINA) Program. The global burden of asthma: Executive summary of the GINA Dissemination Committee report. Allergy 2004;59:469-78.
17. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. J Allergy Clin Immunol 2012;130:647-54.e10.
18. Bentley JK, Chen Q, Hong JY, Popova AP, Lei J, Moore BB, *et al.* Periostin is required for maximal airways inflammation and hyperresponsiveness in mice. J Allergy Clin Immunol 2014;134:1433-42.
19. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, *et al.* Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci U S A 2007;104:15858-63.
20. Bremner PR, de Klerk NH, Ryan GF, James AL, Musk M, Murray C, *et al.* Respiratory symptoms and lung function in aborigines from tropical Western Australia. Am J Respir Crit Care Med 1998;158:1724-9.
21. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, *et al.* Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. J Allergy Clin Immunol 2013;132:305-12.e3.
22. Stenius B, Wide L, Seymour WM, Holford-Strevens V, Pepys J. Clinical significance of specific IgE to common allergens. I. Relationship of specific IgE against *Dermatophagoides* spp. And grass pollen to skin and nasal tests and history. Clin Allergy 1971;1:37-55.

Source of Support: Nil, Conflict of Interest: None declared.