

# Diagnostic value of 8-isoprostane and transforming growth factor- $\beta$ in bronchial asthma patients

Amgad Frahat<sup>a</sup>, Yousef Mansour<sup>a</sup>, Ayman Eldib<sup>a</sup>, Doaa Alsed<sup>b</sup>

**Background** Asthma is an airway inflammatory disease with functional and structural changes, leading to bronchial hyperresponsiveness and airflow obstruction. 8-Isoprostane (8-iso-PGF<sub>2</sub> $\alpha$ ) is considered a marker of oxidative stress specific to lipid peroxidation, transforming growth factor  $\beta$ <sub>1</sub> (TGF $\beta$ <sub>1</sub>) as an important fibrogenic and immunomodulatory factor known to induce structural changes associated with asthma.

**Objective** We aimed to study the diagnostic value of 8-iso-PGF<sub>2</sub> $\alpha$  and TGF $\beta$ <sub>1</sub> in asthmatic patients.

**Patients and methods** Samples of serum and bronchoalveolar lavage fluid from 40 asthmatic patients (20 moderate and 20 severe) and 10 healthy volunteers were assessed for their levels of 8-iso-PGF<sub>2</sub> $\alpha$  and TGF $\beta$ <sub>1</sub>.

**Results** Bronchoalveolar lavage 8-iso-PGF<sub>2</sub> $\alpha$  and TGF $\beta$ <sub>1</sub> was higher in asthmatic patients. It was significantly increased with increased asthma severity.

## Introduction

Asthma is a disease characterized by recurrent attacks of dyspnea and wheezing, which vary in frequency and severity from person to person. Symptoms may occur several times in a day or week in affected patients, and for some people become worse at night or during physical activity. During an asthma attack, the bronchial mucosa swell, causing the airways to narrow and reduce airflow. Recurrent asthma symptoms frequently cause sleeplessness, daytime fatigue, reduced activity levels, and school and work absenteeism [1].

Isoprostanes are prostaglandin-like compounds that are produced by free radical-mediated peroxidation of polyunsaturated fatty acids. There is direct evidence showing that F<sub>2</sub>-isoprostanes can be utilized as a marker of lipid peroxidation due to the mechanism of their formation, chemical stability. An altered generation of F<sub>2</sub>-isoprostanes has been found in a variety of diseases associated with oxidative stress such as bronchial asthma [2].

Transforming growth factor- $\beta$  (TGF $\beta$ ) is an important fibrogenic and immunomodulatory factor known to induce structural changes associated with asthma. TGF $\beta$  is produced in the airways by inflammatory cells infiltrated in the bronchial mucosa, as well as by structural cells of the airway wall including fibroblasts, epithelial, endothelial, and smooth muscle cells [3].

**Conclusion** Increased levels of TGF $\beta$ <sub>1</sub> and 8-iso-PGF<sub>2</sub> $\alpha$  is associated with disease severity. However, there is need for continued exploration on the mechanisms responsible for these structural changes.

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**Keywords:** airflow obstruction, asthma, biomarker, hyperresponsiveness, inflammation, 8-iso-prostaglandin F<sub>2</sub> $\alpha$ , transforming growth factor  $\beta$ <sub>1</sub>

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TGF $\beta$  is increased in asthmatic airways and cells, together with evidence of increased TGF $\beta$  signaling [4,5]. Levels of TGF $\beta$  have been shown as a potential biomarker for asthma [6] as well as a therapeutic target for the modulation of airway remodeling in asthma [5].

## Aim

To evaluate the diagnostic value of 8-isoprostane (8-iso-PGF<sub>2</sub> $\alpha$ ) and TGF $\beta$ <sub>1</sub> in bronchial asthma patients.

## Patients and methods

This study was carried out at the Chest Department of Tanta University Hospital on 40 asthmatic patients and 10 control patients collected from the outpatient clinics starting from October 2014 to September 2015. They were classified into three groups. Group 1 included 10 healthy individuals as control. Group 2 included 20 patients with moderate persistent asthma. Group 3 included 20 patients with severe persistent asthma.

## Inclusion criteria

No smoking within the past 5 years and no current ailments other lung disease. Nighttime symptoms more than once per week but not nightly and FEV<sub>1</sub> more than

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60% but less than 80% in patients with moderate persistent asthma and nightly symptoms and FEV<sub>1</sub> of less than 60% in severe persistent asthma. All patients were subjected to: thorough history taking, complete physical examination, chest radiography to exclude any other lung diseases, complete blood picture, spirometry to classify our patients including forced expiratory volume in the first second (FEV<sub>1</sub>), FEV<sub>1</sub>/percent of forced expiratory volume in the first second (FVC), forced vital capacity (FVC), peak expiratory flow rate (PEFR) before and after B2 agonist inhalation to differentiate asthma from chronic obstructive pulmonary disease, serum level of 8-iso-PGF<sub>2</sub>α and TGFβ<sub>1</sub>, flexible fiberoptic bronchoscope, with bronchoalveolar lavage (BAL) was collected for the estimation of 8-iso-PGF<sub>2</sub>α and TGFβ<sub>1</sub>.

#### Exclusion criteria

Chest diseases other than bronchial asthma, cardiac patients, mechanically ventilated patients with a PaO<sub>2</sub> of less than 70 mmHg, thrombocytopenia with platelets of less than 100 000/ml, patients with psychological impairment, patients receiving immunosuppressive treatment, cancer patients, hepatic cirrhosis, chronic renal failure, autoimmune or connective tissue disorders, and pregnant women.

#### Statistics

Statistical presentation and analysis of the present study was conducted, using the mean, SD, linear correlation coefficient (*r*);  $\chi^2$ , analysis of variance tests was used for comparison among different times in the same group in quantitative data by SPSS, version 17 (SPSS Inc., Chicago, IL USA). A *P* value of less than 0.001 is considered highly significant.

**Table 2 Statistical comparison of the percentage of sex in the studied groups**

	Control (%)	Moderate asthma (%)	Severe asthma (%)	Total (%)	$\chi^2$	<i>P</i> -value
Male	70	40	55	52	2.571	0.276
Female	30	60	45	48		
Total	100	100	100	100		

**Table 3 Statistical comparison of range, mean±SD values of bronchoalveolar lavage fluid levels of 8-isoprostane (ng/ml) in the three studied groups**

Groups	8-Iso-PGF <sub>2</sub> α in BAL		ANOVA	
	Range	Mean±SD	<i>F</i>	<i>P</i> -value
Control	23–27	24.9±1.449	259.336	<0.001*
Mild to moderate	51–77	61.25±6.455		
Severe	73–100	89.2±9.551		
Tukey's test				
Control and mild to moderate		Control and severe		Mild to moderate and severe
<0.001*		<0.001*		<0.001*

ANOVA, analysis of variance; BAL, bronchoalveolar lavage; 8-iso-PGF<sub>2</sub>α, 8-isoprostane. \*Means statistically significant.

## Results

This work was carried out on 40 asthmatic patients and 10 control patients classified into three groups. Group I: 10 nonsmoking healthy volunteers (control) [seven (70%) men and three (30%) women], their ages ranged from 24 to 55 (38 ±8.16) years. Group II: included 20 nonsmoking, moderate intermittent asthmatic patients (eight men 40% and 12 women 60%), their ages ranged from 24 to 55 (39.35±8.23) years. Group III: included 20 nonsmoking severe persistent asthmatic patients [11 (55%) men and nine (45%) women]. Their ages ranged from 30 to 48 (37.4±5.538). On comparing the values of age and sex, they were matched with no significant difference between the three studied groups (Tables 1 and 2).

#### Bronchoalveolar lavage fluid and serum levels of 8-isoprostane (ng/ml)

The mean±SD values of 8-iso-PGF<sub>2</sub>α in BAL were 24.9±1.449, 61.25±6.455, and 89.2±9.55 ng/ml and in serum were 56.4±8.5, 467.7±89.258, and 915±66.132 ng/ml in groups I, II, and III respectively, with significant increase in groups II and III compared with group I and in group III compared with group II (*P*<0.001) (Tables 3 and 4 and Figs 1 and 2).

**Table 1 Statistical comparison of mean±SD values and of age in the studied groups**

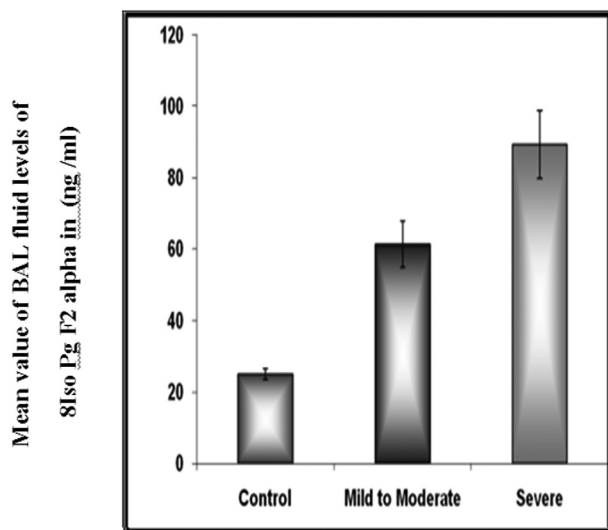
Groups	Age		ANOVA	
	Range	Mean±SD	<i>F</i>	<i>P</i>
Control	24–55	38±8.165	0.372	0.691
Moderate	24–55	39.3±8.235		
Severe	30–48	37.4±5.538		

ANOVA, analysis of variance.

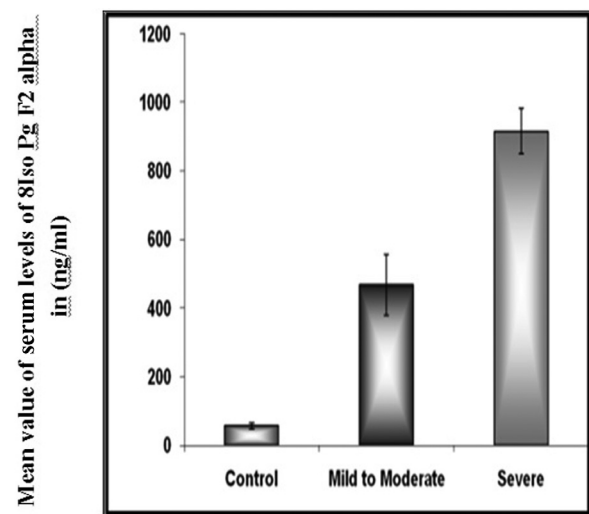
**Table 4** Statistical comparison of range, mean±SD values of serum fluid levels of 8-isoprostane (ng/ml) in the three studied groups

Groups	8-Iso-PGF <sub>2</sub> α in serum		ANOVA	
	Range	Mean±SD	F	P-value
Control	40–70	56.4±8.501	523.046	<0.001*
Mild to moderate	335–630	467.7±89.258		
Severe	800–1000	915.5±66.132		
Tukey's test				
Control and mild to moderate		Control and severe		Mild to moderate and severe
<0.001*		<0.001*		<0.001*

ANOVA, analysis of variance; 8-iso-PGF<sub>2</sub>α, 8-isoprostane. \*Means statistically significant.

**Figure 1**

Statistical comparison of range, mean±SD value of bronchoalveolar lavage fluid levels of 8-isoprostane (ng/ml) in the three studied groups.

**Figure 2**

Statistical comparison of range, mean±SD values of serum fluid levels of 8-isoprostane α (ng/ml) in the three studied groups.

#### Bronchoalveolar lavage fluid and serum levels of transforming growth factor β<sub>1</sub> (ng/ml)

The mean±SD values of TGFβ<sub>1</sub> in BAL were 5.5±0.471, 30.1±2.1 and 43.35±5.8 ng/ml and in serum were 0.25±0.012, 31.40±3.858, and 43.1±3.447 ng/ml in groups I, II, and III, respectively, with significant increase in groups II and III compared with group I and in group III compared with group II ( $P<0.001$ ) (Tables 5 and 6 and Figs 3 and 4).

#### Correlations of 8-isoprostane concentration (ng/ml) in bronchoalveolar lavage and serum and spirometric values

There was significant negative correlation between 8-iso-PGF<sub>2</sub>α concentration in BAL and FEV<sub>1</sub> (percentage of predicted) ( $r=-0.790$  and  $P<0.001$ ), FVC (percentage of predicted) ( $r=-0.529$  and  $P\leq 0.001$ ), PEFR (percentage of predicted) ( $r=-0.682$  and  $P\leq 0.001$ ) as well as there was significant negative correlation between serum 8-iso-PGF<sub>2</sub>α concentration and percentage of predicted FEV<sub>1</sub> ( $r=-0.893$  and  $P<0.001$ ), FVC ( $r=-0.667$  and  $P<0.001$ ), and PEFR ( $r=-0.785$  and  $P\leq 0.001$ ) (Figs 5 and 6).

#### Correlations of transforming growth factor β<sub>1</sub> concentration (ng/ml) in bronchoalveolar lavage and serum and spirometric values

There was significant negative correlation between TGFβ<sub>1</sub> concentration in BAL and FEV<sub>1</sub> (percentage of predicted) ( $r=-0.791$  and  $P<0.001$ ), FVC (percentage of predicted) ( $r=-0.579$  and  $P\leq 0.001$ ), PEFR (percentage of predicted) ( $r=-0.729$  and  $P\leq 0.001$ ) as well as there was significant negative correlation between serum TGFβ<sub>1</sub> concentration and FEV<sub>1</sub> (percentage of predicted) ( $r=-0.782$  and  $P<0.001$ ), FVC (percentage of predicted) ( $r=-0.576$  and  $P<0.001$ ), and PEFR (percentage of predicted) ( $r=-0.719$  and  $P\leq 0.001$ ) (Figs 7 and 8).

#### Discussion

Asthma is considered one of the major causes of chronic morbidity and mortality worldwide. There is evidence that asthma prevalence has increased considerably over the last 20 years. Asthma is a chronic airway disorder characterized by interaction

**Table 5 Statistical comparison of range, mean±SD values of bronchoalveolar lavage fluid levels of transforming growth factor  $\beta_1$  (ng/ml) in the three studied groups**

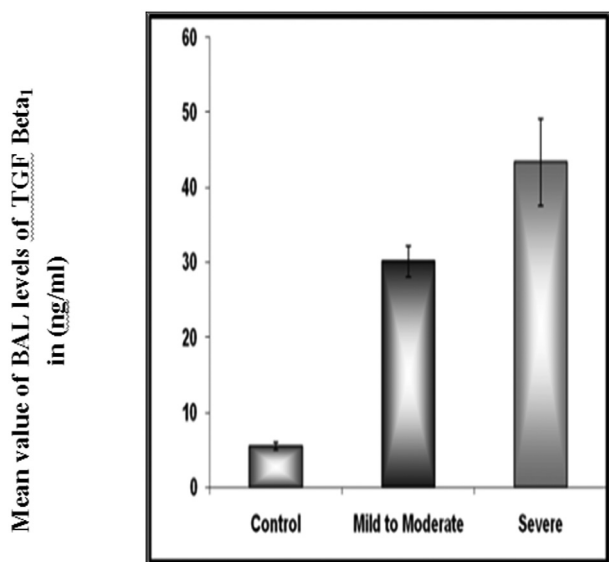
Groups	TGF $\beta_1$ in BAL		ANOVA	
	Range	Mean±SD	F	P-value
Control	5–6	5.5±0.471	309.159	<0.001*
Mild to moderate	25–33	30.1±2.100		
Severe	37–54	43.3±5.806		
Tukey's test				
Control and mild to moderate	Control and severe		Mild to moderate and severe	
<0.001*	<0.001*		<0.001*	

ANOVA, analysis of variance; BAL, bronchoalveolar lavage; TGF $\beta_1$ , transforming growth factor  $\beta_1$ . \*Means statistically significant.

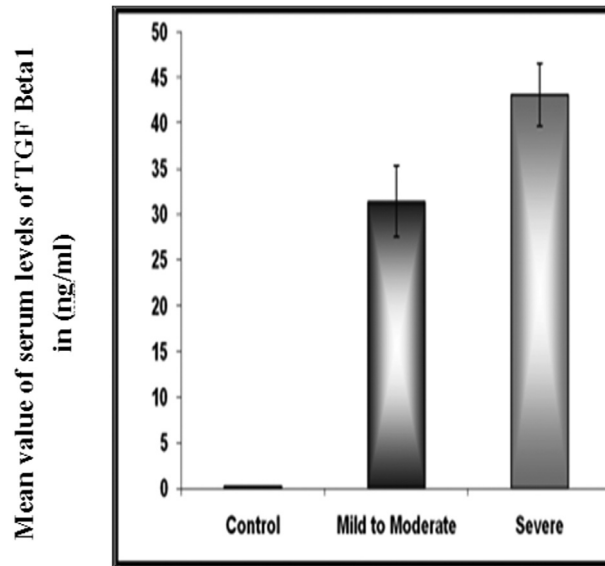
**Table 6 Statistical comparison of range, mean±SD values of serum levels of transforming growth factor  $\beta_1$  (ng/ml) in the three studied groups**

Groups	TGF $\beta_1$ in serum		ANOVA	
	Range	Mean±SD	F	P-value
Control	0.240–0.270	0.252±0.012	569.234	<0.001*
Mild to moderate	25.000–37.000	31.400±3.858		
Severe	38.000–48.000	43.100±3.447		
Tukey's test				
Control and mild to moderate	Control and severe		Mild to moderate and severe	
<0.001*	<0.001*		<0.001*	

ANOVA, analysis of variance; TGF $\beta_1$ , transforming growth factor  $\beta_1$ . \*Means statistically significant.

**Figure 3**

Statistical comparison of range, mean±SD values of bronchoalveolar lavage fluid levels of transforming growth factor  $\beta_1$  (ng/ml) in the three studied groups.

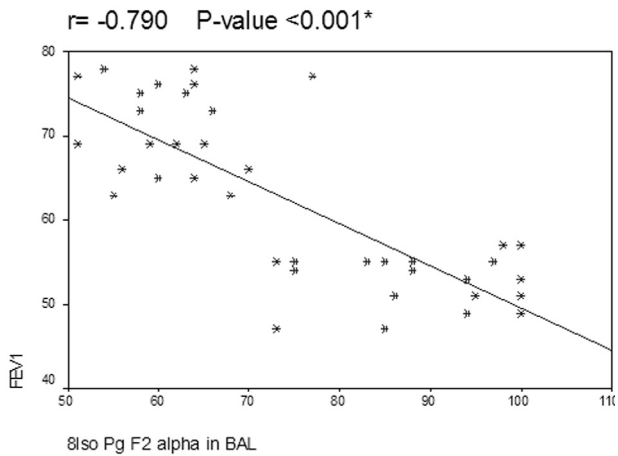
**Figure 4**

Statistical comparison of range, mean±SD values of serum levels of transforming growth factor  $\beta_1$  (ng/ml) in the three studied groups.

of airway inflammation, airway obstruction, and bronchial hyperresponsiveness, which leads to recurrent attacks of chest tightness, dyspnea, wheezing, and coughing [7]. Airway inflammation and remodeling are the two main pathological features of asthma. It is proved that asthma is a nonspecific airway inflammatory disease in which

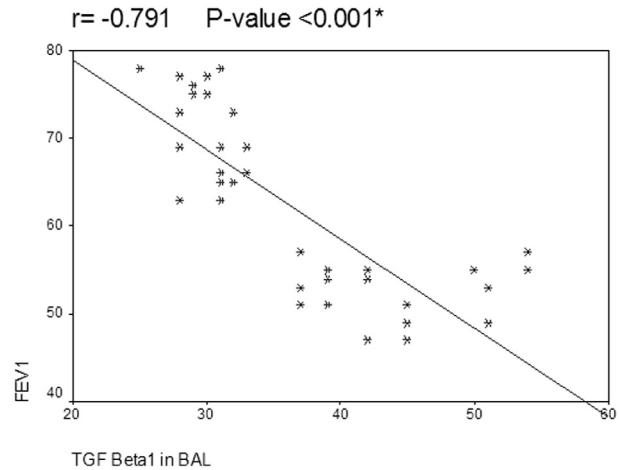
many cells such as eosinophils, T-lymphocytes, neutrophils, epithelial cells, and other cellular components are involved [8]. 8-iso-PGF $_2\alpha$  is considered a marker of oxidative stress specific for lipid peroxidation, which is a stable metabolite of arachidonic acid, synthesized *in vivo* [9]. Previous studies found elevation of 8-iso-PGF $_2\alpha$  levels in exhaled breath condensate, induce sputum, and in

Figure 5



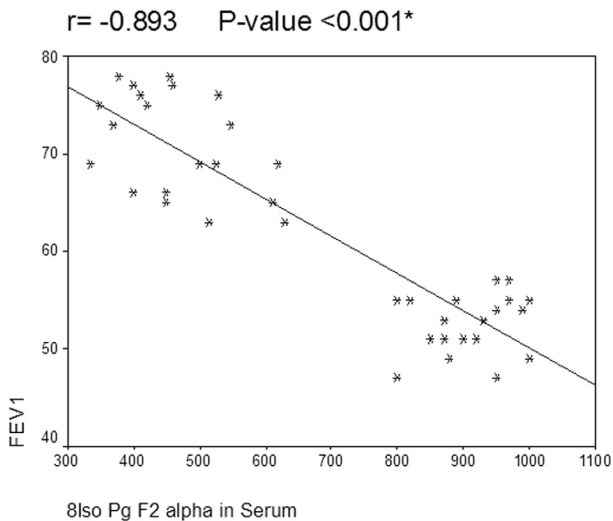
Correlation of 8-isoprostane concentration (ng/ml) in bronchoalveolar lavage and FVE<sub>1</sub>% of predicted.

Figure 7



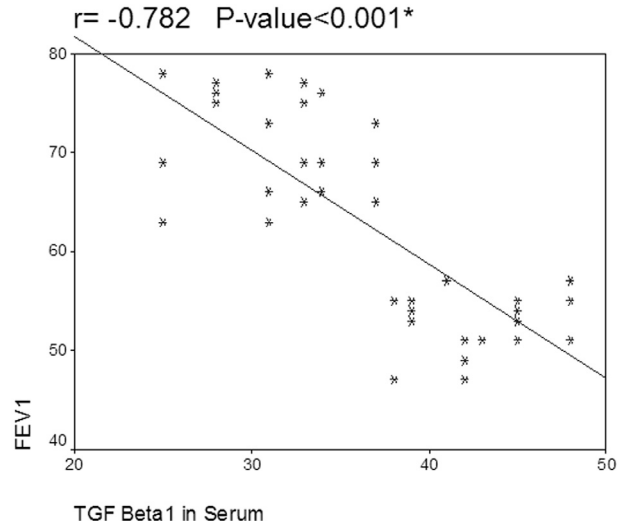
Correlation of transforming growth factor β<sub>1</sub> concentration (ng/ml) in bronchoalveolar lavage and FEV<sub>1</sub>% of predicted.

Figure 6



Correlation of 8-isoprostane concentration (ng/ml) in serum and FEV<sub>1</sub>% of predicted.

Figure 8



Correlation of transforming growth factor β<sub>1</sub> concentration (ng/ml) in serum and FEV<sub>1</sub>% of predicted.

plasma of asthmatic patients [10–12]. The human TGFβ<sub>1</sub> is an important fibrogenic and immunomodulatory factor known to induce structural changes associated with asthma [3]. It is produced in the airways by inflammatory cells infiltrated in the bronchial mucosa, as well as by structural cells of the airway wall including fibroblasts, endothelial, epithelial, and smooth muscle cells. These studies point out that the role for TGF not only as a potential biomarker but as a therapeutic target for the modulation of airway remodeling in asthma [5].

In asthmatic patients the TGFβ<sub>1</sub> levels were found elevated in both bronchoalveolar lavage fluids (BVLFs) and plasma [13]. Recently research

workers' pay considerable attention for airway remodeling in asthmatics. The structural changes observed by light microscopy are multiple. All the changes of remodeling are deviations from what is probably an optimized airways and so it should ideally be reversed by treatment. To date, there is a lack of evidence that the principal changes are indeed reversible but reversal of remodeling is a target goal of existing and future therapies [14].

In the present study, there was significant increase of 8-iso-PGF<sub>2</sub>α in BAL and serum in moderate and severe asthma compared with control, and in severe asthma compared with the moderate one. Kharitonov *et al.* [15], found doubling of

8-iso-PGF<sub>2</sub>α levels of mild asthmatic patients compared with healthy individuals and three times increase in patients with severe asthma, irrespective of treatment with corticosteroids. Dworski *et al.* [16] found elevation of F<sub>2</sub>-isoPGs in BALF after 24 h of allergen instillation compared with its level at baseline. The rise of the concentrations of F<sub>2</sub>-isoPGs in BALF 24 h after allergen challenge was explained by its generation directly in the asthmatic airways in response to allergen exposure. They correlated plasma levels of each of the biomarkers with lung function parameters (FEV<sub>1</sub>, FVC, and FEV<sub>1</sub>/FVC) in acute exacerbation and in remission. They found significant positive correlation between plasma 8-iso-PGF<sub>2</sub>α levels and the severity of airway obstruction detected by pulmonary function parameters. Also, Wood *et al.* [11] recently demonstrated the rise of lipid peroxidation in asthma measured by 8-iso-PGF<sub>2</sub>α concentration which was elevated 3–4 times in persistent asthmatics than the normal group. Judith and Mak [17] found that the plasma levels of 8-iso-PGF<sub>2</sub>α were significantly higher in patients with asthma in acute exacerbation and decreased in remission but remained elevated compared with healthy controls. One explanation could be its resistance to corticosteroids as demonstrated by Montuschi *et al.* [10], who reported that severe asthmatic patients treated with oral prednisolone had higher 8-iso-PGF<sub>2</sub>α levels in their exhaled breath condensates than mild and moderate asthma patients. They found increasing evidence that asthma is a disease associated with increased oxidative stress, with persistent elevated 8-iso-PGF<sub>2</sub>α levels during remission compared with healthy controls. Also, they found that FEV<sub>1</sub>, FVC, and FEV<sub>1</sub>/FVC were positively correlated with plasma 8-iso-PGF<sub>2</sub>α levels. Samitasa *et al.* [18] found increased levels of 8-iso-PGF<sub>2</sub>α in exhaled breath condensates in association with disease severity, in asthmatic adults and children. Keskin *et al.* [19] quantified that the F<sub>2</sub>-isoPGs level in BALF at baseline values was lower than its level 24 h after allergen instillation; moreover, exhaled 8-iso-PGF<sub>2</sub>α level in patients with moderate persistent asthma was higher than in the mild persistent group.

The present study showed that 8-iso-PGF<sub>2</sub>α levels in BALF and serum were higher in asthmatics than in healthy controls. Furthermore, their levels were increasing with asthma severity. These initial results appear to confirm the usefulness of determining these

inflammatory mediators for the diagnosis and evaluation of severity of asthma, and also for investigating the relationship with oxidative stress and possible treatment control. However, further studies are needed to standardize the technique and validate the methods used to measure the mediators in order to define cutoff values for differentiating between healthy patients, asthmatics, and between different degrees of asthma severity.

In our study, we found a significant increase of TGFβ<sub>1</sub> in BAL and serum in moderate and severe asthma compared with control, and in severe asthma compared with moderate asthma. Ozyilmaz *et al.* [20] measured plasma TGFβ<sub>1</sub> level in three groups, 35 atopic, 35 nonatopic asthmatic patients, and 15 healthy control patients. Their levels were significantly higher in the asthmatic groups compared with the control, whereas they were similar among the atopic and nonatopic asthmatics. They found a positive correlation between uncontrolled asthma and plasma TGFβ<sub>1</sub> level. They concluded that plasma TGFβ<sub>1</sub> level may be a systemic marker of asthma control. Manuyakorn *et al.* [21] studied serum TGFβ<sub>1</sub> levels in 31 atopic asthmatic patients and 34 nonatopic controls measured by enzyme-linked immunosorbent assay. Contrary to our results they found an elevation of serum TGFβ<sub>1</sub> in the steroid-naïve mild asthma group in comparison to the moderate asthma group, with no correlations between serum TGFβ<sub>1</sub> levels and pulmonary function test parameters, and also duration of asthma or duration of inhaled corticosteroid treatment. Redington *et al.* [22], measured the levels of TGFβ<sub>1</sub> in BALF from clinically stable asthmatics and healthy control patients. Their levels were significantly higher in asthmatics than control patients. They conclude that basal TGFβ<sub>1</sub> levels in the airways are elevated in asthma. Forno *et al.* [23] also observed elevated TGFβ<sub>1</sub> expression in asthmatics. Joseph *et al.* [24] postulated that the mean value of the plasma TGFβ<sub>1</sub> was significantly higher in nonatopic asthmatic patients compared with control individuals and atopic asthmatic patients. Hong *et al.* [25] reported that the serum expression of TGFβ<sub>1</sub> in asthmatic children was lower than that of the control group. Jarrett *et al.* [26] conducted a study on 74 children with asthma attacks. They found that the TGFβ<sub>1</sub> level in the asthmatic group increased significantly. In contrast to our results, Magnon *et al.* [27] reported decreased TGFβ<sub>1</sub> expression in the asthmatic epithelium. Also, Lommatzsch Xiang and Qiu [28] reported no difference between the levels of serum TGFβ<sub>1</sub> in atopic asthmatic

adults and control patients. Moreover, Ceyhan *et al.* [29] concluded that the TGFβ<sub>1</sub> level in serum had no predictive value in diagnostic use. Although our study confirmed the great role of TGFβ<sub>1</sub> in asthma and assessed the variation in the level of TGFβ<sub>1</sub> in BALF and serum of asthmatic patients, we did not have the information about histopathological changes in bronchial wall in response to TGFβ<sub>1</sub>.

Lastly, further studies need to be directed at the cellular, molecular, and genetic factors that are responsible for determining why only some people develop significant remodeling and why the type of remodeling that occurs can differ from patient to patient. The information that will come from these studies will profoundly affect our understanding of asthma pathogenesis, and will impact the types of strategies we use to combat this increasingly problematic disorder.

## Conclusion

8-Iso-PGF<sub>2</sub>α and TGFβ<sub>1</sub> levels in BAL and serum increase in moderate and severe asthmatic patients than in control normal patients. Their levels are correlated with the severity of asthma.

## Recommendations

Further studies are needed to define cutoff values for differentiating between healthy patients and asthmatics and between different degrees of asthma severity.

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Amgad Frahat contributed for concepts, design, definition of intellectual content, and manuscript review. Yousef Mansour contributed for definition of intellectual content and manuscript review. Ayman Eldib contributed for concepts, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. Doaa Alsed contributed for clinical studies, experimental studies, statistical analysis, manuscript preparation, and manuscript editing.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

- Bousquet J, Mantzouranis E, Cruz AA, Khaled N, Baena-Cagnani CE, Bleecker ER, *et al.* Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. *J Allergy Clin Immunol* 2010; **126**: 926–938.
- Pilacik B, Nofer T, Wasowicz W. F<sub>2</sub>-Isoprostanes biomarkers of lipid peroxidation: their utility in evaluation of oxidative stress induced by toxic agents. *Int J Occup Med Environ Health* 2002; **15**:19–27.
- Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. *Pulm Pharmacol Ther* 2003; **16**:181–196.
- Ghosh B, Sharma S, Nagpal K. *Transforming growth factor beta<sub>1</sub> gene (TGFβ<sub>1</sub>) prediction of susceptibility for immunological disease 2010.* US20107858763.
- Howell JE, McAnulty RJ. TGF-beta: its role in asthma and therapeutic potential. *Curr Drug Targets* 2006; **7**:547–565.
- Jingjing J, Gorge S. TGF-B2 reduces nitric oxide synthase mRNA through a ROCK-dependent pathway in airway epithelial cells. *Curr Drug Targets* 2011; **7**:547–565.
- Mistry V, Doe C, Roach K, Morgan A, Wardlaw A, Pavord I, *et al.* Airway hyper responsiveness is dissociated from airway wall structural remodeling. *J Allergy Clin Immunol* 2008; **122**:335–341.
- Moore W, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, *et al.* Characterization of the severe asthma phenotype by the National Heart, Lung and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; **119**:405–413.
- Morrow JD, Roberts LJ. Isoprostanes: their role as an index of oxidant stress status in human pulmonary disease. *Am J Respir Crit Care Med* 2002; **166**:25–30.
- Montuschi P, Corradi M, Ciabattini G, Nightingale J, Kharitonov SA, Barnes PJ, *et al.* Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999; **160**:216–220.
- Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Garg ML. Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. *Lipids* 2000; **35**:967–974.
- Wood LC, Garg ML, Simpson JL, Mori TA, Croft KD, Wark PA, Gibson PG. Induced sputum 8-isoprostane concentrations in inflammatory airway diseases. *Am J Respir Crit Care Med* 2005; **171**: 426–430.
- Chang CH, Chang CH, Lui S. TGF<sub>1</sub> is associated with asthma and modulates the severity of asthma. *Resp Critical Care* 2013; **58**: 1343–1350.
- Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003; **167**:1360–1368.
- Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensation in asthma patients. *Am J Respir Crit Care Med* 1999; **160**:216–220.
- Dworski R, Murray J, Roberts J, Oates J, Morrow J, Fisher L, *et al.* Allergen-induced synthesis of F<sub>2</sub>-isoprostanes in atopic asthmatics. *Am J Respir Crit Care Med* 1999; **160**:1947–1951.
- Judith C, Mak W. Sustained elevation of systemic oxidative stress and inflammation in exacerbation and remission of asthma. *Allergy* 2013; **29**:561–581.
- Samitasa K, Chorianopoulos D, Vittorakisa S, Zervasa E, Economidou E, Papatheodorou G, *et al.* Exhaled cysteinyl-leukotrienes and 8-isoprostane in patients with asthma and their relation to clinical severity. *Respir Med* 2009; **103**:750–756.
- Keskin O, Balaban S, Keskin M, Kucukosmanoglu E, Gogebakan B, Ozkars MY, *et al.* Relationship between exhaled leukotriene and 8 isoprostane level and asthma severity. *Allergol Immunopathol (Madr)* 2014; **42**:191–197.
- Ozyilmaz E, Canbakan S, Capan N, Erturk A, Gulhan M. Correlation of plasma transforming growth factor beta<sub>1</sub> with asthma control test. *Asian Pac J Allergy Immunol* 2008; **26**:185–189.
- Manuyakorn W, Kamchaisatian W, Atamasirikul K, Sasisakulporn C, Direkwattanachai C, Benjaponpitak S. Serum TGF-beta<sub>1</sub> in atopic asthma. *Asian Pac J Allergy Immunol* 2008; **26**:185–189.
- Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, Howarth PH. Transforming growth factor-beta<sub>1</sub> in asthma. Measurement in bronchoalveolar lavage fluid. *Am J Respir Crit Care Med* 1997; **156**: 642–647.

- 23 Forno E, Lescher R, Strunk R, Weiss S, Fuhlbrigge A, Celedón JC. TGF- $\beta_1$  expression in the asthmatics. *J Allergy Clin Immunol* 2011; **127**:741–749.
- 24 Joseph J, Benedict S, Badrinath P, Wassef S, Joseph M, Abdulkhalik S. Elevation of plasma transforming growth factor  $\beta_1$  levels in stable nonatopic asthma. *Ann Allergy Asthma Immunol* 2005; **91**:472–476.
- 25 Hong JG, Dong WF, Zhou XJ. Effect of montelukast sodium on TGF- $\beta_1$  of peripheral blood mononuclear cells from children with mild persistent asthma. *Eur J Cell Biol* 2011; **87**:679–684.
- 26 Jarrett L, Chen JA, Figen J, Doğu L, Alişan N, Deniz E. Serum TGF- $\beta$ , matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) and tissue inhibitors of metalloproteinase (TIMP-1) levels in childhood asthma. *Turk J Med* 2008; **38**:415–419.
- 27 Magnon L, Fukushima T, Yamasaki A, Harada T. TGF- $\beta_1$  expression in the asthmatic epithelium. *Am J Respir Cell Mol Biol* 2008; **12**:46–50.
- 28 Lommatzsch Xiang S, Qiu D. Changes of CD4+CD25+ regulatory T cells, IL-10 and TGF- $\beta_1$  levels in peripheral blood in asthma. *Am J Respir Crit Care Med* 2009; **179**:356–362.
- 29 Ceyhan N, Ronmark J, Platts-Mills R. TGF- $\beta_1$  slevels in induced sputum samples of asthmatic subjects. *Ann Allergy Asthma Immunol* 2008; **10**:146–148.