Diagnostic value of 8-isoprostane and transforming growth factor-β in bronchial asthma patients

Amgad Frahat[a], Yousef Mansour[a], Ayman Eldib[a], Doaa Alsed[b]

Background Asthma is an airway inflammatory disease with functional and structural changes, leading to bronchial hyperreponsiveness and airflow obstruction. 8-Isoprostane (8-iso-$PGF_{2}\alpha$) is considered a marker of oxidative stress specific to lipid peroxidation, transforming growth factor β_1 ($TGF\beta_1$) 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-$PGF_{2}\alpha$ and $TGF\beta_1$ 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-$PGF_{2}\alpha$ and $TGF\beta_1$.

Results Bronchoalveolar lavage 8-iso-$PGF_{2}\alpha$ and $TGF\beta_1$ was higher in asthmatic patients. It was significantly increased with increased asthma severity.

Conclusion Increased levels of $TGF\beta_1$ and 8-iso-$PGF_{2}\alpha$ 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, hyperreponsiveness, inflammation, 8-iso-prostaglandin $F_{2}\alpha$, transforming growth factor β_1

[a]Chest Department, Faculty of Medicine, Tanta University, [b]Tanta Chest Hospital, Tanta, Egypt

Correspondence to Ayman Eldib, Tanta Chest Hospital, Tanta, Egypt.
e-mail: ADALEX1970@yahoo.com

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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_2-isoprostanes can be utilized as a marker of lipid peroxidation due to the mechanism of their formation, chemical stability. An altered generation of F_2-isoprostanes has been found in a variety of diseases associated with oxidative stress such as bronchial asthma [2].

Transforming growth factor-β (TGFβ) is an important fibrogenic and immunomodulatory factor known to induce structural changes associated with asthma. TGFβ 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].

TGFβ is increased in asthmatic airways and cells, together with evidence of increased TGFβ signaling [4,5]. Levels of TGFβ 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-$PGF_{2}\alpha$) and $TGF\beta_1$ 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 ? included 10 healthy individuals as control. Group ?? included 20 patients with moderate persistent asthma. Group ??? 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_1 more than

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60% but less than 80% in patients with moderate persistent asthma and nightly symptoms and FEV_1 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 (FEV1), FEV1/percent of forced expiratory volume in the first second (FVC), forced vital capacity (FVC), peek expiratory flow rate (PEFR) before and after B2 agonist inhalation to differentiate asthma from chronic obstructive pulmonary disease, serum level of 8-iso-$PGF_2\alpha$ and $TGF\beta_1$, flexible fiberoptic bronchoscope, with bronchoalveolar lavage (BVL) was collected for the estimation of 8-iso-$PGF_2\alpha$ and $TGF\beta_1$.

Exclusion criteria

Chest diseases other than bronchial asthma, cardiac patients, mechanically ventilated patients with a PaO_2 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); χ^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.

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-$PGF_2\alpha$ 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	*F*	*P*
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 2 Statistical comparison of the percentage of sex in the studied groups

	Control (%)	Moderate asthma (%)	Severe asthma (%)	Total (%)	χ^2	*P*-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-$PGF_2\alpha$ in BAL		ANOVA	
	Range	Mean±SD	*F*	*P*-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*

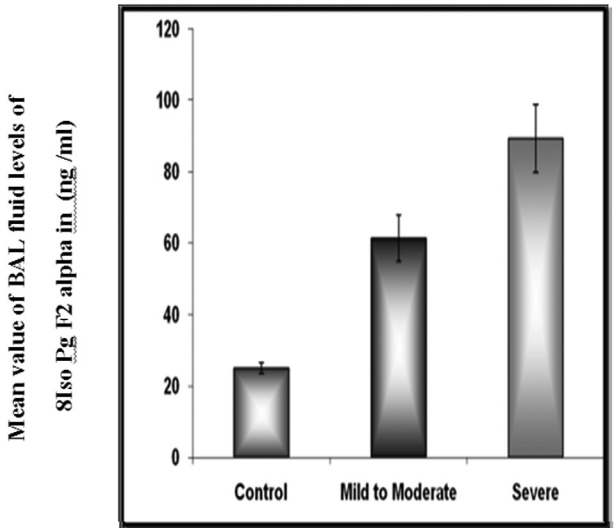
ANOVA, analysis of variance; BAL, bronchoalveolar lavage; 8-iso-$PGF_2\alpha$, 8-isoprostane. *Means statistically significant.

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-$PGF_{2}\alpha$ 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*

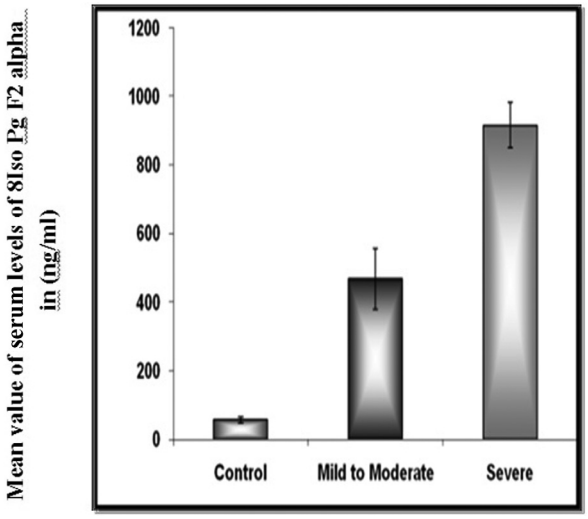
ANOVA, analysis of variance; 8-iso-$PGF_{2}\alpha$, 8-isoprostane. *Means statistically significant.

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_1 (ng/ml)

The mean±SD values of $TGF\beta_1$ 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-$PGF_{2}\alpha$ concentration in BAL and FEV_1 (percentage of predicted) ($r=-0.790$ and $P<0.001$), FVC (percentage of predicted) ($r=-0.529$ and $P\leq0.001$), PEFR (percentage of predicted) ($r=-0.682$ and $P\leq0.001$) as well as there was significant negative correlation between serum 8-iso-$PGF_{2}\alpha$ concentration and percentage of predicted FEV_1 ($r=-0.893$ and $P<0.001$), FVC ($r=-0.667$ and $P<0.001$), and PEFR ($r=-0.785$ and $P\leq0.001$) (Figs 5 and 6).

Correlations of transforming growth factor β_1 concentration (ng/ml) in bronchoalveolar lavage and serum and spirometric values

There was significant negative correlation between $TGF\beta_1$ concentration in BAL and FEV_1 (percentage of predicted) ($r=-0.791$ and $P<0.001$), FVC (percentage of predicted) ($r=-0.579$ and $P\leq0.001$), PEFR (percentage of predicted) ($r=-0.729$ and $P\leq0.001$) as well as there was significant negative correlation between serum $TGF\beta_1$ concentration and FEV_1 (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 $P\leq0.001$) (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 β_1 (ng/ml) in the three studied groups

Groups	TGFβ_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*

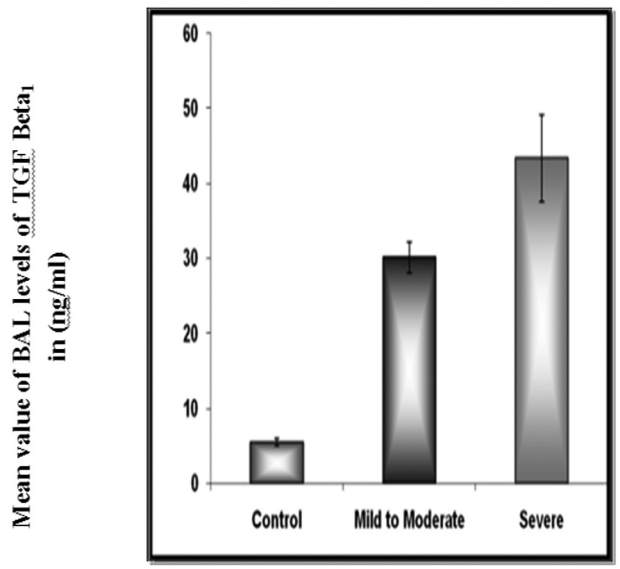
ANOVA, analysis of variance; BAL, bronchoalveolar lavage; TGFβ_1, transforming growth factor β_1. *Means statistically significant.

Table 6 Statistical comparison of range, mean±SD values of serum levels of transforming growth factor β_1 (ng/ml) in the three studied groups

Groups	TGFβ_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*

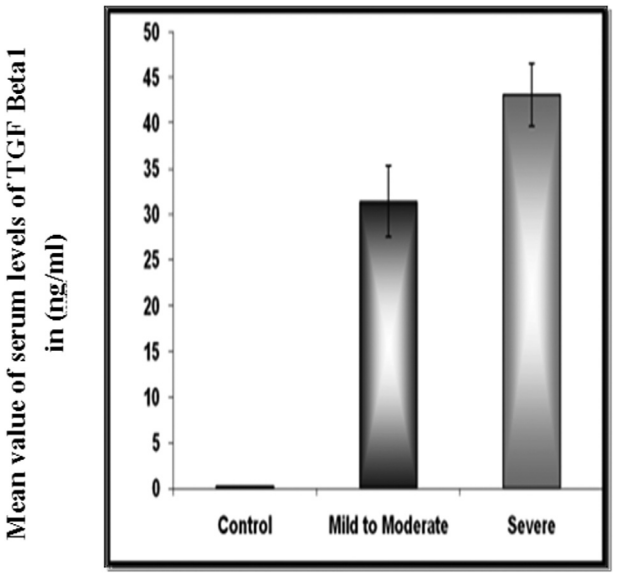
ANOVA, analysis of variance; TGFβ_1, transforming growth factor β_1. *Means statistically significant.

Figure 3



Statistical comparison of range, mean±SD values of bronchoalveolar lavage fluid levels of transforming growth factor β_1 (ng/ml) in the three studied groups.

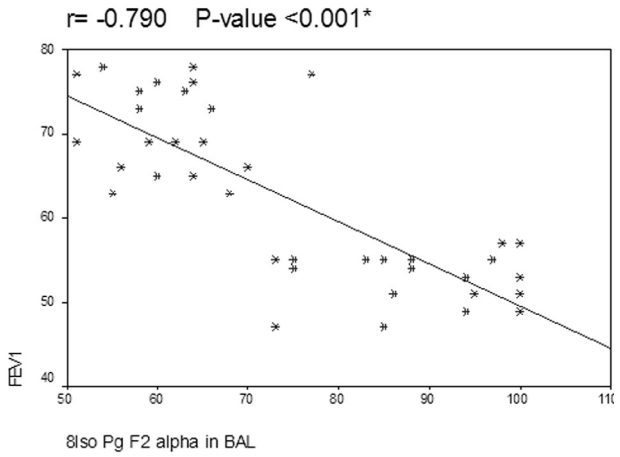
Figure 4



Statistical comparison of range, mean±SD values of serum levels of transforming growth factor β_1 (ng/ml) in the three studied groups.

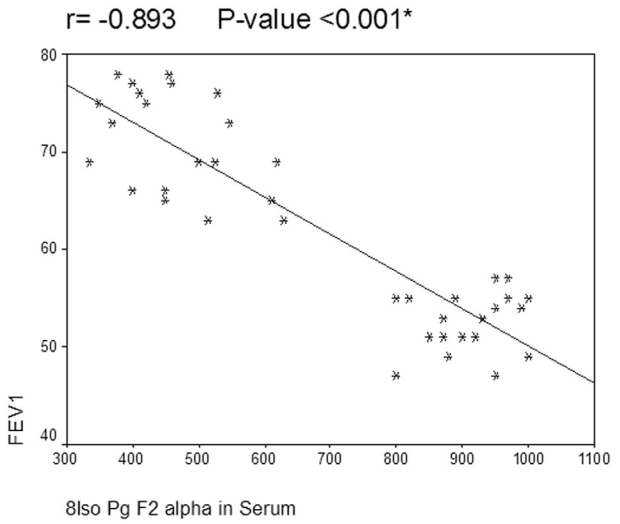
of airway inflammation, airway obstruction, and bronchial hyperresponsiveness, which leads to recurrent attacks of chest tightness, dyspnea, wheezing, and coughing [7]. Airway inflammation and remodeling are the two main pathological features of asthma. It is proved that asthma is a nonspecific airway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



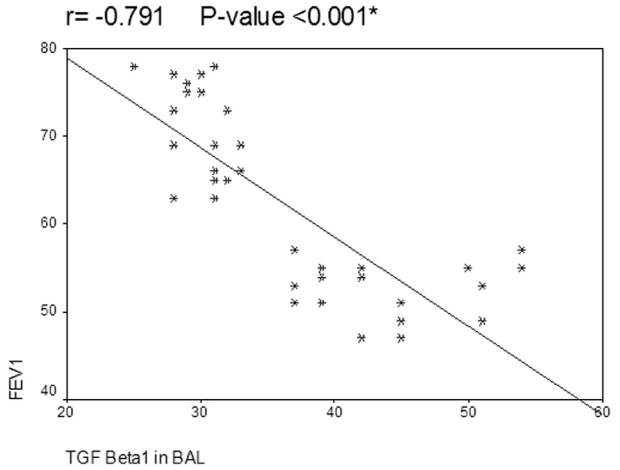
Correlation of 8-isoprostane concentration (ng/ml) in bronchoalveolar lavage and FVE_1% of predicted.

Figure 6



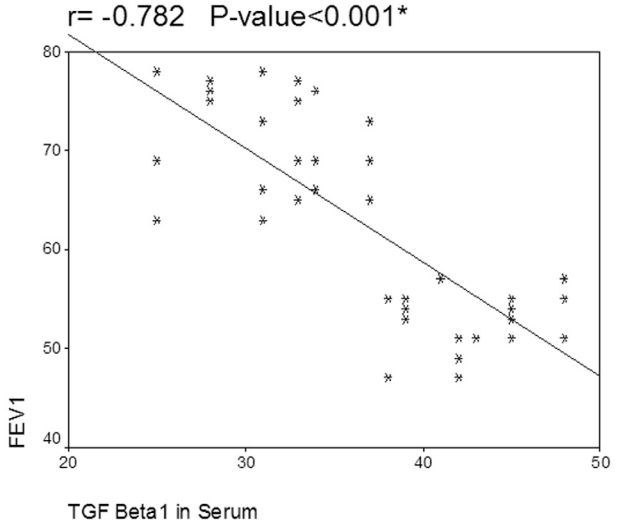
Correlation of 8-isoprostane concentration (ng/ml) in serum and FEV_1% of predicted.

Figure 7



Correlation of transforming growth factor β_1 concentration (ng/ml) in bronchoalveolar lavage and FEV_1% of predicted.

Figure 8



Correlation of transforming growth factor β_1 concentration (ng/ml) in serum and FEV_1% of predicted.

plasma of asthmatic patients [10–12]. The human $TGF\beta_1$ 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 $TGF\beta_1$ 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-$PGF_2\alpha$ 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-$PGF_2\alpha$ 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_2-isoPGs in BALF after 24 h of allergen instillation compared with its level at baseline. The rise of the concentrations of F_2-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_1, FVC, and FEV_1/FVC) in acute exacerbation and in remission. They found significant positive correlation between plasma 8-iso-$PGF_2\alpha$ 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-$PGF_2\alpha$ 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-$PGF_2\alpha$ 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-$PGF_2\alpha$ 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-$PGF_2\alpha$ levels during remission compared with healthy controls. Also, they found that FEV_1, FVC, and FEV_1/FVC were positively correlated with plasma 8-iso-$PGF_2\alpha$ levels. Samitasa *et al.* [18] found increased levels of 8-iso-$PGF_2\alpha$ in exhaled breath condensates in association with disease severity, in asthmatic adults and children. Keskin *et al.* [19] quantified that the F_2-isoPGs level in BALF at baseline values was lower than its level 24 h after allergen instillation; moreover, exhaled 8-iso-$PGF_2\alpha$ level in patients with moderate persistent asthma was higher than in the mild persistent group.

The present study showed that 8-iso-$PGF_2\alpha$ 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 $TGF\beta_1$ 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 $TGF\beta_1$ 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 $TGF\beta_1$ level. They concluded that plasma $TGF\beta_1$ level may be a systemic marker of asthma control. Manuyakorn *et al.* [21] studied serum $TGF\beta_1$ 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 $TGF\beta_1$ in the steroid-naive mild asthma group in comparison to the moderate asthma group, with no correlations between serum $TGF\beta_1$ 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 $TG\beta_1$ in BALF from clinically stable asthmatics and healthy control patients. Their levels were significantly higher in asthmatics than control patients. They conclude that basal $TGF\beta_1$ levels in the airways are elevated in asthma. Forno *et al.* [23] also observed elevated $TGF\beta_1$ expression in asthmatics. Joseph *et al.* [24] postulated that the mean value of the plasma $TGF\beta_1$ 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 $TGF\beta_1$ 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 $TGF\beta_1$ level in the asthmatic group increased significantly. In contrast to our results, Magnon *et al.* [27] reported decreased $TGF\beta_1$ expression in the asthmatic epithelium. Also, Lommatzsch Xiang and Qiu [28] reported no difference between the levels of serum $TGF\beta_1$ in atopic asthmatic

adults and control patients. Moreover, Ceyhan *et al*. [29] concluded that the $TGF\beta_1$ level in serum had no predictive value in diagnostic use. Although our study confirmed the great role of $TGF\beta_1$ in asthma and assessed the variation in the level of $TGF\beta_1$ in BALF and serum of asthmatic patients, we did not have the information about histopathologicol changes in bronchial wall in response to $TGF\beta_1$.

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-$PGF_2\alpha$ and $TGF\beta_1$ 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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Conflicts of interest

There are no conflicts of interest.

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