Diagnostic value of 8-isoprostane and transforming growth factor-β in bronchial asthma patients
Amgad Frahata, Yousef Mansoura, Ayman Eldiba, Doaa Alsedb

Background  Asthma is an airway inflammatory disease with functional and structural changes, leading to bronchial hyperresponsiveness 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$_1$ (TGFβ$_1$) 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 TGFβ$_1$ 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 TGFβ$_1$.

Results  Bronchoalveolar lavage 8-iso-PGF$_{2\alpha}$ and TGFβ$_1$ was higher in asthmatic patients. It was significantly increased with increased asthma severity.

Conclusion  Increased levels of TGFβ$_1$ 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.

Keywords: airflow obstruction, asthma, biomarker, hyperreponsiveness, inflammation, 8-iso-prostaglandin F$_{2\alpha}$, transforming growth factor β$_1$

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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 TGFβ$_1$ 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 FEV₁ 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), peak expiratory flow rate (PEFR) before and after B₂ agonist inhalation to differentiate asthma from chronic obstructive pulmonary disease, serum level of 8-isoprostane and TGFβ₁, flexible fiberoptic bronchoscope, with bronchoalveolar lavage (BVL) was collected for the estimation of 8-isoprostane and TGFβ₁.

Exclusion criteria
Chest diseases other than bronchial asthma, cardiac patients, mechanically ventilated patients with a PaO₂ 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 ($r$); $x^2$, 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 1 Statistical comparison of mean±SD values and of age in the studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (years)</th>
<th>Mean±SD</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24–55</td>
<td>38±8.165</td>
<td>0.372</td>
</tr>
<tr>
<td>Moderate</td>
<td>24–55</td>
<td>39.3±8.235</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>30–48</td>
<td>37.4±5.538</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance.

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

<table>
<thead>
<tr>
<th></th>
<th>Control (%)</th>
<th>Moderate (%)</th>
<th>Severe (%)</th>
<th>Total (%)</th>
<th>$x^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>70</td>
<td>40</td>
<td>55</td>
<td>52</td>
<td>2.571</td>
<td>0.276</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>60</td>
<td>45</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>8-Iso-PGF₂α in BAL</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Control</td>
<td>23–27</td>
<td>24.9±1.449</td>
</tr>
<tr>
<td>Mild to moderate</td>
<td>51–77</td>
<td>61.25±6.455</td>
</tr>
<tr>
<td>Severe</td>
<td>73–100</td>
<td>89.2±9.551</td>
</tr>
</tbody>
</table>

Tukey's test

<table>
<thead>
<tr>
<th></th>
<th>$F$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control and mild to moderate</td>
<td>Control and severe</td>
<td>Mild to moderate and severe</td>
</tr>
<tr>
<td>$&lt;$0.001*</td>
<td>$&lt;$0.001*</td>
<td>$&lt;$0.001*</td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; BAL, bronchoalveolar lavage; 8-isoprostane, 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 transforming growth factor β1 (ng/ml)

The mean±SD values of TGFβ1 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 transforming growth factor β1 concentration (ng/ml) in bronchoalveolar lavage and serum and spirometric values

There was significant negative correlation between TGFβ1 concentration in BAL and FEV1 (percentage of predicted) (r=−0.791 and P<0.001), FVC (percentage of predicted) (r=−0.579 and P≤0.001), PEFR (percentage of predicted) (r=−0.729 and P<0.001) as well as there was significant negative correlation between serum TGFβ1 concentration and FEV1 (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≤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
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-PGF₂α 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-PGF₂α levels in exhaled breath condensate, induce sputum, and in

Table 5 Statistical comparison of range, mean±SD values of bronchoalveolar lavage fluid levels of transforming growth factor β₁ (ng/ml) in the three studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>TGFβ₁ in BAL</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Control</td>
<td>5–6</td>
<td>5.5±0.471</td>
</tr>
<tr>
<td>Mild to moderate</td>
<td>25–33</td>
<td>30.1±2.100</td>
</tr>
<tr>
<td>Severe</td>
<td>37–54</td>
<td>43.3±5.806</td>
</tr>
</tbody>
</table>

Tukey’s test

<table>
<thead>
<tr>
<th>Control and mild to moderate</th>
<th>Control and severe</th>
<th>Mild to moderate and severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; BAL, bronchoalveolar lavage; TGFβ₁, transforming growth factor β₁. *Means statistically significant.

Table 6 Statistical comparison of range, mean±SD values of serum levels of transforming growth factor β₁ (ng/ml) in the three studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>TGFβ₁ in serum</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Control</td>
<td>0.240–0.270</td>
<td>0.252±0.012</td>
</tr>
<tr>
<td>Mild to moderate</td>
<td>25.000–37.000</td>
<td>31.400±3.858</td>
</tr>
<tr>
<td>Severe</td>
<td>38.000–48.000</td>
<td>43.100±3.447</td>
</tr>
</tbody>
</table>

Tukey’s test

<table>
<thead>
<tr>
<th>Control and mild to moderate</th>
<th>Control and severe</th>
<th>Mild to moderate and severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

ANOVA, analysis of variance; TGFβ₁, transforming growth factor β₁. *Means statistically significant.

Figure 3

Statistical comparison of range, mean±SD values of bronchoalveolar lavage fluid levels of transforming growth factor β₁ (ng/ml) in the three studied groups.

Figure 4

Statistical comparison of range, mean±SD values of serum levels of transforming growth factor β₁ (ng/ml) in the three studied groups.
plasma of asthmatic patients [10–12]. The human TGFβ1 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 TGFβ1 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-PGF2α 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 TGFβ$_1$ in BAL and serum in moderate and severe asthma compared with control, and in severe asthma compared with moderate asthma. Ozylmaz et al. [20] measured plasma TGFβ$_1$ 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 TGFβ$_1$ level. They concluded that plasma TGFβ$_1$ level may be a systemic marker of asthma control. Manuyakorn et al. [21] studied serum TGFβ$_1$ 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 TGFβ$_1$ in the steroid-naïve mild asthma group in comparison to the moderate asthma group, with no correlations between serum TGFβ$_1$ 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 TGFβ$_1$ in BALF from clinically stable asthmatics and healthy control patients. Their levels were significantly higher in asthmatics than control patients. They conclude that basal TGFβ$_1$ levels in the airways are elevated in asthma. Forno et al. [23] also observed elevated TGFβ$_1$ expression in asthmatics. Joseph et al. [24] postulated that the mean value of the plasma TGFβ$_1$ 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 TGFβ$_1$ 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 TGFβ$_1$ level in the asthmatic group increased significantly. In contrast to our results, Magnon et al. [27] reported decreased TGFβ$_1$ expression in the asthmatic epithelium. Also, Lommatzsch Xiang and Qiu [28] reported no difference between the levels of serum TGFβ$_1$ in atopic asthmatic
adults and control patients. Moreover, Ceyhan et al. [29] concluded that the TGFβ1 level in serum had no predictive value in diagnostic use. Although our study confirmed the great role of TGFβ1 in asthma and assessed the variation in the level of TGFβ1 in BALF and serum of asthmatic patients, we did not have the information about histopathological changes in bronchial wall in response to TGFβ1.

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-PGF2α and TGFβ1 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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