Study of Plasma Malondialdehyde, Albumin and Bilirubin Levels in Asthmatic Patients as Markers of Oxidative Stress

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ABSTRACT

Asthma is a chronic disorder of the airways with underlying inflammation and oxidative stress. Malondialdehyde (MDA) - the lipid peroxidation product- level increases in inflammatory diseases and used as a common oxidative stress marker. Albumin and bilirubin are components of antioxidant defense system. The aim of this study are, Evaluating oxidative stress as a component of asthma process by measuring MDA (as an oxidative marker), albumin and bilirubin (as biomarkers of antioxidant defense system) in asthmatic patients. Thirty seven asthmatic patients (20

الخلاصة

الربو هو مرض مزمن يصيب المجاري التنفسية الواقعة تحت تأثير الاتهاب والجهد التاكسدي. مستوى المالونديالديهيد (MDA) - ناتج فوق أكسدة الدهن- يزيد في الأمراض الاتهابية ويستعمل كمؤشر عام للجهد التاكسدي. الأبومين والبمروبين هما من مكونات النظام الدفاعي لمنع الأكسدة. إن هدف هذه الدراسة هو تقدير الجهد التاكسدي كمكوّن في عملية الربو وذلك بقياس مDA (كمؤشر أكسدة)، البمروبين والابومين (كمؤشرات حيوية لنظام دفاع منع الأكسدة) في المرضى المصابين بالربو. الدراسة تضمنت سبع وثلاثون مريضا مصاب بالزْبَّو (20 أنثى و17 ذكر) تم مقارنتهم ب 77 من الاصحاء كمجموعة سيطرة. حلت عينات البلازما لقياس MDA، وكذلك الابومين والبمروبين. كانت نتائج الدراسة كالتالي: تركيز MDA في البلازما ازداد بشكل ممحوظ (p<0.05) بينما تركز كل من الابومين والبمروبين في البلازما نقصا بشكل ممحوظ (p<0.05) في المرضى المصابين بالربو ذو مقارنة بمجموعة السيطرة. وهو ما يؤدي الى الاستنتاج بأن المستوى المرتفع للMDA في برزما المرضى المصابين بالربو يشير إلى المستوى العالي للجهد التاكسدي في هؤلاء المرضى. تناقص تركيز كل من الابومين والبمروبين هو بسبب استهلاكهما مما يشير إلى دورهما كجزيئات مانعة للاكسدة في المرضى المصابين بالربو.
females and 17 males) were involved compared with 37 healthy controls. Plasma samples analyzed for total MDA, albumin and bilirubin. The results of underlying study are: plasma MDA was significantly increased (p<0.05) while plasma albumin and bilirubin were significantly decreased (p<0.05) in asthmatic patients compared to control group. Which lead to conclude that elevated levels of MDA in plasma of asthmatic patients indicate high level of oxidative stress in these patients. Decreasing plasma albumin and bilirubin is due to their consumption and indicate their role as antioxidant species in asthmatic patients.

INTRODUCTION

Asthma is a worldwide disease. It is defined as a chronic inflammatory disorder of the airways. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli (1).

Lungs are organs with large epithelial surface area that is at risk for oxidant-mediated attack. The tracheobronchial tree and the alveolar spaces are exposed to reactive oxidizing species in the form of inhaled airborne pollutants, tobacco smoke and product of inflammation. Disequilibrium, either through increased oxidant stress or decrease antioxidant resources, can result in a series of pathophysiological events in the lungs that culminate in cellular death and pulmonary dysfunction(2). It has been shown that inflammation driven by increased oxidative stress (defined as imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates) occurs in the airways of patients with asthma (3,30).

Malondialdehyde (MDA) is produced as a by-product of polyunsaturated fatty acid peroxidation and is the principal and most studied product of polyunsaturated fatty acid peroxidation (4). MDA is one of the most frequently used indicators of lipid peroxidation(5).

Human serum albumin (HSA) is an abundant multi functional non-glycosylated, negatively charged plasma protein, with ascribed ligand-binding, transport properties and antioxidant functions (6). Many antioxidant activities of albumin result from its ligand-binding capacities. Hydroxyl radicals released from Fenton reaction are mostly directed to the protein, sparing more important targets (7). Another indirect antioxidant activity of albumin comes from its ability to transport bilirubin. Such albumin-bound bilirubin was shown to act as an inhibitor of lipid
peroxidation its as well as protection of alpha-tocopherol from damage by peroxyl radicals (8). Human serum albumin (HSA) contains one reduced cysteine residue (Cys34) which, due to the large amount of albumin in plasma, constitutes the largest pool of thiols in the circulation. Through the reduced Cys34, albumin is able to scavenge hydroxyl radicals (7).

Bilirubin (BR) is the yellow breakdown product of normal heme catabolism (9). Bilirubin contains an extend system of conjugated double bonds and a reactive hydrogen atom and thus could possess antioxidant properties. Depending on these ideas stocker et al indicate that bilirubin at micromolar concentrations can scavenge the chain --carrying peroxyl radical

\[
\text{LOO}^{\cdot} + \text{BR} \rightarrow \text{LOOH} + \text{BR}^{\cdot}
\]

BR\cdot may then react with either another peroxyl radical to give rise to a nonradical product, or oxygen as below (10,29):

\[
\text{BR} + \text{LOO}^{\cdot} \rightarrow \text{BR-OOL}
\]

In serum J.Neuzil & R.Stocker demonstrate that lipoprotein-associated and albumin-bound BR can efficiently protect lipid from such peroxidation (8).

**Aim of the study**

Study the behavior of some oxidative stress markers in asthmatic patients and compare them with healthy control group.

**MATERIALS AND METHODS**

Thirty seven asthmatic patients (20 females and 17 males) were evaluated in this study. Diagnosis of asthma was made by a respiratory physician. The study was carried out in Baghdad Teaching Hospital. The exclusion criteria were (i) age less than 15y (ii) vitamin supplements taken in the last 4 weeks, (iii) presence of other diseases known to be associated with elevated oxidative stress (cancer, diabetes, arthritis, etc.). A matching group of 37 healthy volunteer subjects (21 male and 16 female) were considered as a control group.

Each plasma sample was analyzed for total malondialdehyde (MDA), albumin and bilirubin. Plasma albumin and bilirubin were measured by colorimetric method using kits supplied by bioMaghrab Company. MDA lipid peroxidation end product measured spectrophotometrically after adding thiobarbituric acid under acidic conditions (28).
RESULTS AND DISCUSSION

Number and percentage (according to gender) of subjects who's involved in this study are given in Table 1.

Table 1: distribution of study participants according to their health status and gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Study Group</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asthmatic</td>
<td>Control</td>
<td>Total</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>21</td>
<td>38</td>
<td>45.9%</td>
<td>56.0%</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>16</td>
<td>36</td>
<td>54.1%</td>
<td>44.0%</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>37</td>
<td>74</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

MDA concentration in the plasma of asthmatic group was significantly higher than the control group (12.6 ± 0.82 µmol/l and 4.50 ± 0.33 µmol/l respectively), p< 0.01 as shown in table -2.

Table 2: plasma malondialdehyde concentration (µmol/l) in asthmatic patients compared to control subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>MDA* µmol/l Mean ± SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37</td>
<td>4.50 ± 0.33</td>
<td>---</td>
</tr>
<tr>
<td>Asthmatics</td>
<td>37</td>
<td>12.6 ± 0.82</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*MDA= Malondialdehyde

The mean (±SEM) values of plasma albumin levels in 37 healthy controls and 33 asthmatics one are listed in table-3. Data in this table showed that albumin concentration in the plasma of asthmatic group was significantly lower compared with control group (40.29±1.24 g/l and 45.17±1.54g/l respectively), p< 0.05.
Table 3: plasma Albumin (g/l) In asthmatic patients compared to control subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Albumin g/l Mean± SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37</td>
<td>45.17±1.54</td>
<td>---</td>
</tr>
<tr>
<td>asthmatics</td>
<td>33</td>
<td>40.29 ± 1.24</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

The mean (±SEM) values of plasma bilirubin levels in 37 healthy controls and 31 asthmatics one are listed in table-4. Data in this table showed that bilirubin concentration in the plasma of asthmatic group was significantly lower (p< 0.05) compared with that of the control group (0.58± 0.04 mg/dl).

Table 4: plasma bilirubin concentration (mg/dl) in asthmatic patients compared to control subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Bilirubin mg/dl Mean ±SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37</td>
<td>0.58 ± 0.04</td>
<td>---</td>
</tr>
<tr>
<td>asthmatics</td>
<td>31</td>
<td>0.41 ± 0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Asthma is an inflammatory disease with hyperresponsivness. Both of these characteristics produce an oxidative stress situation which is believed to contribute to pathophysiology of asthma. Reactive molecules (particularly free radicals) produced from oxidative stress. These reactive molecules in addition to causing tissue damage by oxidation of biomolecules like DNA, lipids, proteins and sugars (11,12). Malondialdehyde, MDA, is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation (a process of fatty acids oxidation in cell membrane lipids, termed as lipid peroxidation) (13). MDA one of the most frequently used indicators of lipid peroxidation(14).

Table 2 illustrates significant increase in the plasma MDA levels of asthmatic patients compared to control. Similar findings were reported by Ozaras et al, Jacobson et al. and sharma A. et al., all of them, found that MDA was higher in plasma of asthmatic patients compared to controls(15-17).

The increase in the MDA concentration indicates the increased peroxidation of lipids (i.e. oxidative stress) in the asthma disease, where the oxidative stress is involved in asthma pathogenesis and symptoms (18).
High levels of MDA itself is involved in many harmful effects of inflammation (13, 19).

Reactive oxygen species (ROS) production (which cause oxidative stress situation when increased) is strictly regulated by antioxidant defense that protect organism against the potentially destructive effects of ROS. Albumin and bilirubin are members of body defense systems(20).

Table-3- illustrates significant decreasing in the plasma albumin levels of asthmatic patients compared to control. Albumin is found to have an antioxidant properties (7,8). So the decrease in plasma albumin level obtained in the present study could be explained by that albumin antioxidant activity. The results obtained in the present study agree with Vural, et al and Picado, et al , both of them reported a decrease serum albumin level in asthmatic patients (21,22).

Table-4- illustrates significant decrease in the plasma bilirubin levels of asthmatic patients compared to control. Bilirubin is a molecule with effective antioxidant properties due to its structure (10); therefore it is not strangely to exhibit its antioxidative properties that leads to the consumption of this molecules and hence, the low plasma levels.

The result of bilirubin levels in the present study is agree with results obtained by Misso, et al who found a low plasma bilirubin concentration in severe asthma(23) and also with Ohrui T. et al who found remission of asthmatic patients who develop jaundice (24). Neuzil & Stocker demonstrate that lipoprotein-associated and albumin-bound bilirubin can efficiently protect lipid from peroxidation (8).

Many studies stated low bilirubin levels as biomarker of many diseases’ pathology. In peripheral vascular disease, bilirubin levels are lower than in the normal population (24). Hopkins et al compared familial coronary artery disease patients with control subjects. The diseased individuals displayed substantial lower serum bilirubin levels than the control subjects (25). Levinson S. also observed an inverse relationship between bilirubin levels and severity of ischemic heart disease(26). In meta-analysis of 11 studies, Novotny & Vitek found elevated bilirubin levels associated with diminished risk of atherosclerosis (27).

In conclusion, according to this study it is obvious that oxidative stress is an important feature of asthma pathphysiology because of significant elevation of MDA, which is a well-known oxidative stress marker. Also the decreased
plasma albumin and bilirubin levels clearly indicate the antioxidant activities for these molecules. So we recommend use of MDA, albumin and bilirubin measurements as an aid for asthma severity.

REFERENCES


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