Serum Homocystein Level In COPD Patients: Possible Beneficial Effect Of Antioxidants

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Abstract: Chronic obstructive pulmonary disease (COPD) has a dramatic effect on quality of life. Elevated level of homocysteine (HCY) in COPD are risk factors for cardiovascular diseases. Objective: This study was to evaluate the level of HCY and the role of antioxidant supplementation in COPD patients. Methods: Homocysteine and folic acid have been determined by HPLC while, the other parameters have been determined by spectrophotometer. Results: Our study revealed that Paraoxonase and arylesterase activity of Paraoxonase-1 (PON-1), as a high density lipoprotein bound enzyme and its major role is to prevent oxidation of low density lipoprotein, were reduced in the serum of acute and stable COPD patients. HCY level has been elevated in acute and stable COPD as compared with normal healthy control. Vitamin B_{12} (Vit B_{12}), only decreased in acute COPD while Folic acid level has not a significant change. Supplementation of antioxidant combination ameliorated the level of HCY (acute and stable) and Vit B_{12} in acute COPD patients. Conclusion: we concluded that HCY may be elevated as a result of oxidative inflammatory response and co supplementation of antioxidant combination could be of important value in minimizing the risk of high HCY level, especially in acute COPD patients.

Key words: COPD, Homocysteine, Folic acid, Paraoxonase, vitamin B_{12}.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a highly prevalent disease that has a large impact on quality of life for patients and their families and kills millions of people worldwide\textsuperscript{[1-3]}. COPD is defined as a disease state characterized by progressive airflow limitation that is not fully reversible, and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily cigarette smoke\textsuperscript{[2]}. COPD is a chronic inflammatory disease characterized by an increase in neutrophils, macrophages, and T lymphocytes in various parts of the lung, which are driven by inflammatory mediators, particularly cytokines, chemokines, and oxidants\textsuperscript{[9]}. This “abnormal” inflammatory reaction to risk factors is believed to be responsible for the most important pathologic abnormalities of COPD, bronchitis and emphysema\textsuperscript{[3]}. Environmental factors related to COPD\textsuperscript{[6]}, the most important being active\textsuperscript{[7]} and passive\textsuperscript{[4]} cigarette smoking, act through the generation of oxidative stress and/or reduction of antioxidant capacity\textsuperscript{[8]}.

Homocysteine, a nonprotein, sulfur-containing amino acid, and an intermediate in the metabolism of the essential amino acid methionine, were implicated in the development and progression of cardiovascular disease. The mechanisms by which it exerts its effects have not yet been fully elucidated, but cumulative data clearly demonstrate that it affects multiple vascular functions \textit{in vitro} and \textit{in vivo}, such as promoting prothrombotic phenotype of the endothelium by increasing platelet aggregation and activation, stimulating vascular smooth-muscle cell proliferation, and altering endothelial function\textsuperscript{[10,11]}. The leading mechanism suggested for the adverse vascular effects of homocysteine on endothelial function involves oxidative stress and depletion of bioactive nitric oxide (NO)\textsuperscript{[12]}. Paraoxonase (PON) as an ester hydrolase enzyme that is synthesized by the liver. The calcium dependent ester hydrolase activity of PON is found tightly associated with apoA-I in the high density lipoprotein (HDL) particle\textsuperscript{[13]}. Purified PON not only prevents low density lipoprotein (LDL) oxidation but also blocks the ability of mildly oxidized LDL (MM-LDL) to induce monocyte chemotaxis and binding to endothelial cells\textsuperscript{[14]}. The elevated serum homocysteine level and reduced PON activity have been reported in different diseases, therefore we conducted this study to evaluate the activity of PON and serum level of homocysteine in COPD patients. Also, we investigated the level of folic acid and vitamin B_{12} as essential factors in the metabolism of homocysteine. Furthermore, we assessed the effect of antioxidant therapy on their level.
MATERIALS AND METHODS

This study was carried out on 80 human subjects classified as follow:

1. Normal healthy control group: consists of 20 healthy subject (12 male and 8 female) with an age ranging from 17 - 60 years and FEV₁ 92% of predicted values, all subjects had no history of lung diseases.

2. Stable COPD group: consists of 24 patients were stable with no acute exacerbation of COPD for one month prior to the study (18 male and 6 female) with an age ranging from 25 - 75 years.

3. Acute exacerbation COPD group: consists of 20 patients presented by an acute exacerbation (14 male and 6 female) with an age ranging from 30 - 80 years.

4. Treated group: consists of 16 patients presented by an acute exacerbation of COPD (9 male and 7 female) with an age ranging from 32 - 78 years, treated by ordinary therapy of COPD exacerbation with addition of antioxidant supplementation. The antioxidant supplementation consists of β-carotene 2000 IU, vitamin E 45 mg, vitamin C 600 mg, selenium aspartate 25 mg and zinc sulphate 25 mg, in doses of 1 tablet daily for one month[15].

The diagnosis of COPD patient was based on:

a. Chronic cough with sputum production.
b. History of exposure to risk factors.
c. Dyspnea (that is progressive, persistent, worse on exercise) and worse during respiratory infection.
d. FEV₁/FVC < 70 %
e. FEV₁ < 30% predicted or FEV₁ < 50% predicted plus respiratory failure (PaO₂ < 60 mmHg with or without PaO₂ > 50 mmHg) or clinical signs of right heart failure[16]. None of the patients had clinical or radiological evidences of pneumonia. Patients with hypertension, IHD or cardiomyopathy were excluded from the study. All of the patients of COPD were enrolled from respiratory outpatient's clinic of Bab-El-Sheria Hospital, Al-Azhar University. Venous blood samples about 10 CC were taken from all patients and control over night fasting, serum were obtained after centrifugation and prepared for estimation of serum cholesterol[17], triacylglycerol[18], HDL-cholesterol[19], LDL-cholesterol[20] spectrophotometry. Serum homocysteine level was measured by HPLC[21], vitamin B₁₂ and folic acid[22]. Finally serum paraoxonase activity and arylesterase activity was measured spectrophotometricaly[23].

Statistical Analysis: Data are expressed as Mean ± S.E.M. INSTAT version 2.0 (graph pad,ISI software, Philadelphia, PA, USA, 1993) computer program was used to compute statistical analysis. Difference between means was assessed by Student t-test and statistical significance was accepted at P≤0.05.

RESULTS AND DISCUSSIONS

Results: The obtained results demonstrate non significant differences in serum cholesterol (mg/dl), serum triacylglycerol (mg/dl), HDL-cholesterol (mg/dl) LDL-cholesterol (mg/dl). Data in table 1 also clarifies non significant changes in risk ratio 1 and risk ratio 2 between all groups. In respect to serum homocysteine level (µmol/l), the results clarifies a significant increase in serum homocysteine level in both acute and stable COPD groups when compared to normal healthy control group. Treatments were significantly ameliorating serum homocysteine level in relation to acute COPD group (Table 1).

Vitamin B₁₂ and folic acid plays an important role in the metabolism of homocysteine, therefore there are a significant decrease in serum vitamin B₁₂ (Pg/ml) in acute COPD group only compared with normal healthy control group. While, there is no significant changes in serum folic acid (ng /ml) in all studied groups (Table 2).

Although, the data in table 2 reveals a significant decrease in serum paraoxonase activity (U/l) and arylesterase activity (U/ml) in acute COPD group compared to normal healthy control group. On the other hand, both serum paraoxonase and arylesterase activities were significantly increased in stable COPD and treated groups.

Figs. (1, 2 and 3) demonstrate a significant negative correlation between serum homocysteine level and paraoxonase activity, arylesterase activity and vitamin B₁₂ respectively.

Discussion: COPD is a highly prevalent disease that has a large impact on quality of life for patients and their families and kills millions of people worldwide[1-3].

Homocysteine, like other thiols, is a reactive molecule. It is auto-oxidized in the plasma, forming hydrogen peroxide and specifically inhibits glutathione peroxidase activity leading to further increase in hydrogen peroxide that are toxic to endothelial cells[24]. Normally, endothelial cells detoxify homocysteine by releasing NO, which forms S-nitroso-homocysteine adducts by binding to homocysteine[25]. This protective effect of NO is eventually compromised, as long-term exposure to high homocysteine concentrations damages the endothelium, and thus limits NO production. In addition, homocysteine may also decrease the bioavailability of NO by impairing its synthesis[26,27].

Table 1: The obtained results demonstrate non significant differences in serum cholesterol (mg/dl), serum triacylglycerol (mg/dl), HDL-cholesterol (mg/dl) LDL-cholesterol (mg/dl). Data in table 1 also clarifies non significant changes in risk ratio 1 and risk ratio 2 between all groups. In respect to serum homocysteine level (µmol/l), the results clarifies a significant increase in serum homocysteine level in both acute and stable COPD groups when compared to normal healthy control group. Treatments were significantly ameliorating serum homocysteine level in relation to acute COPD group (Table 1).

Table 2: The obtained results reveal a significant decrease in serum paraoxonase activity (U/l) and arylesterase activity (U/ml) in acute COPD group compared to normal healthy control group. On the other hand, both serum paraoxonase and arylesterase activities were significantly increased in stable COPD and treated groups.

Fig. 1: R= -0.5, p<0.01, non significant changes in serum vitamin B₁₂ (Pg/ml) in acute COPD group only compared with normal healthy control group.

Fig. 2: R= -0.5, p<0.01, non significant changes in serum paraoxonase activity (U/l) and arylesterase activity (U/ml) in acute COPD group compared to normal healthy control group.

Fig. 3: R= -0.5, p<0.01, non significant changes in serum folic acid (ng /ml) in all studied groups.

Fig. 4: R= -0.5, p<0.01, non significant changes in serum paraoxonase activity (U/l) and arylesterase activity (U/ml) in acute COPD group compared to normal healthy control group.
Table 1: Lipid profile and risk ratio of all studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Acute COPD</th>
<th>Stable COPD</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>20</td>
<td>20</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>S. Cholesterol (mg/dl)</td>
<td>180 ± 1.12</td>
<td>220 ± 1.7</td>
<td>219 ± 2.3</td>
<td>190 ± 1.3</td>
</tr>
<tr>
<td>S. Triacylglycerol (mg/dl)</td>
<td>152 ± 3.5</td>
<td>172 ± 3.2</td>
<td>169 ± 2.6</td>
<td>141 ± 3.1</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>51.2 ± 0.50</td>
<td>51.3 ± 0.09</td>
<td>50.3 ± 0.90</td>
<td>55.0 ± 1.7</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>129 ± 2.3</td>
<td>140 ± 1.3</td>
<td>139 ± 2.4</td>
<td>120 ± 2.2</td>
</tr>
<tr>
<td>Risk Ratio 1</td>
<td>3.52 ± 0.20</td>
<td>4.29 ± 0.89</td>
<td>4.35 ± 0.85</td>
<td>3.45 ± 0.59</td>
</tr>
<tr>
<td>Risk Ratio 2</td>
<td>2.52 ± 0.08</td>
<td>2.73 ± 0.75</td>
<td>2.76 ± 0.65</td>
<td>2.20 ± 0.70</td>
</tr>
</tbody>
</table>

Table 2: Level of serum homocysteine, vit. B12, folic acid, PON and arylesterase activity in all studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Acute COPD</th>
<th>Stable COPD</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>20</td>
<td>20</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Paraoxonase (U/l)</td>
<td>190 ± 5.24</td>
<td>130 ± 6.40</td>
<td>178 ± 6.9</td>
<td>180 ± 4.95</td>
</tr>
<tr>
<td>Arylesterase (U/ml)</td>
<td>40.75 ± 1.35</td>
<td>24.14 ± 2.27</td>
<td>40.98 ± 3.65</td>
<td>39.29 ± 1.46</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>7.57 ± 0.28</td>
<td>10.52 ± 0.29</td>
<td>9.44 ± 0.26</td>
<td>7.37 ± 0.27</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>6.18 ± 0.67</td>
<td>5.71 ± 0.63</td>
<td>4.85 ± 0.59</td>
<td>5.48 ± 0.70</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>335 ± 12.99</td>
<td>279 ± 11.21</td>
<td>299 ± 9.05</td>
<td>322 ± 11.57</td>
</tr>
</tbody>
</table>

Fig. 1: Correlation between serum paraoxonase activity and serum homocysteine level in acute COPD.

Hyperhomocysteinemia has been reported to be frequent in the elderly. Few studies have adequately controlled for vitamin B12 deficiency, which is associated with elevated level of total homocysteine. Adequate folate and vitamin B12 (both intake and serum levels) act synergistically to decrease the risk of high total plasma homocysteine levels in elderly populations. The higher prevalence of vitamin deficiency contributes to the higher risk of hyperhomocysteinemia. Moreover, Alfons et al., demonstrate vitamins known to decrease the risk of elevated homocysteine level in elderly populations. Although there are non significant differences between all studied groups in lipid parameters, oxidative stress cause significant alteration in enzyme activity of HDL-associated enzyme paraoxonase / arylesterase. PON is in close physical association with HDL which thus acts as its carrier and site of action. HDL was shown to be effective in preventing the oxidative modification of LDL, probably due to a mechanism that is at least partly enzymatic activity including PON, lecithi-
cholesterol acetyltransferase (LCAT), platelet activating factor acetyl hydrolase (PAFAH), proteinase and phospholipase. During an inflammatory responses in COPD, acute phase HDL is formed, which itself become proinflammatory, in contrast to the anti-inflammatory properties of native HDL. This acute phase HDL may be oxidatively modified by free radicals especially peroxynitrite and most often impair the known function of HDL. In addition to paraoxonase / arylesterase activity, PON also hydrolyzes phospholipid hydroperoxide, cholesterol ester hydroperoxide and reduce lipid hydroperoxide to the respective hydroxide as well as degrades hydrogen peroxides. Moreover, PON protect HDL from peroxidation and improve reverse cholesterol transport to the liver.

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REFERENCES