Association between Serum Homocysteine and Oxidative Stress in Elderly Patients with Obstructive Sleep Apnea/hypopnea Syndrome

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Objective: Elderly patients with obstructive sleep apnea/hypopnea syndrome (OSAHS) have a higher risk of cardiovascular and cerebrovascular disease. However, changes of homocysteine (Hcy) as markers of cardiovascular and cerebrovascular disease associated with OSAHS and their mechanism have not been elucidated so far. This study aims to investigate the changes of both serum Hcy and oxidative stress and their possible links with OSAHS in elderly patients.

Methods: Based on polysomnogram (PSG) and age, 83 patients with OSAHS were recruited and divided into elderly-OSAHS (n=32) and non-elderly OSAHS groups (n=51). Fifty two subjects without OSAHS were divided into elderly control (n=29) and non-elderly control groups (n=23). A total of 135 subjects were included in the present study. All subjects were recorded for PSG variables and the contents of homocysteine (Hcy), malonaldehyde (MDA), and glutathione (GSH) which were detected after sleep. Serum homocysteine was measured by cyclophorase. MDA and GSH were measured by spectrophotometer.

Results: (1) The serum levels of Hcy showed significant difference among the four groups (P<0.05). The concentrations of Hcy in elderly OSAHS patients were higher than in other groups, while those in the elderly control group were higher than in the non-elderly control; the concentrations in the non-elderly OSAHS group were higher than in the non-elderly control. (2) The concentrations of MDA and GSH changed at an equal pace with Hcy in the four groups. (3) Multielement linearity regression analysis indicated a statistically significant relationship between Hcy concentration and age, MDA, GSH, and apnea hypopnea index (AHI). Conclusions: (1) The concentrations of Hcy and oxidative stress have increased with advancing age. (2) The concentrations of Hcy and oxidative stress have further increased in the elderly patients with OSAHS. (3) Oxidative stress might cause high-level serum Hcy in the elderly patients with OSAHS.

Key words: Elderly; Obstructive sleep apnea/hypopnea syndrome; Homocysteine; Oxidative stress; Malonaldehyde; Glutathione

INTRODUCTION

High incidence of obstructive sleep apnea/hypopnea syndrome (OSAHS) characterized by repetitive episodes of apnea related hypoxia during sleep and cerebrocardiac vascular thrombotic disease was detected in elderly populations. Recent researches have shown close relationship between OSAHS and cardiovascular complications such as hypertension, coronary heart diseases and others[1-3]. It is known from previous studies that elevated plasma homocysteine (Hcy) levels have been considered as an independent risk factor for cerebrocardiac vascular disease[4] and Hcy is important for the development of atherosclerosis[5-6].

The level of Hcy in elderly patients with OSAHS remains unclear, although some evidence has indicated that OSAHS is associated with the enhancement of Hcy. In addition, little is known about the relationship between serum Hcy and oxidative stress, and their mechanism have never been examined.

Therefore, the aims of the current study are: (1) to evaluate whether the production of both serum Hcy and markers of oxidative stress are increased in elderly patients with OSAHS; (2) to investigate possible mechanism of the changes of serum Hcy in elderly patients with OSAHS.

MATERIALS AND METHODS

Subjects and Design

OSAHS patients and the control in this study were selected from subjects attending the Sleep Disorders Center in the First Affiliated Hospital of 0895-3988/2010
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Biochemical purpose

Soochow University. All participants signed their consent.

Subjects who took drugs affecting psychiatric disorders were excluded (rheumatoid arthritis, chronic renal failure and/or suffered from any chronic disease (chronic obstructive pulmonary disease)). Eighty-three patients with polysomnographically verified OSAHS were divided into an elderly OSAHS group \( (n=32) \), with a mean age of 65.8±7.2 years ranging between 60-82) and a non-elderly OSAHS group \( (n=51) \), with a mean age of 42.7±8.3 years, ranging between 27 and 57). The control group \( (n=52) \) was divided into an elderly control group \( (n=29) \), with a mean age of 69.4±4.2 years, ranging between 61 and 78) and a non-elderly control group \( (n=23) \), with a mean age of 44.7±12.3 years, ranging between 21 and 58). Participants (patient or control) suffered from any chronic disease (chronic obstructive pulmonary disease, liver cirrhosis, thyroid dysfunction, rheumatoid arthritis, chronic renal failure and/or psychiatric disorders) were excluded from the study.

Biochemical Analysis

Fast peripheral venous blood samples were obtained at 6 a.m. just after PSG was performed. Blood was centrifuged and serum was immediately separated in aliquots and stored at -80 °C until assayed. Serum cholesterol, low density lipoprotein cholesterol (LDL-c), total cholesterol (TC), triglyceride (TG), serum creatinine (Scr) and fasting plasma glucose (FPG) concentrations were tested by a full-automatic analyzer (AUS400 Olympus, 1st Chemical Ltd., Japan) on that day. Creatinine clearance (CrCl) was calculated by Cockcroft-Gault: 

\[
\text{CrCl} (\text{mL/min}) = \frac{\left( 140 - \text{age} \right) \times \text{weight (kg)}}{0.818 \times \text{Scr (μmol/L)}} 
\]

\( \text{Female: } x<0.85 \). Serum levels of Hey were measured by cyclophorase (Nine-strong Biological Technology Co., LTD, Shanghai). Levels of MDA and GSH in serum were measured according to the manufacturer’s instructions (Jiancheng Bioengineering Institute, Nanjing) and their absorbencies were measured by a spectrophotometer (DU650, Beckman, USA).

Statistical Analysis

Data were expressed as \( \bar{x} ± s \) for continuous variables. Data with Gaussian distribution were processed with analysis of variance and Student-Newman-Keuls test. Correlation was calculated with Spearman’s rank correlation test. \( \chi^2 \) was used to test distributions of smoking history, hypertension and gender among four groups of patients. Multiple linear regression analysis was used to determine variables that affected homocysteine levels. All statistical analysis was carried out using statistical software (SPSS, v13.0). Differences were considered significant at \( P<0.05 \).

RESULTS

As shown in Table 1, there were no significant differences among four groups concerning the gender, body mass index, smoking history, hypertension and biochemical parameters including total cholesterol, triglyceride, LDL-c, fasting plasma glucose, and creatinine clearance.

The serum levels of Hey, MDA and GSH showed significant difference in the four groups (ANOVA, \( P<0.05 \)). Furthermore, serum concentrations of Hey in 32 elderly OSAHS were significantly higher than that in other three groups. Serum Hey levels in the elderly control and non-elderly OSAHS were both higher than that in the non-elderly control.

Similar results were seen with respect to MDA and GSH. Serum concentrations of MDA in the elderly OSAHS were significantly higher than that in the other three groups. The non-elderly control patients
had lower MDA and GSH levels than the elderly control and non-elderly OSAHS. But no significant difference in GSH was recorded between the elderly OSAHS and elderly control groups (Table 2).

### TABLE 1
Baseline Characteristics and Sleep Study Results in the Four Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Elderly OSAHS (n=32)</th>
<th>Elderly Control (n=29)</th>
<th>Non-elderly OSAHS (n=51)</th>
<th>Non-elderly Control (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.8±7.2</td>
<td>69.4±4.2</td>
<td>42.7±8.3</td>
<td>44.7±12.3</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>30/2</td>
<td>27/2</td>
<td>46/5</td>
<td>20/3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.34±2.36</td>
<td>26.85±2.9</td>
<td>28.36±3.51</td>
<td>25.13±3.61</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>53.13%</td>
<td>55.17%</td>
<td>50.98%</td>
<td>52.17%</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>15.63%</td>
<td>13.79%</td>
<td>13.73%</td>
<td>13.04%</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.29±1.35</td>
<td>4.48±1.43</td>
<td>4.03±0.84</td>
<td>4.10±0.54</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.58±0.72</td>
<td>1.93±1.51</td>
<td>2.26±1.36</td>
<td>1.53±0.49</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>5.9±0.8</td>
<td>5.8±0.5</td>
<td>5.78±1.2</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>110.2±3.4</td>
<td>109.2±3.1</td>
<td>108.3±4.5</td>
<td>105.4±2.4</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.27±0.33</td>
<td>2.58±0.55</td>
<td>2.49±0.62</td>
<td>2.36±0.35</td>
</tr>
<tr>
<td>AHI (events/h)</td>
<td>38.67±21.28abc</td>
<td>2.93±1.09</td>
<td>45.51±25.20a</td>
<td>3.42±2.10</td>
</tr>
<tr>
<td>Lowest SaO₂ (%)</td>
<td>77.33±8.09abc</td>
<td>84.68±2.96</td>
<td>71.07±12.68a</td>
<td>89.84±3.33</td>
</tr>
<tr>
<td>Longest Apnea Time (s)</td>
<td>55.33±12.11abc</td>
<td>16.84±6.34</td>
<td>54.99±24.21a</td>
<td>15.65±6.39</td>
</tr>
</tbody>
</table>

Note. AHI: apnea/hypopnea index; BMI: body mass index; FPG: fasting plasma glucose; LDL-C: low density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride; Ccr: Creatinine clearance. aP<0.05 compared to non-elderly control group; bP<0.05 compared to elderly control group; cP>0.05 compared to non-elderly OSAHS group.

### TABLE 2
Hcy, MDA, and GSH Levels in the Four Groups (x±s)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hcy (μmol/L)</th>
<th>MDA (nmol/mL)</th>
<th>GSH (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elderly OSAHS (n=32)</td>
<td>18.70±4.73abc</td>
<td>6.18±1.23abc</td>
<td>8.79±2.68abc</td>
</tr>
<tr>
<td>Elderly Control (n=29)</td>
<td>11.13±3.05a</td>
<td>5.04±0.69a</td>
<td>6.68±3.13a</td>
</tr>
<tr>
<td>Non-elderly OSAHS (n=51)</td>
<td>10.84±2.56a</td>
<td>5.18±1.51a</td>
<td>6.42±2.00a</td>
</tr>
<tr>
<td>Non-elderly Control (n=23)</td>
<td>8.90±1.23</td>
<td>4.12±1.09</td>
<td>4.18±1.19</td>
</tr>
</tbody>
</table>

Note. aP<0.05 compared to non-elderly control group; bP<0.05 compared to elderly control group; cP<0.05 compared to non-elderly OSAHS group.

Pearson’s correlation analysis identified that the serum Hcy level increased with age (r: 0.28, P=0.009). There was also correlation between Hcy and AHI (r: 0.30, P=0.008), MDA (r: 0.23, P=0.031) and GSH (r: 0.26, P=0.032). Multiple linear regression analysis with the changes of Hcy as the dependent variable, and the above correlated parameter as the independent variables, showed that there was significant relationship (α=0.10) between homocysteine levels and the following four variables: age, MDA, GSH, and AHI (Table 3).

### TABLE 3
Multiple Regression Analysis of the Relationship between Hcy Levels and Various Independent Variables in the Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>AHI</td>
<td>2.00</td>
<td>0.07</td>
</tr>
<tr>
<td>MDA</td>
<td>0.50</td>
<td>0.11</td>
</tr>
<tr>
<td>GSH</td>
<td>0.23</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Note. β=standardized regression coefficient.
DISCUSSION

Elderly patients with OSAHS include the cases that fall ill in their middle age and old age. Epidemiological study has indicated[8] that the morbidity of OSAHS in the general population is 2.0%-4.0% and increases with advancing age. OSAHS as one of the important diseases affecting old people’s health and life quality, has a morbidity of 37.5%-62% among them, and the elderly patients with OSAHS have higher morbidity and mortality in cardiovascular disease. Recent studies have already confirmed that OSAHS is one of the risk factors of hypertension, independent of other causes such as age, weight, obese, smoking, and food, heredity, etc. Relative researches have also shown that OSAHS is an independent risk factor of coronary artery disease. Ci et al.[9] did a ten years follow-up investigation among 3 286 elderly individuals aged from 60 to 90 years, and detected 959 SAHS samples, among which the detected rate of coronary artery disease was 41.3%, while the detected rate of coronary artery disease in the non-SAHS control group was 8.3%. It was clear that the elderly people with SAHS were prone to suffer from cardiovascular disease such as coronary artery disease.

Hcy is a serological marker of cardiovascular disease, and is also a kind of amino acids with sulphydryl group, an intermediate product in the demethylation process of methionine cells. In normal organisms, the production and metabolism of Hcy maintains dynamic balance, which is influenced by hereditary and nutritional factors and is related with age[10]. By comparing the elderly control group with the non-elderly control group, our study has concluded that Hcy level in the former was significantly higher than in the latter, which is similar to previous results. It is clear that Hcy is a marker related with decrepitude. Researchers have reached a consensus that hyperhomocysteinemia (HHcy) caused by Hcy unusual metabolism is an independent risk factor of cardiovascular disease such as arteriosclerosis and coronary artery disease. Clinical and epidemiological studies have shown a clear association between slightly elevated blood Hcy levels and early onset of coronary artery disease, peripheral artery and cerebrovascular diseases, stroke and venous thrombosis[11-13]. It is considered to be an important independent predictor of future cardiovascular events and cerebrovascular disease.

However, the relationship between OSAHS and serum Hcy is uncertain. In recent years, several scholars drew contrast conclusions by adopting different methods. Can et al.[14] reported that serum Hcy levels were significantly increased in the OSAHS group compared with that in the control subjects. Jordan et al.[15] observed that the Hcy levels declined by 30% after CPAP therapy. Kokturk et al.[16] showed that OSAHS patients with and without cardiovascular disease had significantly higher Hcy levels, which was independently associated with severity of OSAHS. In contrast, Svatikova et al.[17] observed that the Hcy level in OSAHS patients neither increased nor decreased by using continuous positive airway pressure (CPAP) therapy. Other studies reported similar mean Hcy values in OSAHS and non-OSAHS groups[18-19]. All the above studies did not divide subjects into groups according to the age.

Our study tested serum Hcy concentration in the elderly OSAHS, non-elderly OSAHS, elderly control and non-elderly control groups. We found that the concentration of Hcy in the elderly OSAHS group was significantly higher than that in the elderly control group (P<0.05), the concentration of Hcy in the non-elderly OSAHS group was higher than that in the non-elderly control group (P<0.05). It indicated that the concentration of Hcy increased in the patients with OSAHS, and much higher in the elderly patients with OSAHS. All the subjects in our study did not have any history of coronary artery disease and the distribution of hypertension and other factors in each group was balanced. The results showed that OSAHS was related with elevated serum Hcy level after these confounding factors were controlled.

The episodes of hypoxia/re-oxygenation during apnea-hypopneic periods in OSAHS patients regarded as a kind of oxidative stress injury to vascular tissue seem responsible for the pathological condition leading to target organ damage, which is similar to the ischemia-reperfusion injury observed in other pathological conditions[20]. Normally, the free radicals are continuously produced and cleared, and oxidation and antioxidation barrier maintain a balanced state. As one of the oxidative defense systems in vivo, the level of GSH is an important factor to assess antioxidating ability. Using MDA, the main product of peroxidation in vivo, as the biomarker of the severity of lipid peroxidation injury. Christou et al.[21] observed that an index of oxidative stress in obstructive sleep apnea patients was higher than in controls and paralleled with the severity of OSAHS. Recent studies[22-23] have shown that oxidative stress existed in patients with obstructive sleep apnea. Meanwhile an alteration of antioxidant defense in patients with OSAS was detected[24-25].

The findings of the present study revealed that serum MDA concentration in elderly OSAHS and non-elderly OSAHS patients was higher than in their respective controls (P<0.01), and that GSH level in non-elderly OSAHS patients was higher than that in
non-elderly control ($P<0.01$). Also, MDA and GSH were both higher in elderly control than in non-elderly control ($P<0.01$). Our findings further confirmed that the redox reactions in vivo was enhanced with age, and the insenscence of the normal body was accompanied by the increasing of oxidative stress level. And oxidative stress also played a role in the occurrence and development of OSAHS. In addition, elevated MDA and GSH levels also increased the risk of HHcy occurrence and oxidative stress was independently associated with serum Hcy level. Review of studies on the mechanisms of cyclical and intermittent hypoxia in OSAHS inducing cardiovascular disease, found that there were some reports about the increasing of free radicals and Hcy level associated with OSAHS,[26], although no authors reported how the enhanced oxidative stress in vivo led to an elevated Hcy level. We can speculate the possible mechanism behind its occurrence according to the research findings and the biochemical pathway.

The following deduction about biochemical pathway might explain why the above conclusion is drawn. In OSAHS patients, oxygen free radicals are generated in an increased number, and in response, GSH as one of antioxidant defenses is increased to resist the injury derived from oxidative stress. Meanwhile, GSH is one of the degradation products of Hcy, its increasing value in vivo is bound to inhibit the continuous degradation of Hcy. Hence the accumulation of Hcy in vivo result in HHcy. The lipid peroxidation and oxidative stress in elderly OSAHS patients are further increased to reach a level which is higher than in younger OSAHS patients. Thereby Hcy level is also further increased in elderly OSAHS patients. Therefore, this mechanism further supports the findings of the present study and it also provides a new clue to explain the high prevalence of cardiovascular disease and clinical sequelae in elderly OSAHS patients.

In conclusion, Hcy levels and oxidative stress index are significantly increased in the elderly OSAHS patients, and both of them present a positive correlation. As to its mechanism, it could be presumed that oxidative stress causes elevated Hcy in elderly OSAHS patients, and that Hcy can theoretically act as a predictor for the development of cardiovascular and cerebrovascular diseases in them and antioxidation treatment may be provided for OSAHS patients to slow down their complications.

REFERENCES


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