Association between Serum Homocysteine and Oxidative Stress in Elderly Patients with Obstructive Sleep Apnea/hypopnea Syndrome[1]

LING WANG, JIE LI[2], YAN XIE, AND XUE-GUANG ZHANG

Laboratory of Clinical Immunology, the First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China

Objective   Elderly patients with obstructive sleep apnea/hypopnea syndrome (OSAHS) has 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

Soochow University and underwent an overnight sleep study from November 2005 to June 2008. Before the examination, information on their general health state, such as age, gender, body height, and weight as well as smoking history and hypertension based on the final clinical diagnosis was collected.

A Respironics Alice 4 polysomnographer (Respironics Co, USA) was used to measure and record overnight polysomnogram (PSG) parameters such as electro-encephalogram, electrocardiogram (ECG), electrooculogram, chin and bilateral anterior tibials electromyogram, chest and abdominal movements by strain gauges and pulse oxyhemoglubin saturation (SpO_2). All tracings were scored manually according to standard criteria. Apnea was diagnosed when a cessation of airflow for more than 10 seconds was detected, while hypopnea was identified when more than 50% reduction in airflow and 3% reduction of SpO_2 were observed in one patient. Sleep apnea/hypopnea syndrome was evaluated with international diagnostic criteria[7]. An event was defined as obstructive one when chest and abdominal respiratory movement was observed during apnea. The apnea/hypopnea index (AHI) is defined by the combined number of apnea and hypopnea events per hour of sleep, with the minimal criteria for diagnosis of OSAHS as AHI≥5. An AHI that was greater than 5/h was diagnosed as OSAHS. Subject whose AHI was equal to or less than 5/h would be placed in the control group.

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. Subjects who took drugs affecting serum Hcy levels such as vitamin B6, vitamin B12, and folic acid were not included either. The study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. All participants signed their consent forms after being fully informed of the purpose and characteristics of 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 ℃ 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(CCr) was calculated by Cockcroft-Gault: CCr(mL/min)=[(140-age)×weight(kg)]/[0.818×SCr (μmol/L)](Female: ×0.85). Serum levels of Hcy 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 $\bar{x} \pm s$ 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. χ^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 Hcy, MDA and GSH showed significant difference in the four groups (ANOVA, $P<0.05$). Furthermore, serum concentrations of Hcy in 32 elderly OSAHS were significantly higher than that in other three groups. Serum Hcy 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

Variables	Elderly OSAHS (n=32)	Elderly Control (n=29)	Non-elderly OSAHS (n=51)	Non-elderly Control (n=23)
Age (years)	65.8±7.2	69.4±4.2	42.7±8.3	44.7±12.3
Sex (males/females)	30/2	27/2	46/5	20/3
BMI (kg/m^2)	23.34±2.36	26.85±2.9	28.36±3.51	25.13±3.61
Smokers (%)	53.13%	55.17%	50.98%	52.17%
Hypertension (%)	15.63%	13.79%	13.73%	13.04%
TC (mmol/L)	4.29±1.35	4.48±1.43	4.03±0.84	4.10±0.54
TG (mmol/L)	1.58±0.72	1.93±1.51	2.26±1.36	1.53±0.49
FBG (mmol/L)	5.9±0.8	5.8±0.5	5.78±1.2	5.4±0.6
Ccr (mL/min)	110.2±3.4	109.2±3.1	108.3±4.5	105.4±2.4
LDL-C (mmol/L)	2.27±0.33	2.58±0.55	2.49±0.62	2.36±0.35
AHI (events/h)	38.67±21.28[a,b,c]	2.93±1.09	45.51±25.20[a]	3.42±2.10
Lowest SaO$_2$ (%)	77.33±8.09[a,b,c]	84.68±2.96	71.07±12.68[a]	89.84±3.33
Longest Apnea Time (s)	55.33±12.11[a,b,c]	16.84±6.34	54.99±24.21[a]	15.65±6.39

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. [a]$P<0.05$ compared to non-elderly control group; [b]$P<0.05$ compared to elderly control group; [c]$P>0.05$ compared to non-elderly OSAHS group.

TABLE 2

Hcy, MDA, and GSH Levels in the Four Groups ($\bar{x} \pm s$)

	Hcy (μmol/L)	MDA (nmol/mL)	GSH (mg/L)
Elderly OSAHS (n=32)	18.70±4.73[a,b,c]	6.18±1.23[a,b,c]	8.79±2.68[a,c]
Elderly Control (n=29)	11.13±3.05[a]	5.04±0.69[a]	6.68±3.13[a]
Non-elderly OSAHS (n=51)	10.84±2.56[a]	5.18±1.51[a]	6.42±2.00[a]
Non-elderly Control (n=23)	8.90±1.23	4.12±1.09	4.18±1.19

Note. [a]$P<0.05$ compared to non-elderly control group; [b]$P<0.05$ compared to elderly control group; [c]$P<0.05$ 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

Variables	β	P Value
Age	0.07	0.01
AHI	2.00	0.07
MDA	0.50	0.11
GSH	0.23	0.09

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 sulfhydryl 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 artherosclerosis 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 insenescence 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

1. Shahar E, Whitney C W, Redline S, *et al.* (2001). Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med* **163**, 19-25.
2. Lopez-Jimenez F, Sert Kuniyoshi F H, Gami A, *et al.* (2003). Obstructive sleep apnea: implications for cardiac and vascular disease. *JAMA* **290**, 1906-1914.
3. Ryan S, Taylor C T, McNicholas W T (2006). Predictors of elevated nuclear factor-kappaB-dependent genes in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* **174**(7), 824-830.
4. Willems F F, Aengevaeren W R, Boers G H, *et al.* (2002). Coronary endothelial function in hyperhomocysteinemia: improvement after treatment with folic acid and cobalamin in patients with coronary artery disease. *J Am Coll Cardiol* **40**(4), 766-772.
5. Lavie L, Perelman A, Lavie P (2001). Plasma homocysteine levels in obstructive sleep apnea: association with cardiovascular morbidity. *Chest* **120**, 900-908.
6. Kokturk O, Ulukavak Ciftci T, Mollarecep E, *et al.* (2006). Serum homocysteine levels and cardiovascular morbidity in obstructive sleep apnea syndrome. *Respir Med* **100**, 536-541.
7. American Academy of Sleep Medicine Task Force (1999). Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. *Sleep* **22**, 667-689.
8. Goldman S E, Hall M, Boudreau R, *et a1*. (2008). Association between nighttime sleep and napping in older adults. *Sleep* **31**, 733-740.
9. CI S P, XU S X, GUO J W, *et al.* (2003). Research of relationship between sleep apnea syndrome and multiple organ disorders. *Practical Geraeology* **17**(6), 304.
10. Varela-Moreiras G, Escudero J M, Alonso-Aperte E (2007). Homocysteine related vitamins and lifestyles in the elderly people: The SENECA study. *Nutr Hosp* **22**(3), 363-370.
11. Nygård O, Nordrehaug J E, Refsum H, *et al.* (2005).Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* **337**, 230-236.
12. Sacco R L, Anand K, Lee H S, *et al.* (2004). Homocysteine and the risk of ischemic stroke in a triethnic cohort: the Northern Manhattan Study. *Stroke* **35**, 2263-2269.
13. Wuillemin W A, solenthaler M (1999). Hyperhomocysteinemia: a risk factor for arterial and venous thrombosis. *Vasa* **28**, 151-155.
14. Can M, Açikgöz S, Mungan G, *et al.* (2006). Serum cardiovascular risk factors in obstructive sleep apnea. *Chest* **129**(2), 233-237.
15. Jordan W, Berger C, Cohrs S, *et al.* (2004). CAPA-therapy effectively lowers serum homocysteine in obstructive sleep apnea syndrome. *J Neural Transm* **111**, 683-689.
16. Kokturk O, Ciftci TU, Mollarecep E, *et al.* (2006) .Serum homocysteine levels and cardiovascular morbidity in obstructive sleep apnea syndrome. *Respir Med* **100**, 536-541.
17. Svatikova A, Wolk R, Magera M J, *et al.* (2004). Plasma homocysteine in obstructive sleep apnea. *Eur Heart J* **25**, 1325-1329.
18. Cheng C H, Huang M C, Liu S C (2006). Traditional cardiovascular risk factors but not homocysteine are associated with obstructive sleep apnea. *Nutr Res* **26**, 59-64.
19. Ryan S, Nolan G M, Hannigan E, *et al.* (2007). Cardiovascular risk markers in obstructive sleep apnoea syndrome and correlation with obesity. *Thorax* **62**(6), 509-514.
20. Lavie L (2003). Obstructive sleep apnoea syndrome-an oxidative stress disorder. *Sleep Med Rev* **7**, 35-51.
21. Christou K, Markoulis N, Moulas A N (2003). Reactive oxygen metabolites(ROMs)as an index of oxidative stress in obstructive sleep apnea patients. *Sleep Breath* **7**,105-110.
22. Suzuki Y J, Jain V, Park A M, *et al.* (2006). Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. *Free Radic Biol Med* **40**, 1683-1692.
23. Yamauchi M, Kimura H (2008). Oxidative Stress in Obstructive

Sleep Apnea: Putative pathways to the Cardiovascular Complications. *Antioxid Redox Signal* **10**, 755-768.

24. Christou K, Moulas A N, Pastaka C, *et al*. (2003). Antioxidant capacity in obstructive sleep apnea patients. *Sleep Med* **4**, 225-228.

25. Barceló A, Barbé F, de la Peña M, *et al*. (2006). Antioxidant status in patients with sleep apnoea and impact of continuous positive airway pressure treatment. *Eur Respir J* **27**, 756-760.

26. Pack A I (2006). Advances in sleep-disordered breathing. *Am J Respir Crit Care Med* **173**(1), 7-15.