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Serum paraoxonase, arylesterase activities and oxidative status in patients with insomnia

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Abstract. – AIM: The aim of this study was to investigate serum paraoxonase (PON) activity, arylesterase (ARE) activity, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) levels in patients with insomnia and to determine whether there was a relationship between oxidative stress and insomnia.

PATIENTS AND METHODS: A total of 29 insomniacs and 25 healthy controls were recruited in this study. Serum PON and ARE activities, TAS and TOS level were determined, and OSI were calculated.

RESULTS: Patients with insomnia had lower PON and ARE activities as compared to healthy controls (PON: 82.0±30.0 U/L vs. 193.5±58.4 U/L, $p < 0.001$; ARE: 143.0±26.7 U/L vs. 175.0±27.1 U/L, $p < 0.001$; respectively). Serum TAS was lower, while TOS and OSI were higher in the insomnia group than in the control group (TAS: 1.13±0.29 mmol Trolox equivalent/L vs. 1.70±0.35 mmol Trolox equivalent /L, $p < 0.001$; TOS: 18.68±5.03 µmol H_2O_2 equivalent/L vs. 10.92±2.21 µmol H_2O_2 equivalent/L, $p < 0.001$; OSI: 1.76±0.74 vs. 0.68±0.23, $p < 0.001$; respectively).

CONCLUSIONS: Patients with insomnia have increased systemic oxidative stress and reduced levels of serum antioxidant enzymes. Oxidative stress appears to be an underlying condition associated with insomnia.

Key Words:
Sleep disorder, Oxidative stress, Biomarker, Serum.

Introduction

Insomnia, by far the most commonly encountered sleep disturbance in medical practice, is characterized by sleep disorder, disrupted sleep, frequent episodes of wakefulness, early wakening, and the reduced quality of life[1]. The prevalence of insomnia in adults in Western countries reportedly ranges from 10% to 30%[2-4], and the similar result was also reported for Asians[5]. To date, the etiology of insomnia remains unknown, but several models have been put forth in the biological, behavioral, and psychological domains to explain the development and maintenance. Epidemiology surveys also indicated that insomnia is associated with psychological distress, impaired daily functioning, and an increased risk of medical and psychiatric morbidity and mortality[6].

Oxidative stress depicts a state of imbalance between the generation of reactive oxygen species (ROS) and antioxidant defenses, either induced by an elevation in ROS production or by a reduction in defense mechanisms resulting in cell and tissue damage[7]. It is well known that oxidative stress is one of the factors that contribute to an increase in the speed of the cell cycle and consequent premature cell death, leading to many degenerative disorders, as well as psychiatric disorders. Moreover, recent research suggests that ROS is an important factor in the pathophysiology of schizophrenia and that the oxidative stress caused by ROS is related to disease severity[8]. Oxidative stress is also implicated in the progression of mood disorders, obsessive-compulsive disorder, and panic disorder[9,10]. Gulec et al[11] showed that the patients with primary insomnia had significantly lower glutathione peroxidase (GSH-Px) activity and higher malonil dialdehyde (MDA) levels compared with the controls. Hachul de Campos et al[12] reported that women complained of insomnia displayed increased plasma thiobarbituric acid reactive substances (TBARS) levels. Thus, both sleep disturbance and oxidative stress have been related to some kinds of psychopathologies.

Paraoxonase 1 (PON1), an antioxidant bioscavenger, is responsible for hydrolyzing lipid peroxides, and also plays a major role in the antioxidant system. PON1 has three known enzymatic molecules, including PON, arylesterase (ARE), and dyazoxonase. Several studies have revealed that increased oxidative stress can lead to reduced

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PON1 activity[13-15]. Furthermore, PON1 activity is decreased in subjects who have had oxidative stress related to diseases, such as coronary artery disease, hypercholesterolemia, type 2 diabetes mellitus, iron-deficiency anemia, and cancer[16-19].

To the best of our knowledge, PON and ARE activities have not been studied as yet in insomnia. The purpose of the present study, therefore, was to determine whether serum PON, ARE, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) levels were altered in insomnia patients as compared to age and gender-matched healthy control subjects; and whether there was a relationship between oxidative stress and insomnia.

Patients and Methods

Study Population

A total of 29 insomniacs were recruited in this study. Patients were diagnosed as having persistent primary insomnia based on DSM-IV-R criteria. Included criteria for insomniacs included a history of difficulty falling asleep (taking 45 or more minutes to fall asleep) and/or staying asleep (obtaining fewer than 6.5 hours of total sleep time) at least 4 nights a week for at least 6 months. In addition, insomniacs had to demonstrate a sleep efficiency of less than 80% during a screening night in the sleep laboratory. Insomniacs were evaluated by a psychiatrist. The control group consisted of 25 age- and sex-matched healthy volunteers, who reported normal sleep for at least 1 year. They were screened with the Pittsburgh Sleep Quality Index (PSQI) for sleep quality.

Exclusion criteria for all subjects included: endocrine abnormalities, respiratory system diseases, diabetes mellitus, hypertension (SBP ≥ 140 and DBP ≥ 90 mm Hg or use of antihypertensive drugs) and other cardiovascular diseases likely to be the cause of the sleep problem; current diagnosis of major depression, psychosis , dysthymia, or anxiety disorders; neurological degenerative diseases; other sleep disorders, including sleep apnea, restless legs syndrome, bruxism, and narcolepsy; body mass index (BMI) ≥ 32 kg/m^2; smoking; alcohol or drug abuse; excessive use of caffeine (> 3 cups per day); unusual sleep schedule (bedtime after midnight and wake time after 09:00) and/or work shift; use of medications affecting the central and/or the autonomic nervous system; history or current treatment with psychotropic drugs, or any antioxidant agents, such as vitamin E or C, et al.

After thorough explanation of the experiment, all subjects gave consent to participate. This work was approved by the Medical Ethics Committee of China Medical University in Shenyang.

Blood Collection

Blood samples in all subjects were collected after an overnight fasting period, and were immediately separated from the cells by centrifugation at 2500 × g for 15 min, stored at −80°C till use.

Measurements of PON and ARE Activites

PON activity measurements were performed both in the absence and presence of NaCl (salt-stimulated activity). The rate of paraoxon hydrolysis (diethylp-nitrophenylphosphate) was measured by monitoring the increase of absorption at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorption coefficient at pH 8.5, which was 18.290[20] M^{-1} cm^{-1}. PON activity was expressed as U/L.

Phenylacetate was used as a substrate to measure the ARE activity. Enzymatic activity was calculated from the molar absorption coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of ARE activity was defined as 1 mol phenol generated per minute under the above conditions and expressed as U/L[21].

Measurement of Total Antioxidant Status (TAS)

Serum TAS levels was measured using a novel automated measurement according to Erel's method[22], involving the production of hydroxyl radical, the most potent of biological radicals. The antioxidant effect of the sample against the potent free radical reactions initiated by the hydroxyl radicals produced is measured. The results are expressed in mmol Trolox equivalents/L.

Measurement of the Total Oxidant Status (TOS)

Serum TOS measurement was performed using the Erel's method[22]. In this method, oxidants that are present in the serum oxidize the ferrous ion-o-dianisidine complex to ferric ion, and glycerol molecules that are abundantly present in the reaction medium enhance the oxidation reaction. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxi-

Table I. Demographic characteristics of the two groups in this study.

	Insomnia group (n=29)	Control group (n=25)	*p*-value
Age (years)	39.2 ± 2.3	38.6 ± 2.9	0.40
Gender (male/female)	10/19	9/16	0.91
Body mass index (kg/m^2)	22.3 ± 2.3	21.6 ± 2.0	0.23
Marital status (married/single)	25/4	22/3	0.84
Education level (years)	14.3 ± 2.1	13.9 ± 1.9	0.45

dant molecules present in the serum. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of mol H_2O_2 equivalent/L.

Calculation of Oxidative Stress Index (OSI)

The OSI is the ratio of the TOS to the TAS. The formula is described as below: OSI= TOS (μmol H_2O_2 equivalent/L / [TAS (mmol Trolox equivalent /L) × 10].

Statistical Analysis

All statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation (SD). Nonparametric continuous variables were compared by the Mann-Whitney U-test. A χ^2 test was used to compare gender distributions and marital status within the controls and the insomnia group. A $p < 0.05$ was considered significant.

Results

Clinical characteristics of all subjects were shown in Table I. There were no significant difference between insomnia patients and healthy controls in terms of gender, age, marital status, BMI and education level ($p > 0.05$).

Figure 1 indicated the serum PON and ARE activities in the examined groups of patients. Patients with insomnia had lower PON and ARE activities as compared to healthy controls (PON: 82.0±30.0 U/L vs. 193.5±58.4 U/L, $p > 0.001$; ARE: 143.0±26.7 U/L vs. 175.0±27.1 U/L, $p > 0.001$; respectively). The results of the serum markers of oxidative stress (TAS, TOS, and OSI) between insomnia group and control group are shown in Figure 2. Serum TAS was lower, while TOS and OSI were higher in the insomnia group than in the control group (TAS: 1.13±0.29 mmol Trolox equivalent/L vs. 1.70±0.35 mmol Trolox equivalent/L, $p < 0.001$; TOS: 18.68±5.03 μmol H_2O_2 equivalent/L vs. 10.92±2.21 μmol H_2O_2 equivalent/L, $p < 0.001$; OSI: 1.76±0.74 vs. 0.68±0.23, $p < 0.001$; respectively).

Discussion

In this study, for the first time in the literature, we investigated the effects of insomnia on certain oxidative stress biomarkers. Our data showed that serum PON and ARE activities were significantly higher in patients with insomnia as compared with healthy controls. Moreover, increased oxidative stress was found in insomnia patients, demonstrated by increased TOS and OSI, and decreased TAS in serum.

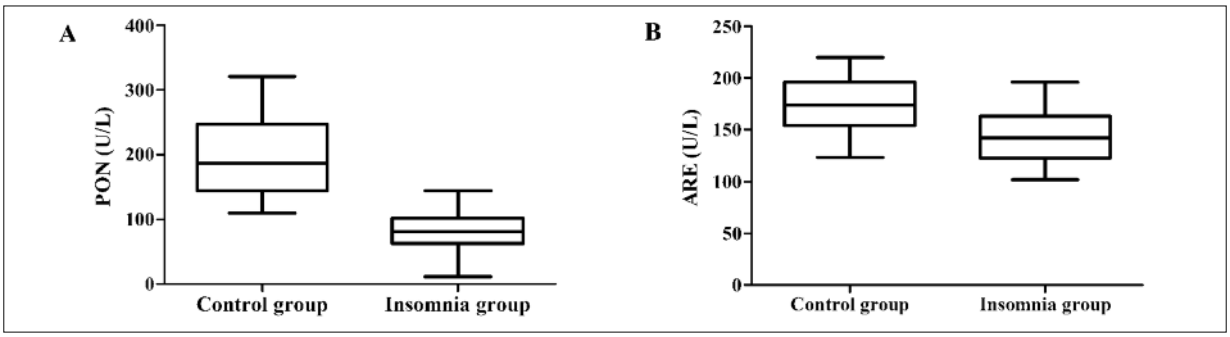

Figure 1. Serum PON and ARE activities between insomnia group and control group. ***A,*** PON activity. ***B,*** ARE activity. PON, paraoxonase; ARE, arylesterase.

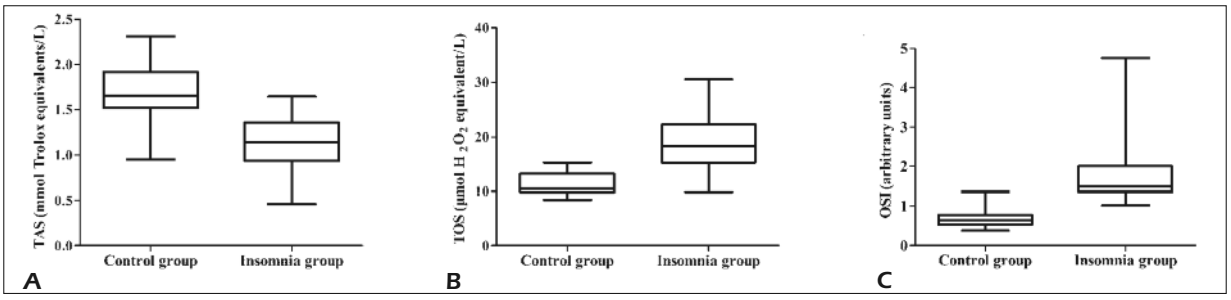

Figure 2. Serum TAS, TOS and OSI between insomnia group and control group. ***A,*** TAS level. ***B,*** TOS level. ***C,*** OSI level. TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

To date, oxidative stress has become a major topic in all areas of medical knowledge. Many studies demonstrated that oxidative stress has been implicated in many diseases, such as cardiovascular disease[23], diabetes mellitus[24], tumorigenesis[25], neurologic and psychiatric diseases[26], and obstructive sleep apnea syndrome[27], etc. The pathogenic role of oxidative stress in insomnia is one of the most challenging hypotheses for sleep researchers. Alzoubi KH et al[28] reported that sleep deprivation and Western diet increases oxidative stress in the hippocampus in animal model, and sleep deprivation alone induces oxidative stress and impairs learning and memory processes[29]. Ramanathan and Siegel[30] demonstrated that short-term insomnia under hypoxia may decrease lipid oxidation, and increase total glutathione, thus serving as an adaptive response to prevent oxidative stress. Apart from that, sleep disorders are associated with an increased rate of various metabolic disturbances, which may be related to oxidative stress and consequent lipid peroxidation. Since liver is responsible for metabolic regulation, PON1, as an antioxidant enzyme produced by liver, may be related to sleep disorder. Our data showed that serum PON and ARE activities decreased in patients with insomnia as compared to healthy controls. The mechanism of the observed decrease in serum PON and ARE activities in insomnia patients remains unclear. This decrease could be related to enhanced lipid peroxidation, since an increased number of lipid and protein oxidation products and decreased number of antioxidant enzymes has been reported to affect the expression and activities of PON1[31,32]. Moreover, in recent studies, reduced serum PON1 activity has been reported to be associated with oxidative stress and inflammation condition[33,34].

Because the effects of antioxidants can be additive and measuring individual antioxidants separately is time consuming and labor intensive, a measurement of the combined activities of all antioxidants or the TAS is often used to estimate the overall antioxidative status[35]. Likewise, TOS is measured to determine a patient's overall oxidation state[22]. Furthermore, the oxidative stress index (OSI), which is calculated as the ratio of TOS to TAS, may be a more accurate index of oxidative stress in the body because it is a comprehensive measurement of TAS and TOS. The determination of TAS, TOS and OSI in serum represents the body oxidative status better than does measurement of the single serum antioxidant. The present data demonstrate that serum TAS decreased, and serum TOS and OSI increased in insomnia patients. The decreased TAS suggests that oxidative stress is severe because it consumes antioxidants and, therefore, decreases their levels. These data imply that increased TOS and OSI levels do not simply play a role in etiopathogenesis, but rather provide information that oxidative stress and the imbalance between oxidants and antioxidants is higher in the patients with insomnia than in the healthy control.

Similar with Reimund's hypothesis, sleep loss represents an oxidative challenge and sleep may have a protective role against oxidative damage[36]. We designed this study to evaluate the effect of insomnia itself on oxidative stress. To eliminate some potential influencing factors, we excluded some case related to oxidative stress disorders, and we matched the patients with healthy controls for age, gender, marital status, BMI, and educational level. Thus, this method mainly removed possible limitations related to the design of the present study.

Conclusions

In the light of the findings of this study, we concluded that oxidative stress is increased, while serum PON and ARE activities are de-

creased in patients with insomnia. In addition, these results indicate that reduced PON and ARE activities are associated with an oxidant–antioxidant imbalance that may contribute to insomnia in patient. Further studies with larger numbers of patients are needed to confirm the mechanisms underlying the association of low PON and ARE activities and the development of insomnia, and they seem to have implications for future sleep research.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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