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Oxidative stress in patients with primary insomnia

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ABSTRACT

Objective: Many physiological and pathological processes, such as infections, environmental toxins, and ionizing radiation increase bodily concentrations of oxidizing substances, known as free radicals, which lead to neurodegenerative disorders. Sleep is one of the most important factors contributing to health; however, insomnia is among the most prevalent health complaints.
Methods: In this study, for the first time in the literature, we investigated the effects of primary insomnia on certain oxidative stress biomarkers. For this purpose, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and myeloperoxidase (MPO) activities and levels of reduced glutathione (GSH) and malondialdehyde (MDA) were measured in 30 patients with primary insomnia and 30 healthy volunteers
Results: Our results show that the patients with primary insomnia had significantly lower GSH-Px activity and higher MDA levels compared with the controls.
Conclusion: These results may indicate the important role of sleep in attenuating oxidative stress.

1. Introduction

Sleep is one of the most important factors contributing to health; however, insomnia is among the most prevalent health complaints in both the general population (LeBlanc et al., 2009; Morin et al., 2006) and some psychiatric samples (Gulec et al., 2011). Approximately one third of the general population suffers from at least one insomnia symptom (Bixler et al., 1979; Klink and Quan, 1987; Klink et al., 1992; Mallon et al., 2000; Quera-Salva et al., 1991; Welstein et al., 1983), and about 6% (Ohayon, 1997, 2001; Ohayon and Sagales, 2010; Ohayon and Smirne, 2002; Ohayon et al., 1997) meet the diagnostic criteria for insomnia of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 4th edition (APA, DSM-IV-TR, 2000). These studies on the epidemiology of insomnia were conducted in North America and various European countries. DSM-IV qualifies primary insomnia here as a complaint of difficulty initiating or maintaining sleep or of poor quality sleep, lasting for a period of at least a month. The diagnosis of primary insomnia requires exclusion of the direct physiological effects of a substance or general medical condition. Moreover, primary insomnia should not occur during the course of a mental disorder or other sleep disorder.

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 (Herken et al., 2001; Savas et al., 2002; Tsaluchidu et al., 2008). Many physiological and pathological processes, such as aging, infections, environmental toxins, emotional or psychological stress, ionizing radiation, cigarette smoke, and alcohol increase the bodily concentration of oxidizing substances, known as free radicals (Ozkol et al., 2011b; Tsaluchidu et al., 2008). Free radicals that originate from molecular oxygen are generally named reactive oxygen species (ROS). These free radicals exert physiological and pathological effects by many different mechanisms, such as activation of phagocytes and the general immune system, lipid peroxidation, the electron transport system in mitochondria, ischemia, and trauma (Halliwell and Gutteridge, 2000). Oxidative stress occurs whenever there is an imbalance between oxidant production and antioxidant defenses, either because the production is increased or because the defenses are decreased, or both (Gopalakrishnan et al., 2004; Ozkol et al., 2011a). Primary antioxidant defense is provided by enzymes that can prevent uncontrolled formation of free radicals, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (Halliwell and Gutteridge, 2000). Because of their short half-lives, free radicals can be evaluated indirectly by measuring some antioxidant enzyme activities, such as SOD, CAT, or GSH-Px, byproducts of lipid

Abbreviations: CAT, Catalase; GSH-Px, Glutathione peroxidase; H_2O_2, Hydrogen peroxide; OH, Hydroxyl radical; MDA, Malondialdehyde; MPO, Myeloperoxidase; PSQI, Pittsburgh Sleep Quality Index; ROS, Reactive oxygen species; GSH, Reduced glutathione; SOD, Superoxide dismutase; O^{2-}, Superoxide radical.

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peroxidation, such as malondialdehyde (MDA), or some transition metal levels, such as copper, zinc, and iron (Leff, 1994).

Both sleep alterations and oxidative stress have been related to some kinds of psychopathologies. Sleep problems here are likely to play an important role in genesis and maintenance of the many psychiatric disorders, especially mood disorders (Gulec et al., 2011). Also, oxidative stress was considered to be associated with schizophrenia, mood disorders, obsessive–compulsive disorder, and panic disorder (Atmaca et al., 2008; Herken et al., 2006). These findings lead to speculation that the central nervous system is vulnerable to oxidative stress- and sleep disturbance-mediated injuries which might underlie the majority of mental disorders.

It has been proposed that cerebral free radicals accumulate during wakefulness and are removed during sleep (Reimund, 1994). Moreover, it has been claimed that removal of excess free radicals during sleep is accomplished by the decreased rate of formation of free radicals and increased efficiency of endogenous antioxidant mechanisms. However, the association between oxidative stress and sleep disorders still remains unclear. Studies of oxidative stress in clinical sleep research primarily have focused on obstructive sleep apnea syndrome (OSAS), a disorder marked by recurrent nocturnal obstruction of the upper airway, which leads to hypoxia and re-oxygenation (Ozturk et al., 2003; Schulz et al., 2000). It is well documented that oxidative stress in sleep apnea is produced by recurrent episodes of ischemia-reperfusion injury (Everson et al., 2005; Katsoulis et al., 2011; Lavie, 2003). To the best of our knowledge, there has not yet been a study evaluating the association between primary insomnia and oxidative stress. Therefore, for the first time in the literature, we aimed to investigate whether there is a correlation between primary insomnia and oxidative stress.

2. Methods

2.1. Patients and study design

The present study was conducted over a 12-month span, from March 2010 until March 2011, in the city of Van, Turkey. The participants of the study were (i) 30 primary insomnia patients and (ii) 30 healthy controls.

The primary insomnia diagnoses of the participants were made according to DSM-IV criteria via structured clinical interviews at the Psychiatric Outpatient Clinic of Yuzuncu Yil University Hospital. Primary insomniac participants were interviewed by an experienced psychiatrist, using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID I), a standardized clinical interview form, in order to evaluate whether they met any axis-I psychiatric disorder. The inclusion criteria were as follows: (1) predominant complaint of difficulty initiating or maintaining sleep, and/or non-restorative sleep for at least 1 month; (2) Clinically significant distress or impairment in social, occupational, or other important areas of functioning caused by the sleep disturbance or associated daytime fatigue. Excluded were those who (1) met the criteria for an axis-I psychiatric disorder other than primary insomnia; (2) had a disease or chronic medical condition likely to be the cause of the sleep problem (e.g., endocrine abnormalities, hypertension, neurological problems, cardiovascular and respiratory system diseases, or chronic pain) or a lifestyle likely to interfere with sleep patterns (e.g., shift work, jet lag); or (3) had used any medications that affect the sleep-wake rhythm for at least 8 weeks prior. All primary insomniac participants were evaluated with the Pittsburgh Sleep Quality Index (PSQI), and those who reported symptoms of sleep disorders other than insomnia on the PSQI, including “have bad dreams, have pain, cough or snore loudly, cannot breathe comfortably, legs twitching or jerking, episodes of disorientation or confusion” were also excluded. The PSQI contains 19 items that are designed to measure different aspects of sleep quality and sleep disturbances during a 1-month period. A cut-off score of 5 was found to correctly identify 88.5% of patients with sleep disturbances. The sum of scores yields one global score, which has a range of 0–21, with higher scores indicating worse sleep quality (Agargun et al., 1996; Buysse et al., 1989).

Healthy controls who reported normal sleep for at least 1 year were recruited from volunteers by local announcements about the study. They were screened with the PSQI for sleep quality. Healthy participants with a PSQI total score over 5 and/or who reported symptoms of insomnia or other sleep disorders were excluded. The control group consisted of 30 healthy participants who had no history of psychiatric, neurological, or serious medical disorders, and they were matched to the primary insomniac participants with regard to age and gender.

Consistent with relevant oxidative stress studies (Atmaca et al., 2008; Herken et al., 2006; Virit et al., 2009), other exclusion criteria were also determined for the primary insomniac participants and the healthy controls, as follows: alcohol and substance abuse or dependence (including tobacco); presence of a severe physical disorder; history or current treatment with glucocorticoids, anticonvulsants, oral contraceptives, psychotropic drugs, or any antioxidant agents, such as vitamin E or C, xanthine oxidase inhibitors (allopurinol, folic acid), and non-steroidal anti-inflammatory drugs; presence of sleep apnea complaints; and presence of obesity, pregnancy, or lactating.

Screening visits were performed to evaluate participants' suitability. They were comprised of physical, psychiatric, and neurological examinations, routine laboratory tests (complete blood count, liver enzymes, blood urea nitrogen, serum creatinine, electrolytes, and urinalysis), and a urine drug screen. Participants who had normal laboratory results and did not meet any of the exclusion criteria were admitted to the study. Whether all participants met the inclusion criteria was confirmed by two experienced internal medicine specialists and two psychiatrists.

The study protocol was approved by the Ethics Committee of the Yuzuncu Yil University Medical Faculty, and written informed consent was obtained from all participants after they received a complete description of the study protocol. The subjects were not paid for their participation.

2.2. Blood sampling

Blood samples were collected in order to determine myeloperoxidase (MPO), SOD, and GSH-Px activities, as well as reduced concentrations of glutathione (GSH) and MDA. Between 8:00 and 9:00 a.m., before breakfast, 2-ml samples of venous blood were taken from each participant and placed into tubes with Ethylenediaminetetraacetic acid. The samples were kept in a cool box, at +4 °C, until they were transferred to the laboratory of the Medical Biology Department. The biochemical analyses were performed under the same conditions after the preparation of all blood samples. Whole blood samples obtained from each subject were hemolyzed with deionized water. After centrifugation (4000×*g* for 10 min at +4 °C), the upper supernatant fluid was separated, and oxidative stress biomarkers (MPO, MDA, GSH-Px, SOD, and GSH) were measured at this stage, consistent with Selvi et al. (2011). Whole blood GSH was determined very shortly after attaining the samples compared to the other oxidative stress markers, because its concentration diminishes as time passes.

2.3. Reduced glutathione (GSH) determination

GSH is a tripeptide that includes glutamate, cysteine, and glycine. It is a necessary compound for maintaining cell integrity, due to its reducing properties and role in cell metabolism. GSH depletion may elevate the risk of oxidative stress (Regoli and Principato, 1995). The GSH content of whole blood was determined at 412 nm, using the method of Sedlak and Lindsay (1968).