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Ratio of serum matrix metalloproteinases and their inhibitors in chronic insomnia patients

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Abstract

Aim: Sleep is an essential part of a healthy life. Many people experience insomnia due to their living conditions. Matrix metalloproteinases (MMPs) play an essential role in remodeling the microenvironment. Tissue inhibitors of MMPs (TIMPs) maintain a balance with MMPs. Disruption of the balance causes various pathologies. The aim of the study is to elucidate the effects of sleep disturbance on matrix metalloproteinases and inhibitors by comparing MMP-2/TIMP-2 and MMP-9/TIMP-1 ratio in the serum of patients with chronic insomnia to healthy controls.

Materials and Methods: This study included 40 adult males diagnosed with chronic insomnia and 40 healthy individuals as a control group. Blood samples were obtained from the brachial vein and subsequently centrifuged at 2,000 rpm for 15 minutes to collect serum samples. MMP-2, MMP-9, TIMP-1, and TIMP-2 levels were analyzed using commercial Enzyme-linked immunosorbent assay (ELISA) kit protocols with the obtained serum samples.

Results: Consequently, in this study it was demonstrated a higher MMP-2/TIMP-2 ratio in chronic insomnia patients when compared to healthy controls, whereas the MMP-9/TIMP-1 ratio remained unchanged.

Conclusion: These results suggest that higher MMP2/TIMP2 ratio may potentially contribute to the pathogenesis of diseases associated with sleep deprivation.

Introduction

Sleep is a reversible altered state of consciousness that covers a significant period of our life [1]. It is known that 7-8 hours of sleep per day is indispensable for human health [2]. In modern times, many individuals experience sleep deprivation due to stress, living and working conditions, and environmental factors [3]. The health issues caused by sleep deprivation have become a growing health problem for many societies. It has been demonstrated that sleep deprivation increases the risk of occupational accidents, diabetes, stroke, and cardiovascular diseases [4]. Reduced melatonin secretion and compromised DNA damage repair capacity because of sleep deprivation are responsible reasons for these increased risks [5] As a result of REM sleep deprivation, one of the stages of sleep, an increase in apoptosis [6] and oxidative stress [7] has been observed in the brain. Increased oxidative stress is believed to potentially play a role in the progression of neurodegenerative diseases [8].

Insomnia is defined as difficulties in initiation, duration, and integrity of sleep despite having sufficient opportunity for sleep, leading to impairment in daytime functions. According to the International Classification of Sleep Disorders (ICSD-3), insomnia is divided into three categories: chronic, short-term, and other. Short-term insomnia lasts between a few days, often triggered by a stressor that diminishes and disappears over time. Chronic insomnia is diagnosed when symptoms persist for at least three months. Studies have indicated that the prevalence of insomnia ranges between 10-40% [9].

The psychobiological inhibition (PI) model in chronic insomnia assumes that sleep is achieved by automaticity and plasticity [10]. Automaticity refers to the involuntary nature of initiating and maintaining sleep, governed by processes such as homeostatic and circadian regulation. In contrast, plasticity refers to the system's ability to adapt to real-world situations. Under normal circumstances, sleep occurs passively. However, acute stressful life events may adversely affect sleep-wake regulation and lead to acute sleep disorder. For most individuals, the "plasticity" of the sleep system allows for temporary changes without

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any chronic changes, and regular sleep patterns return with reduced stress. Chronic insomnia patients exhibit increased in heart and respiratory rate, body temperature, and ectodermal activity related to hyperarousal of autonomic nervous system [11]. When the neuroendocrine system is examined, the increase in urinary cortisol and plasma adrenocorticotropic hormone (ACTH) and cortisol levels in insomnia patients indicates a potential role for increased hypothalamic-pituitary-adrenal axis activity [12]. Neuroimmunological studies have shown a relationship between insomnia, nocturnal sympathetic activation, reduced immunity, and increased levels of interleukins and tumor necrosis factor (TNF). These factors are associated with heightened autonomic arousal and poor sleep quality [13].

The extracellular matrix is a supporting structure for organs and tissues. In addition, it plays a crucial role in the communication of cells, regulation of motility, distribution, and integration of growth factors [14]. Matrix metalloproteinases (MMPs), also known as matrixins, are a group of enzymes that can degrade the extracellular matrix and have a catalytic site that binds Zn++ and Ca++ [15]. MMPs are secreted in latent form; after activation, they play a significant role in remodeling the microenvironment of the cell by degrading the extracellular matrix and cell adhesion molecules. Disruption of the extracellular matrix leads to deficiencies in tissue repair and remodeling processes closely linked to neurodegeneration, cancer progression, and vascular complications [16].

The expression of MMPs is transcriptionally regulated by growth factors, cytokines, chemokines, and excitatory neurotransmitters. The activity of MMPs is controlled by tissue inhibitors of metalloproteinases (TIMPs). TIMPs, endogenous inhibitors of MMPs in many tissues, are crucial in cell reshaping. In humans, four different TIMPs exist (TIMP 1, 2, 3, 4). MMPs and TIMPs also play essential roles in processes such as neurogenesis and cerebral plasticity [17]. Besides their critical functions in the central nervous system during growth and development, MMPs also play significant roles in neuronal repair processes [18]. MMPs and TIMPs maintain a delicate balance, and disruption of this balance contributes to the pathophysiology of numerous diseases [16].

Although many known negative effects of sleep deprivation on health have been proven, the underlying mechanisms remain the subject of this study. MMPs, particularly MMP-2 and MMP-9, and their inhibitors TIMP-1 and TIMP-2 have been extensively studied due to their roles in chronic diseases such as diabetes, cancer, hypertension and neurodegenerative disorders. Our previous study showed that REM sleep deprivation led to changes in the quantity and activity of MMPs [19]. This study aims to elucidate chronic insomnia's effects on matrix metalloproteinases and inhibitors by comparing MMP-2, MMP-9, TIMP-1, and TIMP-2 levels in the serum of chronic insomnia patients to healthy controls.

Materials and Methods

Participants

This study has been approved by the Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee (Approval No: 2023-6/5). Forty adult males aged 20-65 diagnosed with chronic insomnia in Bursa Uludag University Faculty of Medicine Neurology Department, were not diagnosed with cancer, and did not have a known inflammatory or neurodegenerative or chronic cardiovascular disease were included in the study as chronic insomnia patient group. In addition, forty adult males in the same age range who did not have insomnia, were not diagnosed with cancer, and did not have a known inflammatory or neurodegenerative or chronic cardiovascular disease were included as a healthy control group (Table 1). Written informed consent was obtained from patients and volunteers after a detailed explanation of the procedures that they may undergo.

Table 1. General characteristics of study groups and inclusion/exclusion criteria.

	Healthy Control Group	Chronic Insomnia Patient Group
Inclusion Criteria	Age: 20-65 Male	Age: 20-65 Male Diagnosed with; Chronic Insomnia
Exclusion Criteria	Diagnosed with; Insomnia Cancer Inflammatory Disease Neurodegenerative Disease Cardiovascular Disease	Diagnosed with; Cancer Inflammatory Disease Neurodegenerative Disease Cardiovascular Disease

ELISA analysis

Blood samples taken from the brachial vein were centrifuged at 2000 rpm for 15 minutes. The obtained serum was stored at -80oC, and MMP-2, MMP-9, TIMP-1, and TIMP-2 levels were analyzed using commercial ELISA

Table 2. Baseline characteristics of the participants.

	Healthy Control Group	Chronic Insomnia Patient Group
Age (years)		
20-49	90%	75%
50-65	10%	25%
Educational Status		
Primary School Graduate	20%	27.5%
High School Graduate	27.5%	37.5%
University Graduate	52.5%	35%
Employment Status		
Employed	100%	62.5%
Unemployed	-	37.5%
Accommodation Status		
Living alone	15%	17.5%
Living with someone	85%	82.5%

Table 3. Values and ratios of ELISA analyses.

	MMP-2 (ng/ml)	TIMP-2 (ng/ml)	MMP-2/TIMP-2 Ratio	MMP-9 (pg/ml)	TIMP-1 (pg/ml)	MMP-9/TIMP-1 Ratio
Healthy Control Group	295.5 (207.7-648)	20.3 (12-35.9)	15.3 (13.2-20.1)	864.8 (446.7-1832.6)	204.9 (120.6-449.3)	4 (3.3-5)
Chronic Insomnia Patient Group	290 (246.3-370.3)	14.6 (11-17.7)	19.9 (15.9-24.9)**	982.7 (891.3-1311.2)	260.7 (239.6-351.4)	3.7 (3.1-4.7)

Values are represented as median (25^{th}–75^{th} percentiles). **p<0.01 indicates significant differences compared to the Healthy Control group.

(enzyme-linked immunosorbent assay) kit protocols (BT-LAB, Shanghai Korain Biotech Co., Ltd, People's Republic of China) following established procedures.

Statistical analysis

Following the measurements, analyses were performed using Sigma Plot version 12.5. The distribution characteristics of the variables were analyzed using the Shapiro-Wilk test. ELISA results were described using median and interquartile range (25th to 75th percentile), while general characteristics about participants were reported as percentages. Intergroup comparisons of non-normally distributed variables were performed using the Mann-Whitney U test. p-value of less than 0.05 was considered statistically significant.

Results

Considering the baseline characteristics of the Healthy Control group, 90% of the participants were between the ages of 20-49 and 10% were between the ages of 50-65. In addition, 20% of the participants in the study were primary school graduates, 27.5% were high school graduates, and 52.5% were university graduates. All participants were working. While 15% live alone, 85% live with someone. When we examined the baseline characteristics of the participants in the Chronic Insomnia Patient Group, 75% of the participant were between the ages of 20-49 and 25% were between the ages of 50-65. Considering their educational status, 27.5% were primary school graduates, 37.5% were high school graduates and 35% were university graduates. Regarding employment status, 62.5% were employed, while 37.5% were not. Furthermore, 82.5% of participants live with someone, while 17.5% live alone (Table 2). There was a significant increase in serum MMP-2/TIMP2 ratio of Chronic Insomnia Patient group in comparison to Healthy Control group ($p<0.01$). However, there was not any significant difference in serum ratio of MMP-9/TIMP-1 between groups (Table 3).

Discussion

Sleep disorders can be caused by various diseases or can occur alone. In this study, we showed that while MMP-2/TIMP-2 ratio increased in chronic insomnia patients compared to healthy controls, MMP-9/TIMP-1 ratio did not change. It is known that sleep deprivation, which many people experience nowadays, increases the risk of various diseases. It has been shown that the levels of MMPs in the brain change as a result of sleep deprivation [19]. Abnormal modulation of MMPs has been shown to play a role in the pathogenesis of Alzheimer's disease, Parkinson's disease and Multiple Sclerosis (MS) [20]. Therefore, it is argued that their inhibition may be therapeutic in the treatment of diseases [21]. Furthermore, the roles of MMPs and TIMPs in cardiovascular diseases, cancer and the pathogenesis of inflammatory diseases have been demonstrated in numerous studies [16].

Tissue inhibitors of MMPs (TIMPs) maintain a balance with MMPs. Disruption of the balance causes various pathologies. We presented the results of the study as the ratio of MMP-2/TIMP-2 and MMP-9/TIMP-1, as was done in other studies [22], since the disruption of the balance between them is the main reason for the formation of pathologies.

There are many diseases that research the relationship between serum matrix metalloproteinase levels and neurological diseases. When looking at studies on multiple sclerosis, an inflammatory autoimmune disease, it has been shown that serum MMP-3 levels increase during relapse compared to remission periods [23]. Increased intrathecal production of MMP-2 has been observed in MS patients [24]. Increased MMP activity is thought to breach the blood-brain barrier easily, leading to neuroinflammation, demyelination, and neurotoxicity [25].

A recent study showed that sleeping less than 7 hours increases the risk of MS disease [26]. Based on our study, an elevated MMP-2/TIMP-2 ratio may be one of the factors contributing to the increased risk of MS due to sleep deprivation. In a study suggesting that the amount of MMP-9 could be used to monitor the treatment effectiveness, it was stated that the ratio of MMP-9/TIMP-1 decreased with reduced disease activity [27]. MMPs are also considered potential markers for diseases like Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's Disease. For example, increased MMP-2 levels have been observed in ALS patients [28]. MMP inhibitors are thought to be therapeutic [29] and sleep-based therapies are considered neuroprotective for ALS [30]. Insomnia is acknowledged as a risk factor for migraines, increasing their frequency [31]. In a study conducted with migraine patients, it was found that serum MMP-2 activity increased [32]. Our study has demonstrated an elevated MMP-2/TIMP-2 ratio in chronic insomnia patients compared to healthy subjects, suggesting a potential link between insomnia and migraines.

Numerous studies have investigated the relationship between MMP-9 and neurological diseases. It has been shown to play a role in the pathogenesis of ischemia [33] and epilepsy [34]. However, as a result of our study, no significant difference was observed in the MMP-9/TIMP-1 ratio between healthy people and chronic insomnia pa-

tients.

Sleep deprivation is known to contribute to various chronic diseases beyond neurological diseases. Sleep insufficiency is an important risk factor both for acute myocardial infarction risk and coronary artery disease severity [35]. It has been stated that MMP-2 expression is increased in myocardial infarction and hypertensive heart disease diseases, and this increase is related to both the development and progression of the disease [36]. Inhibition of MMP-2 was thought to be therapeutic [37]. As a result of our study, it was determined that the ratio of MMP-2/TIMP-2 increased, which could be one of the pathways in this pathogenesis.

One of the most studied subjects of MMPs is cancer research. Sleep disorders have been observed to increase the risk of cancer [38]. MMP-2 is important in cell migration during some pathological processes such as gastric, pancreatic and breast cancer [39]. It has also been observed that a high serum level of MMP-2 may reflect the severity of breast cancer [40]. It is thought that one of the reasons why sleep disorders increase the risk of cancer may be the changes in the MMP levels.

It is known that sleep deprivation could lead to various neurological and chronic diseases. The pathways leading to these diseases are still under investigation. The extracellular matrix, which is important in the communication of cells, regulation of motility and distribution, and the MMPs, which are important for its regulation, are the most studied subjects nowadays. As MMPs are important in physiological processes, imbalance between MMPs and TIMPs can lead to many diseases. Although the changes in MMP levels due to sleep deprivation have been studied in many brain regions, our study is the first to investigate the serum MMP and TIMP levels in chronic insomnia patients. These results suggest that higher MMP2/TIMP2 ratio may potentially contribute to the pathogenesis of diseases associated with sleep deprivation.

Conclusion

In conclusion, insomnia is a sleep disorder caused by the disturbance of physiological mechanisms related to physiological predisposition, sleep, and wakefulness. While maladaptive neurobehavioral mechanisms lead to the establishment of a disrupted structure, they also create behavioral symptoms of impaired physiology. Insomnia can be considered as a neurobiological disease with these mechanisms.

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Ethical approval

This study has been approved by the Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee (Approval No: 2023-6/5).

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