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Advanced Oxidation Protein Products and the *CLOCK* 3111T/C Single Nucleotide Polymorphism in Menopausal Women with Insomnia

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Abstract

Background: The aim of this study was to assess the dependence of the advanced oxidation protein products (AOPPs) levels on the genotype of the *CLOCK* 3111T/C SNP in Caucasian menopausal women with and without insomnia.

Methods and Results: The study involved 105 Caucasian menopausal women volunteers aged between 45 and 60 years. The Pittsburgh Sleep Quality Index, PSQI, Insomnia Severity Index (ISI), and Epworth Sleepiness Scale (ESS) were employed. The study of the *CLOCK* 3111T/C SNP (rs1801260) was performed by PCR. The blood level of AOPPs was detected by IEMA. Based on the results of a clinical-anamnestic examination, the women were divided into two groups: the main (with insomnia) and the control (without insomnia). Given the small number of women carrying the *CC* genotype of the *CLOCK* 3111T/C SNP, the CC carriers and *TC* carriers were combined into one group as the carriers of the minor 3111C allele.

There were no statistically significant differences in the AOPP levels between carriers of different genotypes (TT genotype and TC+CC genotypes) in controls and patients. A comparative analysis of AOPP levels in the women of the main and control groups showed higher AOPP levels in women with insomnia carrying the TT genotype than in the control of the same genotype (P=0.013).

Conclusion: Insomnia in menopausal women is associated with increased protein oxidation only in carriers of the TT genotype of the *CLOCK* 3111T/C SNP. (**International Journal of Biomedicine. 2020;10(4):352-356.**)

Key Words: *CLOCK* 3111T/C SNP • protein oxidation • insomnia • menopause

Abbreviations

AOPPs, advanced oxidation protein products; **IEMA**, immunoenzymometric assay; **LPO**, lipid peroxidation; **OS**, oxidative stress; **ROS**, reactive oxygen species; **SNP**, single nucleotide polymorphism

Introduction

Many physiological and metabolic processes, including the sleep-wake cycle, are controlled by a circadian system. It is known that this system consists of a central pacemaker, the suprachiasmatic nucleus (SCN), and of peripheral oscillators found in almost all cell types in brain and body that resonate with circadian cues originating from the SCN.[1,2] Disruption of the biological clock function accompanied by the separation of connections between local oscillators in different tissues or the central oscillator, leads to neuroendocrine rhythms and behavior malfunction. Some studies have demonstrated age-related changes in circadian rhythms.[3] Moreover, any circadian system changes increase the risk of developing many pathological conditions, including sleep disorders.[4]

To date, it has been shown that the hormone melatonin plays an important role in the circadian mechanism.[5] Along

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with its regulatory role in the sleep-wake cycle, melatonin is one of the antioxidants that has more pronounced antioxidant properties than vitamin E or glutathione.[6] It is possible that changes in melatonin secretion circadian rhythms in patients with sleep disorders can lead to OS, which is a result of an imbalance between the production of free radicals and the activity of the antioxidant system.[7] In 1994, Reimund proposed the Free Radical Flux Theory of Sleep.[8] Reimund hypothesized that ROS accumulate in the brain during the wake state, and the lower metabolic rate of sleep provides the brain's antioxidant system the opportunity to catch up, neutralizing neuronal ROS down to baseline levels in preparation for the next day's cycle. The role of OS during sleep was later demonstrated not only by experimental investigations[9-13] but also in human studies,[14,15] including menopausal women.[16] The consequences of LPO product accumulation are changes in the metabolism of proteins, fat, carbohydrates, nucleic acids, water, and electrolyte metabolism, which can cause severe tissue damage and reduction in the adaptive capacity of the organism.[7] It can lead to the development of different cardiovascular,[17] endocrine,[18] and mental diseases, sarcopenia,[20] as well as cancer[21] and other pathologies.

In our earlier study, we found that melatonin secretion circadian rhythms are associated with the *CLOCK* 3111T/C SNP in Caucasian menopausal women with insomnia. Early morning hours increased the hormone level registered in TT carriers, which allows considering the 3111T allele as risky in the formation of melatonin circadian rhythm disturbances in these patients.[22] Moreover, it has been shown that some LPO and antioxidant system parameters in Caucasian menopausal women with insomnia depend on the *CLOCK* 3111T/C SNP. However, there is no data about the *CLOCK* 3111T/C SNP and oxidation protein relationships in insomnia.

The aim of this study was to assess the dependence of AOPPs levels on the genotype of the *CLOCK* 3111T/C SNP in Caucasian menopausal women with and without insomnia.

Materials and Methods

Subjects

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. The study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Written informed consent was obtained from each patient.

The study involved 105 Caucasian menopausal women volunteers (the Russian ethnic group) aged between 45 and 60 years, living in Irkutsk city. They were recruited through personal interviews. Once the women had given their written, informed consent to participate in the study, the research program was conducted and included the following methods: clinical-anamnestic, laboratory and statistical.

Inclusion criteria for the perimenopausal group were age of 45–55 years, oligomenorrhea or amenorrhea during 12 months, and ultrasound criteria: (1) endometrial dysfunction (mismatch of structure and thickness corresponding to the first and the second phases of the menstrual cycle); (2) the depletion of ovarian reserve.

Inclusion criteria for postmenopausal group were age of 56–60 years, amenorrhea ≥12 months, follicle-stimulating hormone level >20 iU/ml, index luteinizing hormone/follicle-stimulating hormone<1, and ultrasound criteria: (1) thin non-functional endometrium, endometrial echo thinner than 5 mm; (2) the lack of ovarian reserve.

Exclusion criteria were exacerbation of chronic diseases, hormone replacement therapy, surgical menopause, the presence of chronic sleep disorders in the history before menopause (insomnia, parasomnia, hypersomnia, obstructive sleep apnea syndrome), the use of hypnotic pills in the previous two weeks, and shift work.

Questionnaires

The Pittsburgh Sleep Quality Index,[23] PSQI, Insomnia Severity Index (ISI),[24] and Epworth Sleepiness Scale (ESS) [25] were employed. These questionnaires indicate the presence of insomnia according to the criteria of the International Classification of Diseases (ICD-10) and of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV).

The PSQI questionnaire consists of 19 validated questions for assessment of sleep quality and efficiency. The global PSQI score is calculated from seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction. Each question within the component is scored on a 4-point Likert scale of 0-3, with 3 indicating worse outcomes, and the mean was calculated for each component score. The total score ranged from PSQI 0 to 21 points. A global PSQI score 5 indicates a "good sleeper" and >5 indicates a "poor sleeper."

The ISI questionnaire consists of 7 items and identifies the severity of sleep continuity disturbance, defined as difficulty initiating sleep and staying asleep, early morning awakenings, and related distress. The answer to each of the questions is scored on a 5-point Likert scale of 0 (none) to 4 (very severe) and a total score ranging from 0 to 28 points. The ISI results 0-7, 8-14, 15-21, 22-28 points were interpreted as the norm, slight sleep disorders, moderate, and severe, respectively.

The ESS consists of 8 items about usual chances of dozing off or falling asleep while engaged in 8 different activities. The answer to each of the questions is scored on a 4-point Likert scale of 0 (not at all likely to fall asleep) to 3 (very likely to fall asleep) and a total score ranging is from 0 to 24 points. The ESS results 0-3, 3-9, 9-16, 16-24 points were interpreted as norm, insomnia, obstructive sleep apnea syndrome, and narcolepsy, respectively.

Blood collection

Venous blood was sampled from the cubital vein into two tubes with EDTA tripotassium salt between 8:00 a.m. and 9:00 a.m. after 12-h overnight fasting. Venous blood from the first tube was used for molecular-genetic examination. The sample from second tube was centrifuged for 10 min at 1.500 g at 4°C. Blood plasma was kept frozen at -40°C for up to one month until AOPP determination.

DNA testing

Genomic DNA samples were isolated from the peripheral blood leukocytes using the AmpliPrime DNA-Sorb-B reagent kit (NEKSTBIO, Russia). The study of the *CLOCK* 3111T/C SNP (rs1801260) was performed by PCR on the DTprime amplifier (DNA-Technology, Russia) using reagent sets for genotyping polymorphic markers (TestGen, Russia).

AOPPs determination

AOPPs blood level (nmol/l) was detected by IEMA using ImmunDiagnostik AG kits (Germany) on a Bio Tek EL×808 analyzer (USA).

Based on the results of a clinical-anamnestic examination, the women were divided into two groups: the main (with insomnia) and the control (without insomnia). Given the small number of women carrying the CC genotype of the *CLOCK* 3111T/C SNP, the CC carriers and TC carriers were combined into one group as the carriers of the minor 3111C allele. The basic characteristics of the groups are demonstrated in Table 1.

Statistical processing was carried out using the STATISTICA Version 10 (StatSoft, USA). The normality of distribution of continuous variables was tested by Shapiro-Wilk test. Mean±standard deviation (SD), median (Me), interquartile range (IQR; 25th to 75th percentiles) were calculated. The Mann-Whitney U-Test was used to compare differences between two independent groups. A value of $P<0.05$ was considered significant.

Table 1.

The basic characteristics of the groups

Characteristics	Control		Insomnia	
	3111T/T (n=16)	3111T/C+ 3111C/C (n=20)	3111T/T (n=33)	3111T/C+ 3111C/C (n=36)
	n(%)			
Perimenopause	7(43.75)	8(40)	15(45.45)	16(44.44)
Postmenopause	9(56.25)	12(60)	18(54.55)	20(55.56)
Mean±SD				
Age, yr	53.75±5.68	53.35±5.66	54.85±4.99	54.75±5.01
BMI, kg/m^2	27.45±4.27	26.41±3.57	28.14±1.32	27.16±2.23
PSQI (points)	2.32±0.41	2.11±0.15	16.27±2.33*	15.74±3.04^
ISI (points)	3.71±1.81	4.56±1.18	24.41±1.11*	22.91±1.17^
ESS (points)	1.63±0.28	1.03±0.14	7.42±1.25*	6.65±1.98^

**-<0.05 between 3111T/T carriers in control and insomnia*
^-<0.05 between 3111T/C+3111C/C carriers in control and insomnia

Results

AOPPs levels in menopausal women with insomnia and in the control group, regardless of the *CLOCK* 3111T/C SNP, are presented in Figure 1. No differences between control and main group were found.

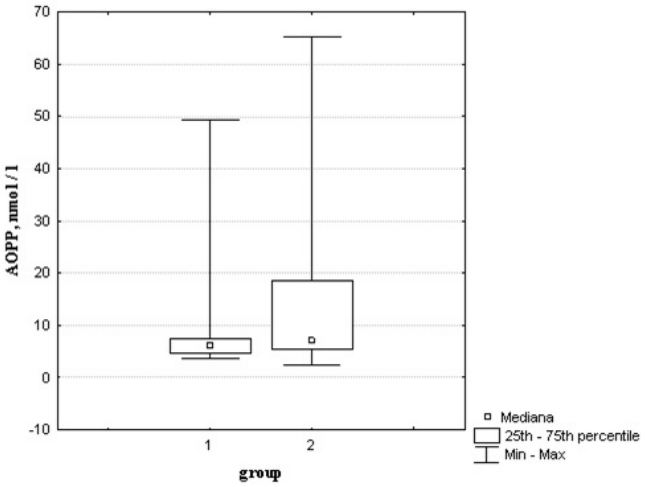

Fig. 1. *AOPP levels in control group and women with insomnia*
Note: 1 - control; 2 – insomnia

AOPPs levels in menopausal women with different genotypes of the *CLOCK* 3111T/C SNP are presented in Figure 2. There were no statistically significant differences in the AOPP levels between carriers of different genotypes (TT genotype and TC+CC genotypes) in controls and patients. A comparative analysis of AOPP levels in the women of the main and control groups showed higher AOPP levels in women with insomnia carrying the TT genotype than in the control of the same genotype (P=0.013).

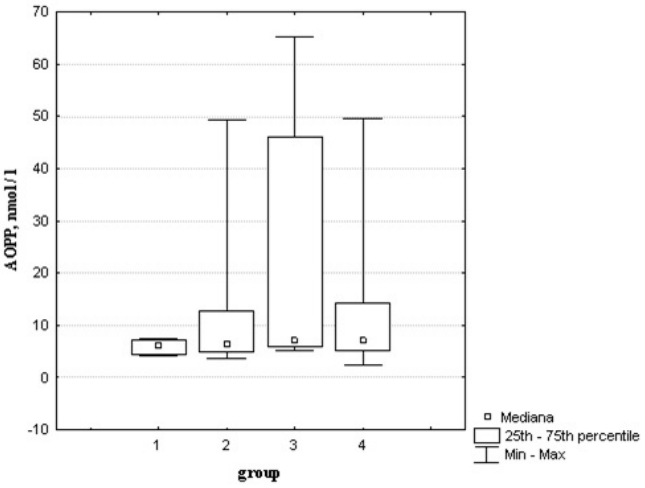

Fig. 2. *AOPPs levels in menopausal women with different genotypes of the CLOCK 3111T/C SNP*
Note: 1 - control, TT-genotype; 2 - control, TC-, CC-genotypes; 3 - insomnia, TT-genotype; 4 - insomnia, TC-, CC-genotypes

Discussion

Proteins are one of the main targets of ROS, and their damage associated with the impossibility of repairing most protein damage exerts a significant effect on cell viability.[26] Damaged proteins can cross-link and be involved in the pathogenesis of various diseases. Intensified oxidative modification of

proteins and increased concentration of AOPPs are confirmed by many experimental investigations in different pathological states, especially those with well-known participation of OS in pathogenesis.[27]

Previously ambiguous results about the relationship between OS and insomnia have been shown in some experimental [12,13] and human studies.[14,15] The results of the studies involving menopausal women with insomnia have demonstrated increased levels of TBARS and similar outcomes with control antioxidant system parameters in patients.[16,28] However, further research showed that higher TBARS levels were identified in menopausal women with insomnia who were TT carriers of the *CLOCK* 3111T/C SNP, while carriers of the minor *C* allele had only primary LPO products at a high level.[29]

The results of our study demonstrated that protein oxidation in menopausal women with insomnia also depends on the *CLOCK* 3111T/C SNP. These data confirm the development of OS in TT carriers with insomnia. A possible reason for this may be a shift in the melatonin secretion rhythms in these patients.[22] In addition, an increase in AOPP levels combined with a decrease in melatonin levels has been demonstrated in several studies.[30] Another possible reason for the development of OS could be differences in activities of proteasome and lysosomal systems for irreversibly damaged protein utilization and amino acid reuse for continuous protein synthesis in the body. It is known that their proteolytic activity significantly decreases with age.[26]

Based on our results, it can be assumed that the 3111C allele is protective for excessive protein oxidation in menopausal women with insomnia. Insomnia in menopausal women is associated with increased protein oxidation only in carriers of the TT genotype of the *CLOCK* 3111T/C SNP. Preventing OS leading to AOPP accumulation and various pathological conditions is important for menopausal women carrying the TT genotype of the *CLOCK* 3111T/C SNP.

Competing Interests

The authors declare that they have no competing interests.

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