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Original Article

Serum level of melatonin in patients with osteoarthritis and its relation with 8-hydroxy-2-deoxyguanosine and vitamin D

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Abstract

Introduction: This study aimed to evaluate the relationship between the serum level of melatonin, 8-hydroxy-2-deoxy guanosine, and vitamin-D in patients with osteoarthritis (OA).
Methods: This study enrolled 47 patients with OA and 40 healthy controls. Serum levels of melatonin and 8-hydroxy-2-deoxy guanosine (8-OH-dG) were assessed. Also, serum levels of bone turnover biomarkers such as calcium, phosphorus, vitamin D, and alkaline phosphatase (ALP) were measured in OA patients and controls.
Results: The serum level of melatonin was significantly lower in OA patients than the controls (6.18 ± 2.25 vs. 11.57 ± 3.87 pg/mL, $P<0.05$). In contrast, the serum level of 8-OH-dG was significantly increased in OA patients compared to controls (65.21 ± 16.12 vs. 22.51 ± 5.3 ng/dL, $P<0.001$). There was a negative correlation between serum melatonin and 8-OHdG levels in OA patients ($P<0.05$). There was a positive correlation between serum melatonin and vitamin D levels in OA patients ($P<0.05$). We found decreased calcium and vitamin D levels, and increased phosphorus and ALP levels in OA patients compared to controls ($P<0.05$).
Conclusion: Decreased levels of melatonin and elevated levels of 8-OH-dG might play a role in the pathogenesis of OA. Therefore, melatonin might be involved in decreasing DNA damage and exerting a preventive function in OA.

Introduction

Osteoarthritis (OA) is one of the most prevalent degenerative joint diseases.[1,2] According to the World Health Organization (WHO), approximately 9.6% of men and 18.0% of women aged over 60 have symptomatic OA.[2] OA is a multifactorial disease, and aging, obesity, joint instability, sedentary life, genetic predisposition, bone density, occupational injury, trauma, and gender are among the most critical factors affecting the occurrence and extent of this chronic disease.[2] Despite increased focus on elucidating the underlying mechanisms, the exact etiology of the OA remains unknown.[3] The recent studies have shown that reactive oxygen species (ROS), and reactive nitrogen species (RNS) including nitric oxide (NO•), superoxide (O•−2), peroxynitrite (ONOO−), and hydrogen peroxide (H2O2) play critical roles in OA pathogenesis.[3] Previous studies demonstrated that OA progression is significantly related to oxidative stress and ROS.[4,5] Also, inflammation and mechanical stress are associated with increased ROS and RNS production in OA.[6,7] More importantly, an increased level of DNA damage is indicated in the OA chondrocytes in comparison to non-OA chondrocytes.[8] Additionally, increased production of ROS and RNS such as NO or ONOO- in OA patients leads to DNA damage in chondrocytes.[7] It has been reported that levels of DNA damage are highest in the later stages of OA.[9] 8-Hydroxy-2-deoxyguanosine (8-OH-dG), as an oxidative product of DNA, is one of the most studied markers of DNA damage whose tissue expression and serum levels markedly change.[10] Determination and analysis of 8-OH-dG can be performed in animal organs and human samples (urine, human organs, leukocyte DNA) as a biomarker of oxidative stress, aging, and carcinogenesis.[11,12] Previous studies have reported increased levels of 8-OH-dG and decreased levels of its repair enzyme 8-oxoguanine DNA glycosylase (Ogg1) in OA chondrocytes, but not in normal chondrocytes, as well as in OA model rabbits and patients with OA.[8] Melatonin

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(N-acetyl-5-methoxytryptamine) is the main endogenous product of the pineal gland, with a wide distribution within highly developed creatures to elementary organisms.[13] It has been identified that melatonin exists in many extra pineal tissues and organs independently of the pineal gland.[14] Functions of melatonin can be divided into chronobiotic and non-chronobiotic functions.[15] Chronobiotic effects are mediated by the daily rhythm of melatonin in the circulation due to nocturnal pineal synthesis.[16] Extra pineal melatonin, which is independent of the light/dark cycle and exerts non-chronobiotic effects, includes anti-oxidant, oncostatic, anti-aging, immune-modulatory, and anti-inflammatory properties.[17] The cytoprotective and therapeutic potentials of melatonin are well established in preclinical and clinical OA settings. Growing data showed that melatonin and vitamin D might exert several anti-inflammatory activities in the situation of meta-inflammation in OA patients.[18] Therefore we aimed to measure the serum levels of melatonin, 8-OH-dG, and vitamin D in patients with OA in the present study. Based on the role of melatonin, we assumed that melatonin might be involved in the decrease of DNA damage and have a role in preventing OA.

Methods

In this case-control study, 47 volunteer OA patients (22 males, 25 females; mean age 62.2 ± 5.5 years) were recruited from the Department of Orthopaedic Surgery, School of Medicine, and Shohada educational hospital, Tabriz University of Medical Sciences, Tabriz, Iran. All of the patients had OA without any other bone diseases. Diagnostic criteria included clinical, laboratory and radiographic diagnostic factors (erythrocyte sedimentation rate <45 mm/h; rheumatoid factor <1.40; synovial fluid and pain plus osteophytes signs of OA.)

Forty healthy subjects having no bone illnesses (19 males, 21 females; mean age 60 ± 6.5 years) were chosen as the control group. Individuals in the control group were randomly recruited during a detailed check-up examination in Shohada educational hospital. None of the patients or controls were smoker, consumed alcohol or had any other chronic diseases. Individuals with any type of lymphoproliferative disorders and other neoplasms, arterial hypertension, metabolic syndrome, dyslipidemia, diabetes, renal pathology, coronary artery disease, mental retardation, depression treated with drugs, pulmonary hypertension, autoimmune diseases, smoking, and alcohol consumption were excluded in both groups. None of the patients in either the case or control group had taken NSAIDS or undergone hormone therapy.

Blood samples were collected after an overnight fasting from all the participants. Venous blood samples were placed immediately on ice, centrifuged for 10 minutes at 3000 g within 30 minutes of the sampling kept frozen at −80°C until testing. Serum samples were not stored longer than two months and thawed at room temperature only once at the time of trial.

The serum contents of melatonin, 8-OH-dG, and vitamin D were measured by the enzyme immunoassay procedure and enzyme-linked immunosorbent assay (ELISA). Melatonin ELISA kit (Abcam, Cambridge, MA, USA, ab-213978(was used to analyse melatonin concentration in the samples. The intra- and inter-assay coefficients of variation were 4.31% and 7.36%, respectively. The measurement of 8-OH-dG was performed using an 8-OH-dG ELISA kit (Abcam, Cambridge, MA, USA, ab-201734). The intra- and inter-assay coefficient of variation was below 4.31% and 7.36%, respectively. The measurement of vitamin D was performed using a vitamin D ELISA kit (Monobind Inc, USA). The absorbance was determined at 450 nm using a microplate reader (State Fax, 2100; Awareness Technology Inc, Palm City, FL, USA(. The total melatonin, 8-OH-dG, and vitamin D in the samples were measured using the standard curve, and the results were expressed in ng/mL, ng/mL, and nmol/L.

In addition, the levels of calcium, phosphorus, and alkaline phosphatase (ALP) were measured by an enzymatic colorimetric method (Pars Azmoon Co, Tehran, Iran) with an automated chemical analyser (BT 3000, Rome, Italy).

Statistical analysis

SPSS (version16) was used for data analyses. The normality of the quantitative variables was assessed by the Kolmogorov-Smirnov test. Chi-square test and independent sample *t* test were used for qualitative and quantitative data analysis, respectively. All results were expressed as mean ± standard deviation (SD) and percentage (%); correlations between parameters were studied using Pearson's test. The statistical significance was considered as $P < 0.05$.

Results

Patients in the two groups were similar in age, sex, height, body weight, and body mass index (BMI), as shown in Table 1. Serum melatonin concentrations were significantly lower in the OA patients than in the healthy controls (6.18 ± 2.25 vs. 11.57 ± 3.87 ng/mL, $P < 0.05$; Figure 1),

Table 1. Basic characteristics of the groups

	Healthy controls	OA patients	*P* value
Number	40	47	-
Age (y) **	60 ± 6.5	62.2 ± 5.5	>0.05
Male/female (%)*	19(48%)/21(52%)	22(47%)/25(53%)	-
BMI (kg/m^2) **	24.58 ± 2.92	25.08 ± 1.61	>0.05
Total ALP (IU/L)	59.94 ± 21.39	173.48 ± 47.11	<0.001
Calcium(mg/dL)*	10.26 ± 0.93	7.17 ± 0.9	<0.001
Phosphorus(mg/dL)*	3.39 ± 0.35	5.41 ± 0.4	<0.001
Vitamin D (nmol/L)*	44.7 ± 14.87	28.43 ± 12.49	<0.001

BMI: body mass index, ALP: alkaline phosphatase
**P* value is based on independent samples *t* test. ***P* value is based on the chi-square test.

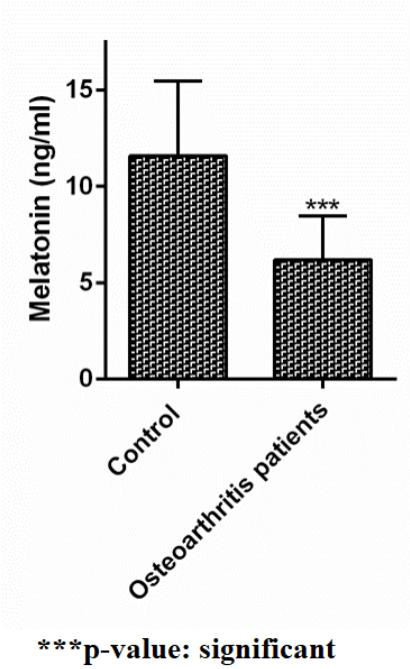

Figure 1. Melatonin levels in serum of patients with osteoarthritis in comparison to controls.

while 8-OH-dG concentrations were significantly higher in the OA patients in comparison with healthy controls (65.21 ± 16.12 *vs.* 22.51 ± 5.3 ng/mL, $P < 0.001$; Figure 2). Also, the serum levels of calcium were significantly lower in OA patients compared to healthy controls (7.17 ± 0.9 vs. 10.26 ± 0.93 mg/dL, $P < 0.001$; Table 1). Phosphorus concentrations were higher in OA patients in comparison to healthy controls (5.41 ± 0.4 vs. 3.39 ± 0.35 mg/dL, $P < 0.001$; Table 1). Moreover, ALP levels were higher in OA patients than healthy controls (173.48 ± 47.11 vs. 59.94 ± 21.39 IU/L, $P < 0.001$; Table 1). Vitamin D levels were significantly lower in OA patients than healthy controls (28.43 ± 12.49 vs. 44.7 ± 14.87 nmol/L, $P < 0.001$; Table 1).

In OA group, correlation analysis revealed a negative correlation between melatonin and 8-OH-dG levels ($r = -0.601$; $P < 0.001$; Figure 3), a positive correlation between levels of melatonin and Vitamin D ($r = 0.453$; $P < 0.05$; Figure 3), a positive correlation between levels of melatonin and calcium ($r = 0.529$; $P < 0.05$; Figure 3), a negative correlation between levels of melatonin and phosphorus ($r = -0.643$; $P < 0.05$; Figure 3), and a negative correlation between levels of melatonin and ALP ($r = -0.550$; $P < 0.05$; Figure 3).

Discussion

The results of the present study indicated that serum levels of 8-OH-dG were higher in OA patients. Also, melatonin levels were significantly lower in OA patients with a negative correlation with 8-OH-dG. There were substantial differences in serum levels of vitamin D, calcium, phosphorus, and ALP in the OA and control groups, as the markers of bone turnover. Increased bone turnover, and enhanced metabolic activity of subchondral bone are two typical events in the OA patients, as demonstrated by previous studies.[19] Additionally, low serum levels of vitamin D appear to be associated with an increased risk of progression of OA of the knee.[20-22] With this in mind, in agreement with previous studies, this study showed a dramatic decrease in the serum levels of bone turnover markers, including calcium, phosphorus, and ALP, as well as vitamin D in OA patients compared to healthy controls.

The importance of melatonin in OA pathogenesis is evident from studies reporting the beneficial therapeutic effects of this hormone in alleviating OA-related symptoms in different models of OA.[6] For example, in the rabbit model of OA, melatonin attenuated H_2O_2- induced increase in the inflammatory response and oxidative stress.[23] Therefore, the anti-inflammatory and anti-oxidative functions of melatonin are demonstrated to be contributed to the positive effects on the OA.

An increase in oxidative stress with aging may also contribute to the development of chronic inflammation and disease.[24] On the other hand, it is well-known that aging is one of the most critical factors leading to OA development.[3,25,26] Melatonin prevents the translocation of NF-κB to the nucleus and its binding to DNA, and reduces the upregulation of a variety of pro-inflammatory cytokines.[27] Also, melatonin treatment produces beneficial effects on mitochondrial morphology and dynamics by mitofusin2 (Mtf2) and the intrinsic apoptotic cascade modulation,[28] and preserves complex I and III activity, inhibits mitochondrial permeability transition

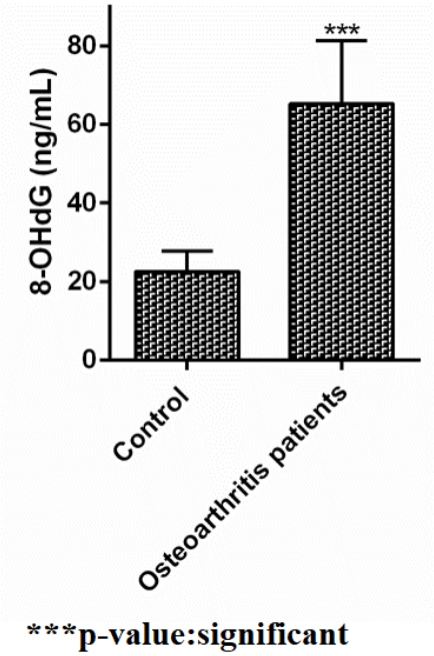

Figure 2. 8-OH-dG levels in serum of patients with osteoarthritis in comparison to controls.

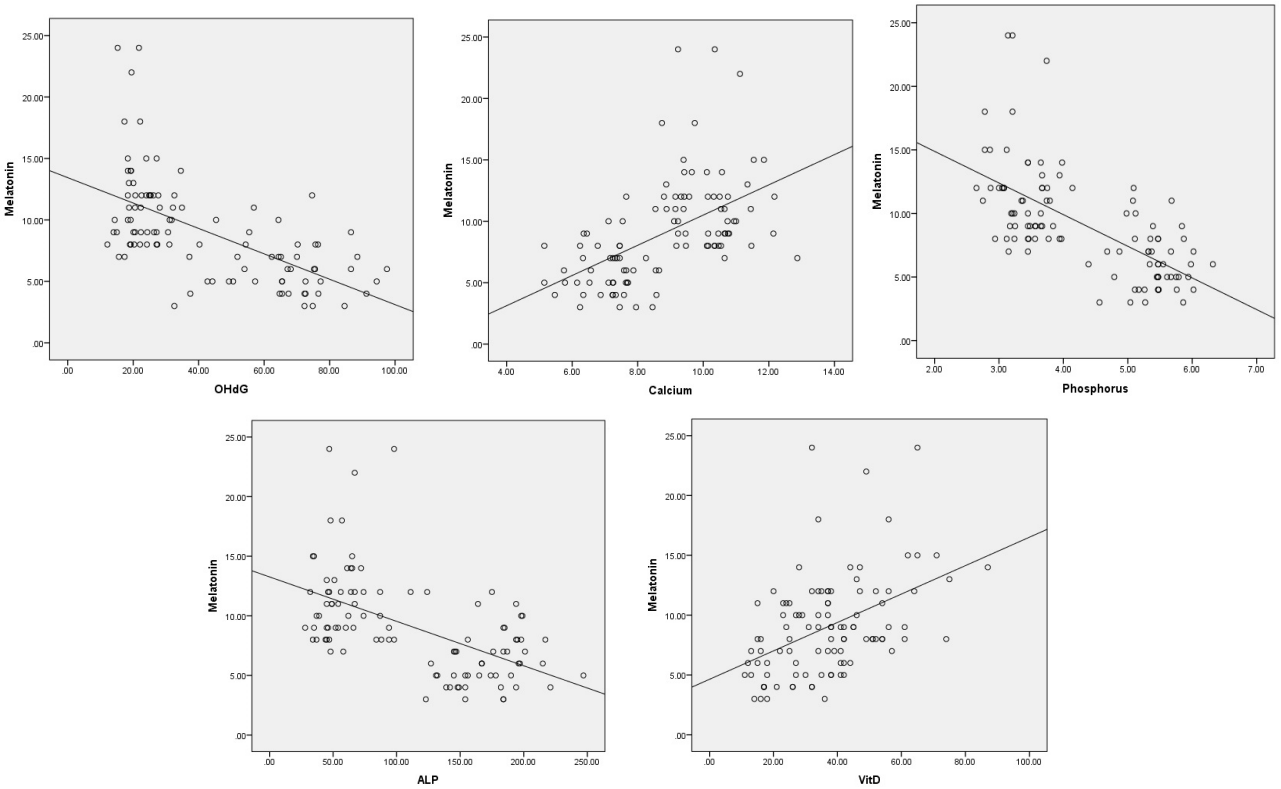

Figure 3. Correlation between melatonin and serum levels of 8-OH-dG, vitamin D, calcium, ALP and phosphorus in serum of patients with osteoarthritis.

pore opening, and cytochrome c release.[29] Melatonin suppresses TNF-α expression, which is responsible for dynamin-related protein 1-mediated mitochondrial fission.[30] Moreover, it reduces the phosphorylation of NF-κB in the adipose tissue,[25,31] and inhibits the NLRP3 pathway downstream. Studies have shown that exogenous melatonin ameliorates apoptotic-associated speck-like protein containing a caspase recruitment domain, and thereby caspase-1 and IL-1β.[32] According to the mentioned studies, melatonin can reduce the effects of aging on anti-oxidant and anti-inflammatory properties. In our study, we showed that melatonin production levels were significantly lower in patients with OA in comparison with healthy controls. Therefore, due to potent functions of melatonin in improving inflammation and scavenging free radicals, its low levels may be rational in OA patients, and may be related to the pathogenesis of this disease.

Investigations have shown that the 8-OH-dG level increases when ROS rise in the cell. ROS can affect DNA and cause DNA damage. Thus, an increased level of the oxidative stress biomarker, 8-OH-dG, is an evidence of oxidative DNA damage.[33] During the aging process as well as age-related diseases, mtDNA and nDNA undergo several damages like mutation,[34] leading to 8-OH-dG production.[35] Increased levels of 8-OH-dG are also reported in OA chondrocytes by Chen et al. They showed that 8-OH-dG levels were significantly higher in OA chondrocytes, but not in normal chondrocytes in the porcine model of OA.[8] Also, the serum levels of 8-oxoguanine DNA glycosylase (Ogg1), an enzyme that recognizes and hydrolyzes oxoguanine in the DNA backbone, was significantly depleted in degenerated articular cartilage and participated, at least in part, in the development of cartilage degeneration in OA.[8] Our study revealed that the 8-OH-dG levels were significantly higher in OA patients than the controls. According to these data, it can be considered that since ROS and RNS levels increase in OA patients, DNA degradation rises as well. As a result, 8-OH-dG levels increase in OA patients. We observed that there is a negative correlation between melatonin and 8-OH-dG levels in OA patients. It means that decreased levels of melatonin are accompanied by increased 8-OH-dG levels. In other words, reduced melatonin levels and subsequent decreased anti-oxidative function in chondrocytes resulted in increased oxidative DNA damage and increased production of 8-OH-dG.

Conclusion

In conclusion, our results showed a negative correlation between melatonin and 8-OH-dG, as a DNA damage marker, in the serum of patients with OA. Decreased levels of melatonin and elevated levels of 8-OH-dG might play a role in the pathogenesis of OA. Taken together, our findings indicated that melatonin might be involved in decreasing DNA damage and exerting a preventive function in OA. Moreover, it can be a potentially useful therapeutic agent

Study Highlights

What is current knowledge?

- According to the World Health Organization, approximately 9.6% of men and 18.0% of women aged over 60 have symptomatic OA.

What is new here?

- Melatonin might be involved in decreasing DNA damage and exerting a preventive function in OA.

for patients with OA. However, this study was limited by application of a single analysis method to measure each laboratory parameter, a relatively small sample size, and lack of outcome data from the patients. Consequently, this study requires a larger sample size to validate prognostic and diagnostic values of these biomarkers.

Conflict of interest

The authors declare that there are no conflicts of interest.

Ethics Approval

All study procedures performed were in accordance with the ethical standards of the institutional and national research committee and followed 1964 Helsinki declaration and its later amendments. This study was approved by the Ethics Committee of the Tabriz University of Medical Sciences (TBZMED). All participants provided written and informed consent. The study was granted by the ethical committee of Tabriz University of Medical Sciences (Ethical code: IR.TBZMED.REC.1396.940), and the informed written consent was obtained from all the participants.

Authors' Contribution

Mo and MAR participated in experiments preforming. NK and MoM participated in the sampling and manuscript drafting. MaM participated in data analysis. AS and BY participated in the study design, revising, and data interpretation.

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