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ORIGINAL ARTICLE

Presence of Peripheral Neuropathy Does Not Affect Urine 6-Sulfatoxymelatonin Levels in Type 2 Diabetics

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Abstract

BACKGROUND: Oxidative stress plays a crucial role in the pathogenesis of diabetic peripheral neuropathy. Melatonin is one of the most powerful antioxidant substances and its role in the pathogenesis of diabetes has been the focus of much research. However, no data exist on melatonin levels in diabetic peripheral neuropathy. We investigated how levels of urinary 6-sulfatoxymelatonin, the main metabolite of melatonin, differed in diabetic peripheral neuropathy.

METHODS: A total of 127 participants were enrolled into 3 groups: diabetic neuropathy (n=43), diabetes but no neuropathy (n=44), and controls (n=40). Neuropathy was diagnosed using the Michigan Neuropathy Screening Instrument. Melatonin level was evaluated by measuring 24-hour urine 6-sulfatoxymelatonin levels.

RESULTS: We found significant differences in urinary 6-sulfatoxymelatonin levels between the 3 groups (p=0.023). The distribution of 6-sulfatoxymelatonin among all diabetic participants was significantly lower than in the control group (p=0.006). However, there was no difference in diabetics with and without neuropathy (p=0.792). 6-sulfatoxymelatonin levels were negatively and weakly correlated with plasma glucose(r = −0.211, p=0.017) and positively and weakly correlated with microalbuminuria (r= 0.209, p=0.023). Regression analysis was found a significant relationship between age (B = 0.826, 95% Cl=0.227 to 1.426), insulin use (B = 14.584, 95% CI= 3.857 to 25.311), glomerular filtration rate (B = 0.248, 95% CI= 0.018 to 0.478) and 6-sulfatoxymelatonin levels. 6-sulfatoxymelatonin levels in insulin users were significantly higher than they were in nonuser diabetics (p=0.001).

CONCLUSION: Urinary 6-sulfatoxymelatonin levels were lower in diabetics but the presence of neuropathy did not affect 6-sulfatoxymelatonin levels. Insulin may improve melatonin levels in diabetics.

BACKGROUND

Diabetic peripheral neuropathy (DPN), also known as distal symmetrical polyneuropathy (DSPN), is a microvascular complication of type 2 diabetes mellitus (DM) and is quite common in diabetics. Neuropathy may have in an insidious course like DM as well as remain asymptomatic for long periods. Moreover, it can cause neuropathic pain in the lower extremities as well as serious problems that can lead to foot amputation through sensory loss (Pop-Busui *et al.* 2017). Oxidative stress and inflammatory processes play crucial roles in the pathogenesis and progression of type 2 DM and DPN. Increased glucose levels lead to the use of glucose in the polyol and hexosamine pathways. This change causes an increase in reactive oxygen species (ROS) and subsequent inflammation. As a result, mitochondrial damage occurs first followed by nervous system dysfunction (Oguntibeju 2019). The effects of endogenous antioxidants on the development of DM and its complications as well as how they are affected by the diabetic process are critical topics that must be investigated.

Melatonin, also known as N-acetyl 5-methoxytryptamine, is a hormone secreted from the pineal gland with increased secretion in darkness. It is one of the most powerful endogenous antioxidants, an anti-inflammatory substance, and also acts as an internal synchronizer. It performs a wide range of essential physiological functions, including regulation of the circadian rhythm, functions in cell regeneration, strengthening of the immune system, glucose homeostasis, regulation of thermoregulation, seasonal reproduction of animals, and ovarian functions (Claustrat *et al.* 2005, Yeğin *et al.* 2009). Melatonin exhibits antioxidant properties both by direct action, the induction of antioxidant enzymes, and the inhibition of prooxidant enzymes (Reiter 2003).

Tab. 1. Distribution of age, BMI and laboratory values of all participants

	Median (min-maX)
Age(year)	53.0 (35.0-73.0)
BMI (kg/m^2)	27.8 (20.5-51.5)
HbA1c (%) (mmol/mol)*	7.1 (5.3-11.7) 54 (34-104)
FPG (mg/dl)	124.1 (83.4-344.0)
Creatinin (mg/dl)	0.81 (0.48-1.42)
GFR (ml/dk/1.73m^2)	94.1 (45.2-148.7)
ALT (u/L)	20.7 (5.1-66.8)
Vitamin B12 (pg/ml)	364.1 (134.0-1670.0)
TSH (μIU/ml)	1.9 (0.4-22.9)

Abbreviations: SD = Standart deviation, min = Minimum, max = Maximum, BMI = Body mass index, HbA1c = Glycosylated hemoglobin, FPG = Fasting plasma glucose, GFR = Glomerular filtration rate, ALT = Alanine transaminase, TSH = Thyroid stimulating hormone

Melatonin synthesis starts from tryptophan, which is an essential amino acid. Tryptophan is taken up by the pineal gland with active transport and transformed into melatonin through participating in 4 consecutive enzymatic reactions (Amaral & Cipolla-Neto 2018). Its half-life is limited to 30 to 50 minutes. Melatonin metabolism mainly occurs through hydroxylation and conjugation in the liver (Auld *et al.* 2017). The main urinary metabolite is 6-sulfatoxymelatonin (6-SOM), which exhibits a strong correlation with plasma melatonin levels (Tordjman *et al.* 2017).

The relationship of melatonin with DM and its complications has been the subject of many studies. However, little is known about the relationship between melatonin and DSPN in humans. We aimed to investigate whether melatonin secretion is affected in type 2 DM patients with DPN by measuring 24-hour urine levels of 6-SOM.

METHODS

The participants of this study were randomly selected from individuals who applied to a university hospital between October 2019 and July 2020. In total 127 participants between the ages of 25 and 75 years were included in our study and enrolled into 3 main groups: those who were diabetic and had DSPN [DM(+)NP(+) group], those who were diabetic but did not have DSPN [DM(+)NP(-) group], and those who did not have any chronic disease but applied for general health control (control group).

Diabetic patients were selected from patients who had previously been diagnosed with type 2 DM or those who were newly diagnosed.

All participants were investigated for neuropathy using the Michigan Neuropathy Screening Instrument (MNSI). The control group was composed of participants who did not meet the criteria for DM and neuropathy.

Ethics committee approval was obtained from the local clinical research ethics committee. Informed consent was obtained from all participants.

Exclusion Criteria

Individuals with a chronic disease other than DM or drug use, which has the potential to affect plasma melatonin concentration, were excluded from our study. Patients with renal failure [glomerular filtration rate (GFR) <45 mL/min/1.73 m^2], respiratory system disease, gastrointestinal system disease, genitourinary system disease, cancer, active autoimmune or rheumatological disease, chronic infectious disease, allergic disease, and sleep or psychiatric disorders, as well as patients using β-blockers or selective serotonin reuptake inhibitors in the last 6 months, were not included. Pregnant women were also not included.

Also, we excluded patients with diabetic proliferative retinopathy because it may affect melatonin levels.

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Tab. 2. Distribution of age, BMI, MNSI scores and laboratory values of the participants by groups

	Control Mean +- SEM (N)	Diyabetes Mellitus Group		*p**
		NP(-)	NP(+)	
	Median (min-max)	Median (min-max)	Median (min-max)	
Age (year)	47.0 (35.0-67.0)	55.5 (37.0-70.0)	57.0 (40.0-73.0)	***0.0001***
BMI (kg/m²)	26.5 (20.5-51.5)	27.8 (22.8-47.9)	28.4 (21.1-38.8)	0.065
MNSI A. (puan)	1.0 (0.0-4.0)	1.0 (0.0-3.0)	3.0 (0.0-8.0)	***0.0001***
MNSI B. (puan)	0.0 (0.0-1.0)	1.0 (0.0-2.0)	3.0 (2.5-10.0)	***0.0001***
DM duration(year)**	-	5.5 (0.0-43.0)	11.0 (1.0-30.0)	***0.0001****
HbA1c (%) (mmol/mol)**	-	6.8 (5.3-10.7) 51(34-93)	8.1 (5.9-11.7) 65(41-104)	**0.001****
FPG (mg/dl)	95.0 (83.4-112.3)	132.3 (90.7-342.3)	158.8 (91.9-344.0)	**0.0001**
T.chol (mg/dl) 207.9 (124.2-341.0)		184.0 (95.6-301.1)	193.8 (121.0-298.2)	0.153
LDL (mg/dl)	142.0 (82.3-280.7)	129.1 (52.6-228.8)	143.0 (62.4-224.7)	0.118
HDL (mg/dl)	52.0 (31.2-87.6)	46.2 (14.6-112.2)	50.1 (36.3-85.4)	0.069
Trigliserid (mg/dl)	123.8 (36.4-1394.4)	157.1 (45.9-740.0)	154.8 (60.5-484.4)	0.094
Creatinin (mg/dl)	0.75 (0.54-1.25)	0.83 (0.53-1.26)	0.81 (0.48-1.42)	0.448
GFR (ml/dk/1.73m²)	95.4 (47.3-134.3)	91.9 (46.9-128.3)	94.9 (45.2-148.7)	0.444
ALT (u/L)	19.1 (8.2-47.8)	26.2 (10.7-57.6)	18.8 (5.1-66.8)	***0.014***
Vitamin B_{12} (pg/ml)	345.7 (149.0-672.3)	346.7 (134.0-1670.0)	499.4 (183.7-1650)	***0.015***
TSH (µIU/ml)	2.1 (0.4-14.6)	1.8 (0.4-22.9)	1.9 (0.7-7.4)	0.683

Abbreviations: NP = neuropathy, BMI = Body mass index, MNSI = Michigan neuropathy screening instrument, HbA1c = Glycosylated hemoglobin, FPG = Fasting plasma glucose, T.chol = Total cholesterol, LDL = Low density lipoprotein, HDL = High density lipoprotein, GFR = Glomerular filtration rate, ALT = Alanine transaminase, TSH = Thyroid stimulating hormone
p *: Kruskal-Wallis test between groups [Control. DM(+)NP (-). DM(+)NP(+)]
**: Mann-Whitney U test [between DM (+) NP (-). DM (+) NP (+) groups]

Besides, individuals out of our selected age range and those who did not provide informed consent were excluded from the study. Patients with neuropathy, diagnosed vitamin B12 deficiency, and thyroid dysfunction were also not included. Those with a clinical presentation of atypical neuropathy (motor loss more severe than sensory loss, asymmetry in symptoms and signs, and rapid progression) were also excluded.

Data Collection and Evaluation of Participants

First, 24-hour urine samples were collected at home from all participants. Participants did not collect their first urine void in the morning but proceeded to collect all their urine during the day and night. They were told to sleep in a dark room without eating that night and collect night urine, and then to collect their first urine void the next morning. The amount of 24-hour urine they collected was measured and noted; 100 cc of collected urine was poured into a sample container and stored at −80°C until the date of evaluation.

Height and weight measurements of all participants were performed. Blood samples were taken from all participants between 08:00 and 10:00 AM after at least 8 hours of fasting.

Fasting plasma glucose (FPG) was measured using the photometric method; LDL, HDL, total cholesterol (T.chol), triglyceride, and ALT were measured using the enzymatic colorimetric method; creatinine was measured using the kinetic colorimetric method; and urine microalbumin was measured using the immunoturbidimetric method on a clinical chemistry analyzer (cobas c6000 c501 device, Roche Hitachi, Switzerland). Vitamin B12 (197-771 pg/mL) and thyroid-stimulating hormone (TSH) (0.27-4.20 µIU/mL) were measured using the electrochemiluminescence immunoassay method on an immunochemistry analyzer (cobas 6000 e601 device, Roche Hitachi, Switzerland). Glycosylated hemoglobin (HbA1c) was measured on a glycohemoglobin analyzer (ADAMS A1c HA-8160, Arkray) according to the principles of high-performance liquid chromatography. Participants' GFR levels were calculated using the Modification of Diet in Renal Disease Study formula.

An information form containing medical history, medications, other diseases, and complications for all participants was filled in.

Tab. 3. Distribution of 6-SOM (µg / day) values of the participants by groups

	6-SOM Median (min-max)	*p*
Control	51.7 (7.8-98.2)	
DM(+)NP(-)	34.9 (3.3-74.6)	**0.023***
DM(+)NP(+)	29.3 (6.4-76.6)	
All Diabetics	32.0 (3.3-76.6)	**0.006****

Abbreviations: 6-SOM = 6- sulfatoxymelatonin, SD = Standart deviation, min = Minimum, max = Maximum, DM = Diiabetes mellitus, NP = Neuropathy
*: Kruskal-Wallis test between groups [Control, DM(+)NP (-), DM(+)NP(+)]
**: Mann-Whitney U test [between Control and All diabetics groups]

Evaluation of 6-Sulfatoxymelatonin in Urine Samples

Urine samples were stored at −80°C until the date of evaluation. The study began after the kit content and urine samples reached room temperature. Measurement of 6-SOM was performed using an ELISA kit (Cat No 40-371-25006, GenWay Biotech Inc., USA) with an ELISA microplate reader (ELx800, Biotek, USA).

Michigan Neuropathy Screening Instrument

The MNSI is widely used for DSPN evaluation in DM. It consists of 2 parts: a self-administered questionnaire (MNSI A.) and a physical examination (MNSI B.). In the first part, patients answer 15 questions about their neuropathy symptoms, and abnormal results are scored. In the second part, a physical examination is performed, which includes inspection of both lower extremities, evaluating ankle reflex, toe–toe vibration perception, and monofilament perception; abnormal findings are scored (Feldman *et al.* 1994).

The MNSI was applied to all participants, including those in the control group, by the same and experienced clinician.

Those with a total score of 7 points or more out of 15 questions in the first part (questionnaire) were accepted as having DSPN. In the second part, 2.5 points (out of 10 points) and above was accepted as DSPN.

Statistical Analysis

Statistical analysis of the data was performed using SPSS 20. Number, percentage, mean, standard deviation, median, and minimum and maximum levels were used in the presentation of descriptive data. Before the comparison of continuous variables, a normal distribution test (Kolmogorov–Smirnov test) was used first. Then, Mann–Whitney U and Kruskal–Wallis tests were used to compare continuous variables. The Mann–Whitney U test with Bonferroni correction was used to compare paired groups (post hoc test) in the Kruskal–Wallis test. Correlation analysis of 6-SOM levels with age, body mass index (BMI), MNSI scores, duration of DM, and other laboratory levels was performed with Spearman correlation analysis. HbA1c and DM duration data were used only for diabetic participants. Factors affecting the 6-SOM levels of participants were

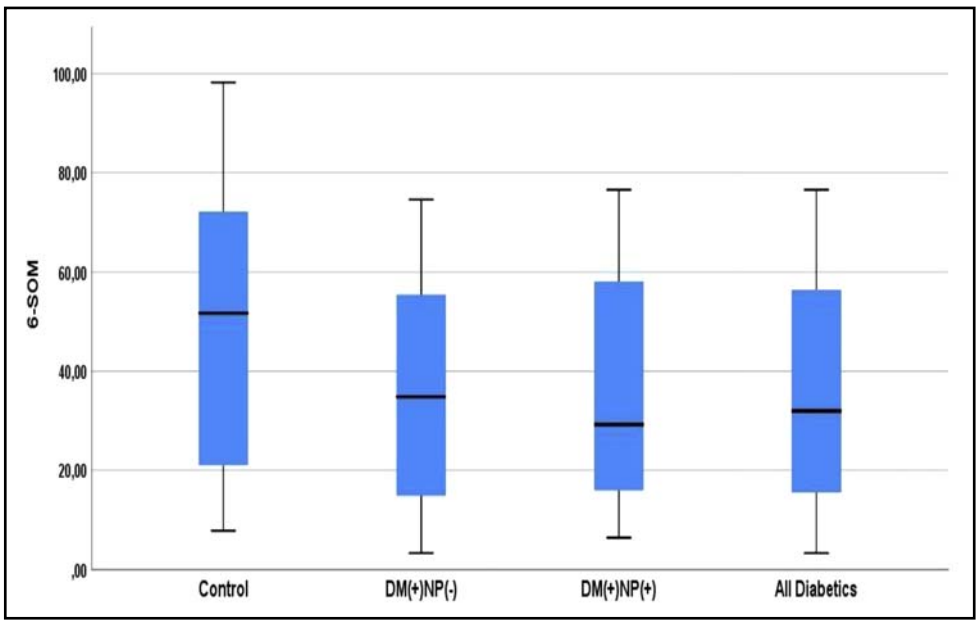

Fig. 1. Distribution of 6-SOM (µg / day) levels of the participants by groups
Abbreviations: 6-SOM = 6-sulfatoxymelatonin, DM = Diabetes mellitus, NP = Neuropathy

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Tab. 4. Distribution of diabetic participants by neuropathy groups according to antidiabetic drug use

	OAD (n)	Insulin±OAD* (n)	Total
DM(+)NP(-)	35	9	44
DM(+)NP(+)	24	19	43
Total	59	28	87
p**		***0.018***	

Abbreviations: OAD = Oral antidiabetic drug, DM = Diabetes mellitus, NP = Neuropathy
*: insulin users with or without OAD
**: Chi-square test

evaluated using multiple linear regression analysis. In the analysis, 6-SOM value was taken as the dependent variable, whereas age, BMI, use of antidiabetic medication, FPG, GFR, HbA1c, and MNSI B. score were taken as the independent variables. The enter method was used in the analysis, revealing R = 0.481, R2 = 0.232, and Durbin–Watson = 2.246. Regarding statistical significance, $P < 0.017$ in the post hoc tests and $P < 0.05$ in other tests were accepted.

RESULTS

A total of 127 participants were examined in this study; 40 (31.5%) were in the control group, 44 (34.6%) were in the DM(+)NP(-) group, and 43 (33.9%) were in the DM(+)NP(+) group. Moreover, 44.1% of the participants were men and 55.9% were women. Their median age was 53.0 years (35.0 to 73.0). Their median BMI was 27.8 (20.5 to 51.5) kg/m^2. The median level of HbA1c (for diabetics) was 7.1% (54 mmol/mol), that of FPG was 124.1 mg/dl, that of creatinine was 0.81 mg/dl, that of GFR 94.1 was mL/min/1.73 m^2, that of ALT was 20.7 u/L, that of vitamin B12 was 364.1 pg/mL, and that of TSH was 1.9 µIU/mL (Table 1).

The distribution of age, BMI, MNSI scores, laboratory values, DM duration, and HbA1c levels of diabetic participants according to the groups are presented in Table 2. A significant difference was found between the 3 groups in median age, MNSI A. and B. scores, and FPG, ALT, and vitamin B12 values ($p < 0.05$). Significant differences also existed in median DM duration and HbA1c values between the DM(+)NP(-) and DM(+)NP(+) groups ($p < 0.05$). It was observed that FPG levels increased toward the control, DM(+) NP(-), and DM(+)NP(+) groups, respectively ($p = 0.0001$). MNSI scores were higher in the DM(+)NP(+) group compared with other groups ($p = 0.0001$, MNSI A.; $p = 0.0001$, MNSI B.). Considering the ages of the groups in our sample, higher median values were found in the diabetic groups compared with the control group ($p = 0.0001$). DM duration and HbA1c medians were higher in the DM(+)NP(+) group compared with the DM(+)NP(-) group ($p = 0.0001$, DM duration; $p = 0.001$, HbA1c).

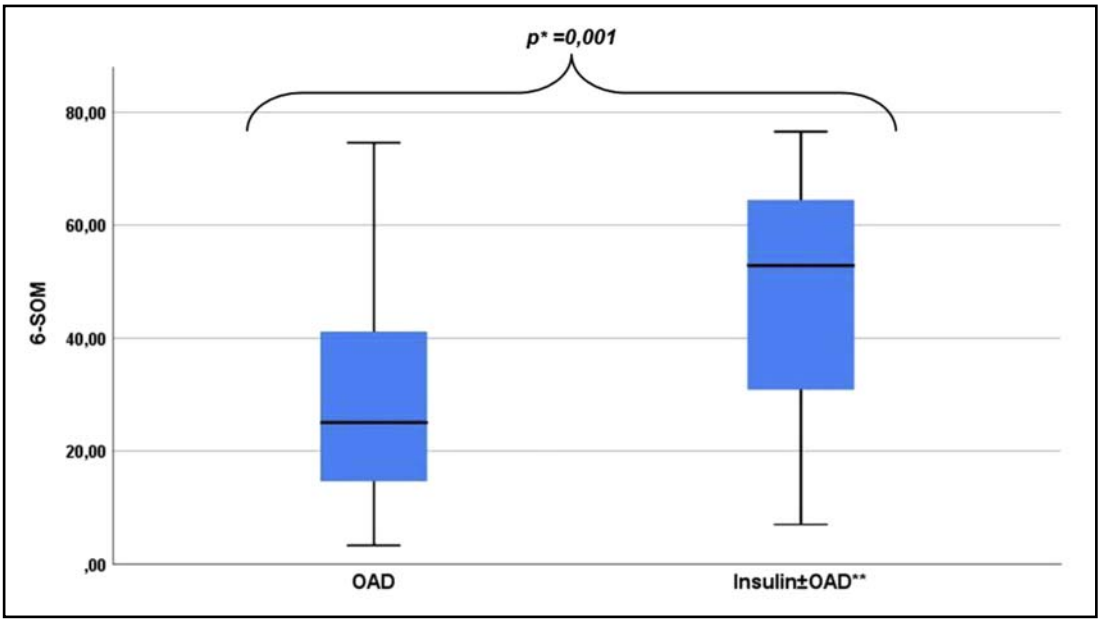

Fig. 2. Distribution of 6-SOM (µg/day) levels in diabetics according to the antidiabetic drug group
Abbreviations: 6-SOM = 6-sulfatoxymelatonin, OAD = Oral antidiabetic drug

Tab. 5. Correlation analysis of 6-SOM (µg / day) levels of all participants with other variables

	6-SOM	
	r*	P
Age (year)	0.019	0.832
Body Mass Index (kg/m^2)	-0.101	0.258
MNSI A. (point)	-0.087	0.333
MNSI B. (point)	-0.047	0.603
DM duration (year)**	0.028	0.798
HbA1c (%)**	0.134	0.215
FPG (mg/dl)	***-0.211***	***0.017***
LDL (mg/dl)	0.034	0.706
Trigliserid (mg/dl)	0.033	0.715
HDL (mg/dl)	0.075	0.410
T.chol (mg/dl)	0.136	0.133
Creatinin (mg/dl)	-0.101	0.258
GFR(ml/dk/1.73m2)	0.079	0.377
ALT (u/L)	-0.058	0.515
Vitamin B_{12}(pg/ml)	0.130	0.156
TSH(µIU/ml)	0.110	0.219
Microalbuminuria (mg/day)	***0.209***	***0.023***

Abbreviations: 6-SOM = 6-sulphatoxymelatonin, MNSI = Michigan neuropathy screening instrument, DM = Diabetes mellitus, FPG = Fasting plasma glucose, LDL = Low density lipoprotein, HDL = High density lipoprotein, T.chol = Total cholesterol, GFR = Glomerular filtration rate, ALT = Alanine transaminase, TSH = Thyroid stimulating hormoner
*: Spearman correlation analysis (Correlation coefficient)

6-Sulfatoxymelatonin Levels

A significant difference was found in the distribution of 6-SOM levels of participants according to their groups (p = 0.023). The median level of the control group was 51.7 µg/day, whereas that of the DM(+)NP(-) group was 34.9 µg/day and that of the DM(+)NP(+) group was 29.3 µg/day (Table 3). The medians of the 6-SOM values of the groups are plotted in Figure 1. The comparison conducted with post hoc tests revealed that the difference originated between the control and DM(+)NP(-) groups (p = 0.011). No significant difference existed between the other pairwise comparisons of the groups [p = 0.792, DM(+)NP(-) and DM(+)NP(+); p = 0.026, control and DM(+)NP(+)].

The median 6-SOM level of all 87 diabetic participants was 32.0 µg/day, which was significantly lower than that of the control group (p = 0,006) (Table 3). The median 6-SOM values of the diabetic and control groups are plotted in Figure 1.

Of all the diabetic participants, 59 (67.8%) used only oral antidiabetic drug (OAD), while 28 (32.2%) used insulin with or without OAD (insulin ± OAD). The number of insulin users was 9 in the DM(+)NP(-) group and 19 in the DM(+)NP(+) group; the number of patients using only OADs was 35 in the DM(+)NP(-) group and 24 in the DM(+)NP(+) group. The number of participants using insulin was significantly higher in the DM(+)NP(+) group (p = 0.018) (Table 4).

A significant difference existed in the distribution of 6-SOM levels of diabetic participants according to insulin usage. The median of the group using only OAD was 25.1 µg/day, whereas that of the group using insulin was 52.9 µg/day; 6-SOM levels were significantly higher among insulin users (p = 0.001) (Figure 2).

The correlation between the 6-SOM levels of all participants and other variables was examined. No significant correlation was found between 6-SOM levels and age, BMI, DM duration, HbA1c, MNSI A. and B. scores, LDL, HDL, T.chol, triglyceride, creatinine, GFR, ALT, vitamin B12, and TSH levels. There was a weak negative correlation between 6-SOM and FPG (r = −0.211, p = 0.017) and a weak positive correlation between 6-SOM and microalbuminuria (r = 0.209, p = 0.023) (Table 5).

In the regression analysis, which examined the factors affecting the 6-SOM levels of the participants,

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Tab. 6. Evaluation of the factors affecting the 6-SOM (µg / day) levels of the participants by multiple linear regression analysis

	B	Beta	%95 CI	*p**
Constant	**-62.140**	**---**	**(-120.371 - -3.908)**	**0.037**
Age (year)	***0.826***	***0.296***	***(0.227 - 1.426)***	***0.007***
Insulin usage	***14.584***	***0.305***	***(3.857 - 25.311)***	***0.008***
MNSI B	-0.561	-0.048	(-3.141 – 2.020)	0.667
VKİ (kg/m^2)	-0.055	-0.013	(-0.922 – 0.811)	0.900
FPG (mg/dl)	-0.067	-0.176	(-0.176 – 0.042)	0.226
GFR (ml/dk/1.73m^2)	***0.248***	***0.229***	***(0.018 – 0.478)***	***0.035***
HbA1c (%)	3.039	0.206	(-1.547 – 7.626)	0.191

Dependent variable: 6-SOM value
Independent variable: Age, İnsulin usage, Michigan neuropathy screening instrument (MNSI B).
Abbreviations: BMI = Body mass index , FPG = fasting plasma glucose, GFR = glomerular filtration rate , HbA1c = glycosylated hemoglobin,CI = confidence interval, *p**: Multiple linear regression (enter method)

the independent variables were determined as age, BMI, FPG, HbA1c, GFR, MNSI B. score, and insulin use status. The analysis determined that R = 0.481, R2 = 0.232, and Durbin–Watson = 2.246. The regression analysis revealed that age [B = 0.826, 95% confidence interval (CI) = 0.227 to 1.426], insulin use (B = 14.584, 95% CI = 3.857 to 25.311) and GFR (B = 0.248, 95% CI = 0.018 to 0.478) variables and 6-SOM levels were significantly correlated. Each unit increase in age and GFR caused increases of 0.826 and 0.248 units in 6-SOM level, respectively. It was determined that the use of insulin caused an increase of 14.584 units in 6-SOM level compared with the use of OAD alone (Table 6).

DISCUSSION

We found that the amount of urinary 6-SOM, the main metabolite of melatonin, was significantly lower in diabetic patients compared with controls; however, the presence of peripheral neuropathy did not cause a difference in the amount of urinary 6-SOM in diabetic patients.

Melatonin is a difficult molecule to measure. When the aim is to evaluate serum melatonin levels, it is necessary to examine night serum measurements several times, which is quite challenging. As an alternative method to obtain an idea about plasma melatonin levels, the main urinary metabolite 6-SOM can be evaluated. Urine 6-SOM values correlate with plasma melatonin values (Nowak *et al.* 1987, Bojkowski & Arendt 1990, Pääkkönen *et al.* 2006). Many studies aiming to evaluate melatonin levels have been conducted using urine 6-SOM measurements (Hidalgo *et al.* 2011, Abeysuriya *et al.* 2018, Pérez-Caraballo *et al.* 2018, Reutrakul *et al.* 2018).

In some of these studies, the 6-SOM/creatinine ratio in spot urine in the morning was used, whereas in others measurements were made by collecting urine for 24 hours as we did (Chen *et al.* 2014, Caumo *et al.* 2019). We prefer to use this urine collection method as it directly provides the daily 6-SOM level without creatinine correction.

The relationship between melatonin and DM was examined long ago by O'Brien (O'Brien *et al.* 1986) in 8 diabetic patients with autonomic neuropathy, 8 diabetic patients without neuropathy, and 7 healthy participants. It was found that the night melatonin peak was significantly lower in all diabetic participants than that in the control group. On the other hand, no significant difference existed between diabetic participants' melatonin levels according to the presence of autonomic neuropathy.

Tutuncu (Tutuncu *et al.* 2005) examined melatonin levels in the serum of 36 participants with type 2 DM and 13 healthy controls at certain hours, and found that nighttime melatonin levels were lower in diabetic patients and daytime melatonin levels were similarly low in both groups. Melatonin levels were also compared between 18 diabetic patients diagnosed with autonomic neuropathy and 7 without autonomic neuropathy. Melatonin levels were found to be significantly lower in diabetics with autonomic neuropathy than in diabetics without neuropathy.

In these 2 studies, unlike our study, melatonin was measured at certain hours from the serum of participants instead of urinary 6-SOM measurement. Although different from our study in terms of method, the findings of O'Brien's study were similar to ours. However, we investigated the relationship between melatonin and DSPN rather than autonomic neuropathy. Studies investigating the relationship between DPN and melatonin are scant. To our knowledge, this is the first human study to examine the relationship between DPN and melatonin.

Tutuncu (Tutuncu *et al.* 2005) examined the relationship between melatonin levels and autonomic neuropathy and found that it was significantly lower in diabetics

with autonomic neuropathy than in those without neuropathy. However, they only examined 18 patients with neuropathy and 7 without neuropathy. The small study sample is a crucial limitation of the present study.

The experimental study findings concerning the relationship between melatonin and DM were similar to human reports that melatonin levels were lower in DM (Do Carmo Buonfiglio *et al.* 2011, Amaral *et al.* 2014, Cardinali & Vigo 2017, Mok *et al.* 2019, Pourhanifeh *et al.* 2020).

In addition, numerous animal studies have suggested that melatonin administration can contribute to neuroprotective mechanisms. Negi *et al.* (Negi *et al.* 2010) showed that melatonin administration improved neuronal pain parameters and corrected some neuronal functions in streptozotocin-induced diabetic rats. They associated these improvements with the inhibition of ROS-related damage and peroxynitrite, which is responsible for apoptotic neuronal damage. In another experimental diabetic neuropathy model (Negi *et al.* 2011), it was shown that melatonin administration decreased the level of NF-κB (responsible for inflammation) and increased the level of Nrf2, a molecule that increases antioxidant expression. The same study found that nerve conduction velocity and blood flow increased after melatonin treatment.

Metwally *et al.* (Metwally *et al.* 2018) evaluated the therapeutic potential of melatonin in diabetic central neuropathy in a streptozotocin-induced diabetic rat model. They suggested that melatonin administration exhibited therapeutic effects against neurodegeneration caused by hyperglycemia.

Seyit *et al.* (Seyit *et al.* 2016) studied the effects of melatonin and α-lipoic acid administration on the tibial nerve in diabetic rats and found that both significantly increased nerve conduction velocity and amplitude.

Another experimental animal study (Onphachanh *et al.* 2017) stated that silencing PINK1 expression due to hyperglycemia caused increased mitochondrial ROS production as well as activation of the apoptotic process. Furthermore, the study suggested that melatonin administration stimulated PINK1 expression and prevented neuronal cell apoptosis under hyperglycemic conditions.

Although melatonin administration has been found to possibly have beneficial effects on neuropathy in most animal models, none have addressed melatonin levels in neuropathic rats. Besides, human research on this subject is lacking.

We found a weak negative correlation between urinary 6-SOM levels and FPG. As FPG increases, the decreasing 6-SOM levels may indicate that patients in diabetic processes may be deprived of the antioxidant and anti-inflammatory effects of melatonin.

Kurhaluk (Kurhaluk *et al.* 2017) investigated the relationship between melatonin and glucose homeostasis. The experimental study showed that melatonin administration lowers HbA1c and protects the liver, kidney, and muscle cells from lipid peroxidation and oxidant damage. In a clinical study conducted by Reutrakul, low urinary 6-SOM levels were found in diabetic patients with obstructive sleep apnea syndrome (OSAS). In the same study, it was stated that a relationship exists between long DM duration and high HbA1c levels, and low urinary 6-SOM levels (Reutrakul *et al.* 2017). However, it is known that high HbA1c levels are detected in patients with OSAS (Tasali *et al.* 2008), and that evaluating the relationship between DM duration and high HbA1c and low melatonin levels independently of OSAS is difficult. Unlike these studies, we did not find a significant correlation between DM duration, HbA1c levels, and urinary 6-SOM levels.

Some experimental studies have shown that insulin administration may enhance melatonin levels, but no such data exists on humans. In one experimental study, low retinal melatonin and arylalkylamine N-acetyltransferase activity was observed in diabetic rats, and this decrease was prevented by insulin treatment (Do Carmo Buonfiglio *et al.* 2011). Our regression analysis demonstrated that the use of insulin was related to a significant increase in 6-SOM levels. Furthermore, 6-SOM levels in insulin users were approximately twice as high as those in diabetics who did not use insulin. The effect of insulin use on melatonin should be elucidated with more comprehensive human studies.

Contrary to the positive effect of insulin on melatonin, many studies have shown that the administration of melatonin may reduce insulin levels (Peschke *et al.* 2013). Melatonin exerts its regulatory effect on insulin secretion through MT1 and MT2 membrane receptors on pancreatic β cells, and melatonin may be responsible for the formation of the daily circadian rhythm of insulin (Mayo *et al.* 2018). A recent experimental study showed that oral melatonin decreases insulin levels in prediabetic rats but does not affect plasma glucose levels (Wang *et al.* 2018).

Wang *et al.* suggested that melatonin protects the glomeruli from diabetic and oxidative damage in rats and prevents fibrosis in the kidneys (Djordjevic *et al.* 2018). A few animal models have demonstrated that the application of melatonin may improve some changes in diabetic nephropathy (Ebaid *et al.* 2020, Pourhanifeh *et al.* 2020). It is difficult to find research about the relationship between GFR and 6-SOM in the literature. We found that as urinary 6-SOM levels increased in humans, GFR levels also increased. Increased GFR levels can be explained by the protective effects of melatonin. However, in our study, the amount of microalbuminuria was weakly and positively correlated with 6-SOM levels in the correlation analysis, which is difficult to explain. Special studies on diabetic nephropathy may be more revealing in this regard.

One limitation of our study was that our participants collected their 24-hour urine at home. Written and verbal information about the urine collection was

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given to all of the participants, but some may not have collected urine in accordance with the instructions. Another limitation was the abnormal age distribution between the groups due to randomization during recruitment. The median ages of the DM(+)NP(-) and DM(+)NP(+) groups were similar and higher compared with that of the control group. It has been reported that melatonin secretion decreases with advancing age (Karasek 2004). However, in our study, the age difference was not large, and it may not have had a significant effect on melatonin levels. Moreover, multiple regression analysis demonstrated that 6-SOM levels were weakly and positively correlated with increasing age.

It can be said that lower 6-SOM levels result in diabetics not being affected by age, as we observed increasing 6-SOM levels with increasing age in our multiple regression analysis. The median ages of the DM(+)NP(-) and DM(+)NP(+) groups were similar, and the age variable had no effect in the comparison of 6-SOM levels between these 2 groups. In conclusion, the effect of age can be ignored when comparing 6-SOM levels in the diabetic neuropathic patients in our sample.

CONCLUSION

We concluded that the diabetic process and hyperglycemia in humans reduce melatonin levels, but the presence of neuropathy in diabetic patients does not affect melatonin levels. Insulin may improve melatonin levels in diabetics.

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Author Contributions

Kemal Kurt and Ersen Karakilic designed the study and enrolled patients. Ozgul Ocak performed MNSI. Suat Cakina performed measurement of 6-SOM. All Authors managed data and wrote/reviewed/edited the manuscript together.

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Conflicts of interest/Competing interests

The authors have no multiplicity of interest to disclose

Ethical approval

The study was approved by the Research Ethics Committee of our institution.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Availability of data and material

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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