ORIGINAL RESEARCH

The Effects of Melatonin Supplementation on Parameters of Mental Health, Glycemic Control, Markers of Cardiometabolic Risk, and Oxidative Stress in Diabetic Hemodialysis Patients: A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective: This study evaluated the effects of melatonin supplementation on parameters of mental health, glycemic control, markers of cardiometabolic risk, and oxidative stress in diabetic hemodialysis (HD) patients.

Design: A randomized, double-blind, placebo-controlled clinical trial was conducted in 60 diabetic HD patients, 18-80 years of age. Participants were randomly divided into 2 groups to take either melatonin (2 x 5mg/day) (n = 30) or placebo (n = 30) 1 hour before bedtime for 12 weeks. The effects of melatonin on mental health, metabolic status, and gene expression related to metabolic status were assessed using multiple linear regression adjusting for age and BMI.

Results: Melatonin supplementation significantly decreased Pittsburgh Sleep Quality Index ($P = .007$), Beck Depression Inventory index ($P = .001$), and Beck Anxiety Inventory index ($P = .01$) compared with the placebo. Additionally, melatonin administration significantly reduced fasting plasma glucose ($\beta = -21.77$ mg/dL, 95% CI −33.22 to −10.33, $P < .001$), serum insulin levels ($\beta = -1.89$ μIU/mL, 95% CI −3.34 to −0.45, $P = .01$), and homeostasis model of assessment-insulin resistance ($\beta = -1.45$, 95% CI −2.10 to −0.80, $P < .001$), and significantly increased the quantitative insulin sensitivity check index ($\beta = 0.01$, 95% CI 0.007-0.02, $P < .001$) compared with placebo treated subjects. In addition, melatonin administration resulted in a significant reduction in serum high sensitivity C-reactive protein ($\beta = -1.92$ mg/L, 95% CI −3.02 to −0.83, $P = .001$) and plasma malondialdehyde ($\beta = -0.21$ μmol/L, 95% CI −0.36 to −0.06, $P = .005$); also, significant rises in plasma total antioxidant capacity ($\beta = 253.87$ mmol/L, 95% CI 189.18-318.56, $P < .001$) and nitric oxide levels ($\beta = 2.99$ μmol/L, 95% CI 0.71-5.28, $P = .01$) were observed compared with the placebo.

Conclusion: Overall, melatonin supplementation for 12 weeks to diabetic HD patients had beneficial effects on mental health, glycemic control, inflammatory markers, and oxidative stress.

Introduction

DIABETES MELLITUS IS a major risk factor for chronic kidney diseases and many affected people develop diabetic nephropathy, the leading cause of end-stage renal disease (ESRD).[1] Approximately 95% of diabetic patients, especially type 2 diabetes mellitus, with ESRD receive hemodialysis (HD).[1] Cardiovascular disease (CVD) remains the most common cause of death in these patients.[2] In addition, malnutrition, increased biomarkers of inflammation, and oxidative damage are the most

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prevalent consequences in HD patients.[3] Prior studies have documented that both malnutrition and inflammation are correlated with atherosclerosis progression and with an increased risk of all-cause morbidity and mortality in HD patients.[4] Moreover, metabolic disorders including insulin resistance, hyperinsulinemia, dyslipidemia, and increased oxidative stress are prevalent in HD patients.[5,6]

Melatonin is an endogenous indoleamine that is synthesized and secreted by the pineal gland.[7] Both animal and human studies suggest that melatonin administration may improve components of metabolic syndrome, such as elevated glucose and insulin resistance, hypertension, dyslipidemia, and obesity.[8,9] In addition, epidemiologic studies have reported an inverse relationship between melatonin secretion and insulin resistance.[10,11] Some studies have also demonstrated the anti-inflammatory and antioxidant effects of melatonin.[12,13] In a study conducted by Quiroz et al.,[14] melatonin intake reduced oxidative stress, inflammation, proteinuria, and the progression of renal damage in rats with renal mass reduction. Oxidative stress and its constant companion, inflammation, play key roles in the pathogenesis of the progression of kidney injury.[15] Melatonin directly scavenge free radicals and stimulates the activity and expression of antioxidant enzymes.[16] It also decreases gene expression related to inflammatory markers.[17] On the other side, circadian rhythms have been demonstrated to be considerably disrupted in HD subjects.[18] Interestingly, melatonin production declines as renal function decreases[19] and in the majority of HD people, the nighttime surge of serum melatonin is even absent.[20]

Given the antioxidant and anti-inflammatory effects of melatonin, we hypothesized that melatonin might be beneficial in diabetic HD patients. The present study was, therefore, performed to evaluate the effects of melatonin administration on parameters of mental health, metabolic status, and gene expression related to metabolic status in these patients.

Methods

Trial Design and Participants

The current study is a randomized, double-blind, placebo-controlled clinical trial which was conducted with the aid of 60 diabetic HD patients, 18-80 years between February 2018 and June 2018. This intervention was done in accordance with the Declaration of Helsinki and informed consent was taken from all participants. Patients with infectious, inflammatory, and malignant diseases, those taking melatonin supplements, antioxidant, and/or anti-inflammatory supplements within 3 months prior to enrollment in the study, the night shift workers, and subjects taking immunosuppressive and antibiotic medications were not included in the current study.

Study Design

At the onset of the study, after balanced blocked randomization, all participants were allocated into two groups to take either melatonin or placebo. Patients were requested to continue their routine physical activity, and not to take any anti-inflammatory and antioxidant medications or supplements that might influence their nutritional status during the 12-week treatment. Consumption of melatonin and placebos throughout the study was checked by asking subjects to return the medication containers. In addition, a short message was sent to the cell phones of all patients every day to remind participants to use the desired supplements. A 3-day food records and physical activity records were completed by all participants at weeks 0, 4, 7, and 12 of the intervention. To obtain macro- and micronutrient intakes of participants based on these 3-day food diaries, Nutritionist IV software (First Databank, San Bruno, CA) was used.

Intervention

Patients were allocated into 2 groups to take either melatonin capsule (2×5 mg/day) ($n = 30$) or placebo ($n = 30$) 1 hour before bedtime for 12 weeks. Melatonin and its placebo were produced in the same shape and package by Zahravi Pharmaceutical Company (Tabriz, Iran).

Assessment of Anthropometric Measures

Body weight and height were quantified in an overnight fasting status using a digital scale (Seca, Hamburg, Germany) at baseline and after the 12-week intervention. BMI was calculated by weight and height measurements [weight (kg)/height (m^2)].

Clinical Measures

Quality of sleep was determined using Pittsburgh Sleep Quality Index (PSQI).[21] Beck Depression Inventory (BDI) was assessed using a self-compiled questionnaire of 21 items in multiple choice format.[22] Anxiety was measured using the Beck Anxiety Inventory (BAI)-21 questionnaire developed by Beck et al.[23] to determine the frequency of anxiety symptoms in adults.

Assessment of Outcomes

Insulin levels, the homeostasis model of assessment-insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI) were considered as primary outcomes and parameters of mental health, lipid profiles, and biomarkers of inflammation and oxidative stress were considered secondary outcomes. Before the onset and after the end of the intervention, 15 mL blood samples before dialysis session after weekend were obtained from each patient in an early morning after an overnight fast. Fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), and lipid profiles were quantified on the day of blood collection. Then, the samples were stored at $-80°C$ before analysis. Serum insulin and high sensitivity C-reactive

protein (hs-CRP) levels were quantified by the use of an enzyme-linked immunosorbent assay kit (DiaMetra, Milano, Italy; LDN, Nordhorn, Germany) with inter- and intra-assay coefficient variances (CVs) of lower than 7%. HOMA-IR and QUICKI were determined according to the standard formula.[24] HOMA-IR was calculated using the following formula[24]: [FPG (mg/dL) × fasting serum insulin (mU/mL)/405]. QUICKI was calculated using the following formula[24]: [1/log fasting serum insulin (mU/mL) + log FPG (mg/dL)]. HbA1c values in whole blood were quantified by Glycomat kit (BiocodeHycel, Massy, France) using the method of exchange chromatography. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify FPG, lipid profiles with inter- and intra-assay CVs less than 5%. The plasma total nitrite was estimated using the Griess method, total antioxidant capacity (TAC) by the method of ferric reducing antioxidant power developed by Benzie and Strain, total glutathione (GSH) using the method of Beutler et al.,[25] and malondialdehyde (MDA) concentrations were determined by the thiobarbituric acid reactive substances spectrophotometric test with inter- and intra-assay CVs of lower than 5%. Systolic and diastolic blood pressure was determined via a sphygmomanometer (ALPK2, Zhejiang, China). Blood pressures were quantified between 08:00 and 09:00 AM by the same investigator each time. Dialysis Kt/V was calculated using the 1993 Daugirdas equation.[26]

The questions of subjective global assessment questionnaire were also asked by the same person after 12 weeks of the intervention. Then, the subjective global assessment classifications were converted to numerical equivalents: a score of <10 points was regarded as well nourished; 10-17 points, at risk for malnutrition or mildly to moderately malnourished; and more than 17 points, severely malnourished.[27]

Isolation of Lymphocytes

Lymphocytes were extracted from participants' blood samples using 50% percoll (Sigma-Aldrich, Dorset, UK). Cell count and viability test were conducted using trypan blue, RNA, and DNA extraction.

RNA Extraction and Real-Time Polymerase Chain Reaction

RNX-plus kit (Cinnacolon, Tehran, Iran) was used to extract RNA from blood samples. RNA suspension was frozen at −20°C until complementary DNA was derived. Following the extraction of total RNAs from each sample, RNA quantification was performed using an ultra violet spectrophotometer. Each sample optical density 260/280 ratio was considered to be between 1.7 and 2.1, demonstrating no contamination with either protein or DNA. The isolated RNA was reverse transcribed to complementary DNA library, using moloney murine leukemia virus reverse transcriptase. Gene expressions of peroxisome proliferator-activated receptor gamma (PPAR-γ), low-density lipoprotein receptor (LDLR), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and transforming growth factor beta (TGF-β) were assessed by quantitative real-time polymerase chain reaction in peripheral blood mononuclear cells (PBMCs), using the Light Cycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection and Amplicon Kit (Table 1). Glyceraldehyde-3-phosphate dehydrogenase primers were used as a housekeeping gene. Primer Express Software (Applied Biosystems, Foster City, CA) and Beacon designer software (Takaposizt, Tehran, Iran) were used to design primers. Relative transcription levels were calculated using the method of Pffafi.

Sample Size

In this investigation, we used a randomized clinical trial sample size calculation formula where type 1 (α) and type 2 errors (β) were 0.05 and 0.20 (power = 80%), respectively. According to the previous trial,[9] we used 2.20 as the standard deviation and 1.75 as the change in mean (d) of HOMA-IR as a primary outcome. Based on the formula, we needed 25 participants in each group; after allowing for 5 dropouts in each group, the final sample size was 30 persons in each group.

Randomization

Randomization assignment was conducted using computer-generated random numbers. Randomization and allocation were hidden from the researchers and patients until the final analyses were completed. The randomized allocation sequence, enrolling participants, and allocating them to interventions were conducted by a trained nutritionist at the dialysis clinic.

Statistical Methods

The Kolmogorov-Smirnov test was done to determine the normality of data. To detect the differences in anthropometric measures, dietary intakes, and gene expression between 2 groups, we used the independent-samples t-test. Multiple linear regression model was used to assess treatment effects on study outcomes after adjusting for confounding parameters including age and BMI. The effect sizes were presented as the mean differences with 95% confidence intervals. P-values <.05 were considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL).

Results

In the melatonin group, a total of 4 participants were excluded from the study: 2 patients were excluded because of changes in treatment protocol and 2 patients were unwilling to continue for personal reasons. In the placebo group, 3 patients were excluded: 1 patient was excluded because of changes in treatment protocol and 2 patients were unwilling to continue for personal reasons. Finally,

Table 1. Specific Primers Used for Real-Time Quantitative PCR

Gene	Primer	Product Size (bp)	Annealing Temperature (°C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG R: TCTTCCTCTTGTGCTCTTGCTGG	126	61.3
TNF-α	F: GTCAACCTCCTCTCTGCCAT R: CCAAAGTAGACCTGCCCAGA	188	52
IL-8	F: GCAGAGGGTTGTGGAGAAGT R: ACCCTACAACAGACCCACAC	150	56
TGF-β	F: TTGAGACTTTTCCGTTGCCG R: CGAGGTCTGGGGAAAAGTCT	227	56
PPAR-γ	F: ATGACAGACCTCAGACAGATTG R: AATGTTGGCAGTGGCTCAG	210	54
LDLR	F: ACTTACGGACAGACAGACAG R: GGCCACACATCCCATGATTC	223	57

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-8, interleukin-8; LDLR, low-density lipoprotein receptor; PCR, polymerase chain reaction; PPAR-γ, peroxisome proliferator-activated receptor gamma; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta.

53 patients [melatonin (n = 26) and placebo (n = 27)] completed the study (Fig. 1). The compliance rate was high; more than 90% of capsules were taken during the course of the trial in both groups. No side effects were reported following the consumption of melatonin supplements in diabetic HD patients throughout the study. During the 12-week intervention period, no adverse event such as hypotension, CVD, and hyperglycemia has been reported.

Distribution of gender, mean age, height, baseline weight, and BMI as well as their means after intervention, and years of dialysis of study participants were not statistically different between the 2 groups (Table 2).

Based on the 3-day dietary records obtained throughout the treatment period, we found no significant change in dietary macro- and micro-nutrient intakes (data not shown).

After the 12-week intervention, melatonin supplementation significantly decreased PSQI ($\beta = -2.41$, 95% confidence interval [CI] −4.13 to −0.69, $P = .007$), BDI ($\beta = -3.52$, 95% CI −5.50 to −1.55, $P = .001$), and BAI ($\beta = -1.92$, 95% CI −3.51 to −0.33, $P = .01$) compared with the placebo (Table 3). Additionally, melatonin supplementation significantly reduced FPG ($\beta = -21.77$ mg/dL, 95% CI −33.22 to −10.33, $P < .001$), serum insulin levels ($\beta = -1.89$ μIU/mL, 95% CI −3.34 to −0.45, $P = .01$), HOMA-IR ($\beta = -1.45$, 95% CI −2.10 to −0.80, $P < .001$), and HbA1c ($\beta = -0.58$%, 95% CI −1.16 to −0.002, $P = .04$), and significantly increased QUICKI ($\beta = 0.01$, 95% CI 0.007-0.02, $P < .001$) compared with the placebo. In addition, melatonin administration resulted in a significant reduction in serum hs-CRP ($\beta = -1.92$ mg/L, 95% CI −3.02 to −0.83, $P = .001$) and plasma MDA ($\beta = -0.21$ μmol/L, 95% CI −0.36 to −0.06, $P = .005$); also, significant rises in plasma TAC ($\beta = 253.87$ mmol/L, 95% CI 189.18-318.56, $P < .001$) and NO levels ($\beta = 2.99$ μmol/L, 95% CI 0.71-5.28, $P = .01$) were observed compared with the placebo. Melatonin supplementation did not change other metabolic parameters.

Melatonin supplementation upregulated gene expression of PPAR-γ ($P = .03$), and downregulated gene expression of IL-8 ($P = .03$) and TNF-α ($P = .008$) in PBMCs of subjects with diabetic HD, compared with the placebo; melatonin did not affect gene expression of LDLR and TGF-β (Fig. 2).

Discussion

In the current study, we investigated the effects of melatonin supplementation on metabolic profiles among diabetic HD subjects. We found that melatonin supplementation for 12 weeks to diabetic HD patients had favorable effects on BDI, BAI, PSQI index, FPG, insulin levels, HOMA-IR, HbA1c, QUICKI, hs-CRP, total nitrite, TAC and MDA, and gene expression of IL-8, TNF-α, and PPAR-γ, but did not affect other metabolic profiles and gene expression of LDLR and TGF-β. Several studies evaluated the effects of melatonin supplementation on metabolic profiles in patients with metabolic disorders. Although the efficacy of melatonin has been examined in diabetic patients with CHD,[9] we are aware of no study examining the effects of melatonin supplementation on metabolic profiles in diabetic HD subjects. However, we have previously shown that melatonin intake at a dosage of 10 mg/day for 12 weeks in diabetic patients with CHD had beneficial effects on plasma GSH, total nitrite, MDA, serum hs-CRP levels, glycemic control, HDL-cholesterol, total-/HDL-cholesterol ratio, blood pressures, and parameters of mental health,[9] in the current study, we demonstrated that melatonin supplementation for 12 weeks to diabetic HD subjects had favorable effects on BDI, BAI, PSQI index, FPG, insulin levels, HOMA-IR, HbA1c, QUICKI, hs-CRP, total nitrite, TAC, and MDA levels. In the present study, we did not observe any significant effect of melatonin intake on lipid profiles and blood pressures. Raygan et al.[9] did not examine the effect of melatonin supplementation on gene expression related to insulin, lipid, and inflammatory cytokines, while we

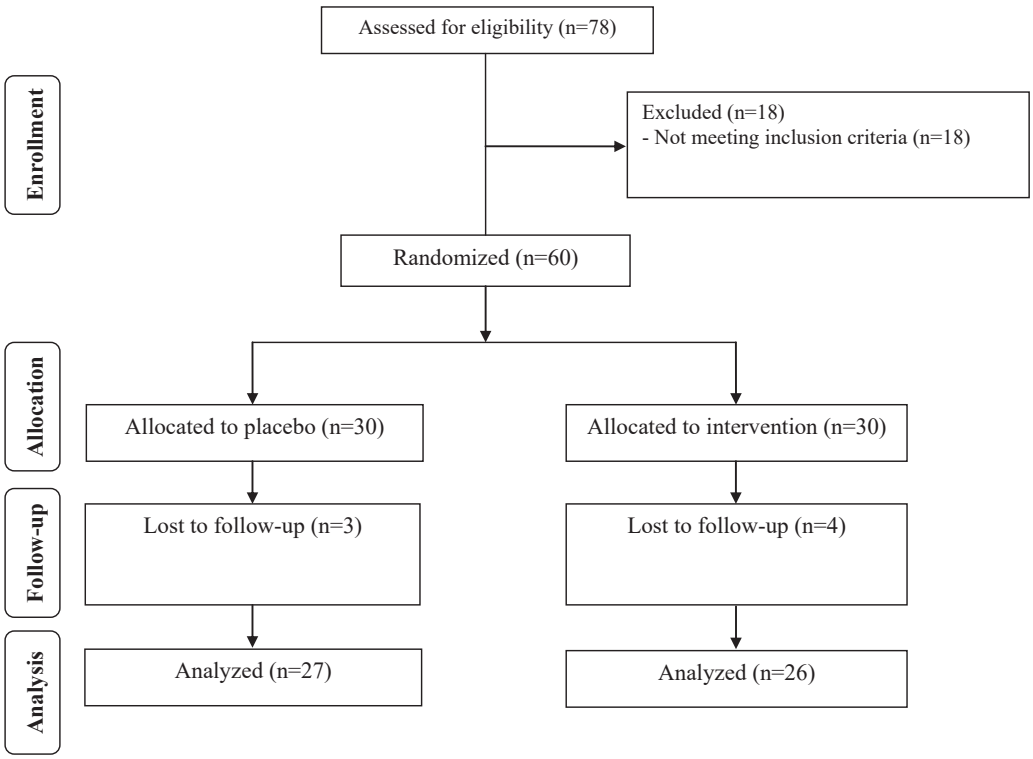

Figure 1. Summary of patient flow diagram.

assessed the effect of melatonin supplementation on these biomarkers as well.

Effects on Glycemic Control and Lipid Profiles

This study documented that melatonin administration for 12 weeks to diabetic HD patients significantly decreased FPG, insulin concentrations, HOMA-IR, and HbA1c, and significantly increased QUICKI while it did not affect lipid profiles. In addition, melatonin supplementation upregulated gene expression of PPAR-γ in PBMCs of patients with HD, but did not influence gene expression of LDLR. Both animal and human studies have previously investigated the effects of melatonin supplementation on glucose metabolism and lipid profiles. In a rat model of DM, melatonin administration for 30 weeks attenuated the development of hyperinsulinemia, hypertriglyceridemia, and hyperleptinaemia.[28] In addition,

Table 2. General Characteristics of Study Participants

	Placebo Group (n = 27)	Melatonin Group (n = 26)	*P* Value*
Gender (%)			
Male	20 (74.1)	18 (69.2)	.69†
Female	7 (25.9)	8 (30.8)	
Age (y)	64.1 ± 8.2	65.6 ± 13.1	.60
Height (cm)	168.9 ± 7.1	169.9 ± 8.5	.64
Weight at study baseline (kg)	75.0 ± 13.3	75.5 ± 14.8	.89
Weight at end-of-trial (kg)	74.9 ± 13.0	75.4 ± 14.7	.88
Weight change (kg)	−0.1 ± 1.1	−0.05 ± 1.3	.89
BMI at study baseline (kg/m^2)	26.4 ± 5.9	26.4 ± 4.7	.84
BMI at end-of-trial (kg/m^2)	26.4 ± 5.4	26.1 ± 4.6	.85
BMI change (kg/m^2)	−0.04 ± 0.4	−0.02 ± 0.4	.80
Dialysis *Kt/V* urea	1.9 ± 0.6	2.1 ± 0.7	.45
Residual renal function at study baseline (mL/min)	3.0 ± 0.6	3.2 ± 0.8	.41
Dialysis duration per week (h)	10.3 ± 2.4	10.6 ± 2.5	.68
Years on dialysis	3.7 ± 1.3	3.6 ± 1.2	.71

BMI, body mass index; SD, standard deviation.
Data are represented as mean ± SD.
*Obtained from independent *t*-test.
†Obtained from Pearson chi-squared test.

Table 3. Metabolic Profiles, Biomarkers of Inflammation, and Oxidative Stress at Study Baseline After the 12-Week Intervention in Patients With Diabetic Hemodialysis That Received Either Melatonin Supplements or Placebo

	Placebo Group (n = 27)		Melatonin Group (n = 26)		Difference in Outcome Measures Between Melatonin and Placebo Treatment Groups*	
Variables	Baseline	Wk 12	Baseline	Wk 12	β (95% CI)	*P* Value†
PSQI	10.7 ± 3.2	9.9 ± 3.4	11.1 ± 3.2	7.4 ± 2.8	−2.41 (−4.13 to −0.69)	.007
BDI score	26.2 ± 4.9	24.8 ± 5.0	27.3 ± 5.1	21.9 ± 3.9	−3.52 (−5.50 to −1.55)	.001
BAI score	18.6 ± 3.9	18.1 ± 4.5	20.8 ± 4.7	17.9 ± 4.3	−1.92 (−3.51 to −0.33)	.01
FPG (mg/dL)	125.3 ± 63.8	131.4 ± 62.5	127.1 ± 40.5	112.1 ± 36.7	−21.77 (−33.22 to −10.23)	<.001
Insulin (μIU/mL)	14.0 ± 5.0	14.7 ± 5.1	13.9 ± 4.2	12.6 ± 4.1	−1.89 (−3.34 to 0.45)	.01
HOMA-IR	4.0 ± 1.5	4.6 ± 2.2	4.4 ± 2.0	3.5 ± 1.7	−1.45 (−2.10 to −0.80)	<.001
QUICKI	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.02	0.32 ± 0.02	0.01 (0.007-0.02)	<.001
HbA1c (%)	6.9 ± 1.6	7.0 ± 1.8	7.2 ± 0.9	6.7 ± 1.0	−0.58 (−1.16 to −0.002)	.04
Triglycerides (mg/dL)	132.2 ± 59.7	125.7 ± 60.1	133.5 ± 69.6	115.4 ± 73.8	−11.50 (−28.16 to 5.15)	.17
VLDL-cholesterol (mg/dL)	26.4 ± 11.9	25.1 ± 12.0	26.7 ± 13.9	23.1 ± 14.8	−2.30 (−5.63 to 1.03)	.17
Total cholesterol (mg/dL)	124.6 ± 20.4	122.3 ± 24.7	128.7 ± 39.3	114.7 ± 39.7	−9.45 (−24.86 to 5.96)	.22
LDL-cholesterol (mg/dL)	67.0 ± 18.4	65.8 ± 23.6	71.2 ± 28.5	60.0 ± 32.3	−7.78 (−21.96 to 6.39)	.27
HDL-cholesterol (mg/dL)	31.2 ± 4.9	31.4 ± 5.3	30.8 ± 6.0	31.6 ± 6.1	0.64 (−1.29 to 2.58)	.50
hs-CRP (mg/L)	5.7 ± 3.2	5.8 ± 3.6	5.4 ± 2.8	3.7 ± 2.5	−1.92 (−3.02 to −0.83)	.001
Total nitrite (μmol/L)	33.1 ± 4.8	32.8 ± 6.1	36.9 ± 3.9	38.9 ± 4.0	2.99 (0.71-5.28)	.01
TAC (mmol/L)	786.5 ± 68.5	760.5 ± 73.9	889.92 ± 103.2	1064.5 ± 131.6	253.87 (189.18-318.56)	<.001
GSH (μmol/L)	498.0 ± 82.7	497.8 ± 86.7	479.7 ± 62.5	497.6 ± 62.8	9.81 (−22.81 to 42.44)	.54
MDA (μmol/L)	2.9 ± 0.6	3.0 ± 0.7	2.7 ± 0.2	2.6 ± 0.3	−0.21 (−0.36 to −0.06)	.005
SGA score	10.9 ± 2.0	11.3 ± 2.1	10.7 ± 2.4	10.5 ± 2.7	−0.59 (−1.38 to 0.20)	.20
Creatinine (mg/dL)	8.2 ± 3.0	8.1 ± 2.8	7.5 ± 3.0	7.4 ± 2.5	−0.12 (−0.61 to 0.36)	.61
BUN (mg/dL)	56.9 ± 18.4	56.2 ± 12.6	60.1 ± 27.9	59.4 ± 27.1	2.15 (−8.75 to 13.05)	.69
SBP (mm Hg)	124.8 ± 11.6	122.0 ± 8.6	123.3 ± 10.9	120.4 ± 9.9	−0.65 (−4.43 to 3.12)	.72
DBP (mm Hg)	74.6 ± 7.5	75.5 ± 7.2	77.5 ± 6.2	76.9 ± 7.2	0.60 (−3.33 to 4.50)	.75

BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; BMI, body mass index; BUN, blood urea nitrogen; CI, confidence interval; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GSH, total glutathione; HDL-cholesterol, high density lipoprotein-cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; hs-CRP, high sensitivity C-reactive protein; LDL-cholesterol, low density lipoprotein-cholesterol; MDA, malondialdehyde; PSQI, Pittsburgh Sleep Quality Index; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; SD, standard deviation; SGA, subjective global assessment; TAC, total antioxidant capacity; VLDL-cholesterol, very low density lipoprotein-cholesterol.

Data are represented as mean ± SD.

*"Outcome measures" refer to the change in values of measures of interest between baseline and week 12. β [difference in the mean outcome measures between treatment groups (melatonin group = 1 and placebo group = 0)].

†Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age, and baseline BMI).

melatonin supplementation in combination with exercise significantly improved insulin resistance and hypertension via upregulation of GLUT-4, PPAR-γ coactivator 1a, and mitochondrial biogenesis in type 2 diabetes mellitus rats.[29] Sun et al.[30] also demonstrated that taking melatonin at a dosage of 3 mg/day for 12 weeks by obese people significantly decreased insulin levels and HOMA-IR score. Recently, we documented that consuming melatonin for 12 weeks by diabetic patients with CHD had beneficial effects on glycemic control, HDL-cholesterol, and total-/HDL-cholesterol ratio.[9] In a meta-analysis, melatonin intake had significant effects on triglycerides and total cholesterol concentrations, but did not influence other lipid profiles.[31] However, supplementation with 6 mg/day of melatonin for 2 weeks increased VLDL-cholesterol, but did not change HDL- and LDL-cholesterol levels in postmenopausal women.[32] This difference in the results of various studies could be correlated with the differences in the type of studied diseases, dosage of melatonin used, and duration of the intervention. Based on previous clinical trials and meta-analyses, high dose (≥8 mg) and/or long period (>8 weeks) melatonin supplementation might show more benefits in improving glycemic indicators, serum lipids, inflammatory markers, and oxidative stress parameters,[33,34] while low dose and/or short period administration of melatonin did not affect metabolic profiles.[35-37] Due to lack of evidence about the appropriate dosage of melatonin for diabetic HD patients, we used dosage and duration of melatonin based on a previous study in diabetic patients with CHD.[9] Therefore, higher dose of melatonin supplementation or a longer intervention period in diabetic HD patients might ameliorate lipid profiles and other metabolic outcomes. Melatonin receptors (MT1 and MT2) have been documented to be present on the adipocytes and the beta cells of the human pancreatic islets. The effects of melatonin on insulin secretion may be mediated through these receptors.[38] Melatonin can decrease insulin secretion by inhibiting cyclic adenosine monophosphate and cyclic guanosine monophosphate pathways.[39]

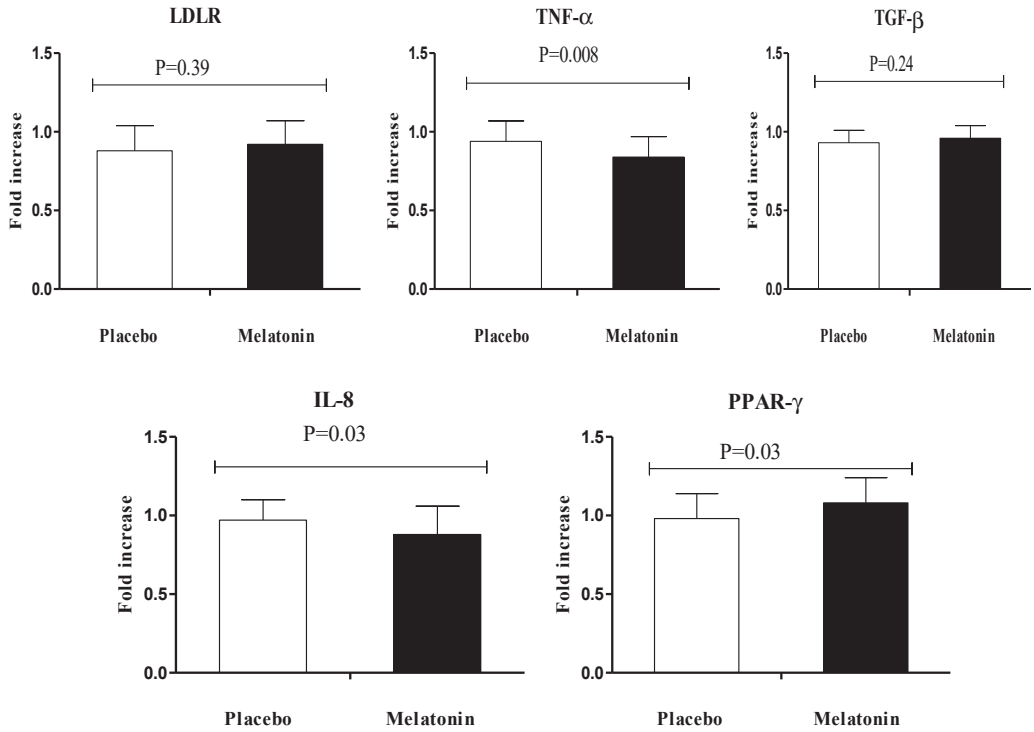

Figure 2. Effect of the 12-week supplementation with melatonin or placebo on expression ratio of PPAR-γ, LDLR, TNF-α, IL-8, and TGF-β gene in PBMCs of diabetic HD patients. HD, hemodialysis; IL-8, interleukin-8; LDLR, low-density lipoprotein receptor; PBMCs, peripheral blood mononuclear cells; PPAR-γ, peroxisome proliferator-activated receptor gamma; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta.

Effects on Parameters of Mental Health, Biomarkers of Inflammation, and Oxidative Stress

We found that taking melatonin by diabetic HD patients for 12 weeks resulted in a significant reduction in BDI, BAI, PSQI, hs-CRP, and MDA, and a significant elevation in TAC and total nitrite levels, but melatonin did not affect GSH levels. Moreover, melatonin administration downregulated IL-8 and TNF-α gene expression in PBMCs of HD patients, but did not influence gene expression of TGF-β. Interventional studies have demonstrated that melatonin has antioxidant and anti-inflammatory functions,[40] but the results have not always been consistent. In a study conducted by Hussein et al.,[41] melatonin administration (1 mg/kg/day) to obese rabbits for 4 weeks significantly increased TAC levels. Melatonin consumption at a dosage of 5 mg/day among subjects with metabolic syndrome after 8 weeks reduced MDA and increased catalase activity.[13] Recently, we reported that consuming melatonin for 12 weeks by diabetic patients with CHD had beneficial effects on some inflammatory factors, oxidative stress, and parameters of mental health.[9] Leppamaki et al.[42] found that melatonin administration at a dosage of 20 mg/day for 3 weeks significantly improved the quality of sleep, vitality, and quality of life in subjects with weather-associated syndrome. However, melatonin consumption (6 mg/day for 40 days) in obese women did not affect TAC and MDA levels.[12] In addition, melatonin intake did not affect cognition, well-being, or psychosocial functioning in people with severe mental illness.[43] Increased oxidative stress and inflammation which presented in ESRD patients can intensify each other and are associated with a large number of ESRD complications including protein energy wasting, atherosclerosis, and all-cause and CVD mortality.[44] Therefore, the finding of a beneficial impact of melatonin administration on inflammatory status and oxidative damage has great implications in the context of ESRD and there may be important advantages for HD people including preventing cardiovascular events, reducing protein energy wasting, and even mortality rate.[45–47] Melatonin may improve parameters of mental health, inflammatory markers, and oxidative damage through regulating the activities of antioxidative enzymes,[48] stimulating glutathione synthesis[49] and suppressing nuclear factor-kappaB activation.[50,51]

Prior human and animal studies documented that melatonin has a safety profile without any serious adverse effects, even in high doses. Only minimal side effects such as sleepiness, dizziness, nausea, and headache have been reported.[52] It must be kept in mind that the time of melatonin consumption is very important. Administration of melatonin in the morning and evening increases insulin-resistant and impairs glucose tolerance,[53] whereas nighttime melatonin intake can decrease the release of insulin in response to a rise in circulating glucose levels.[9] Furthermore, melatonin

has sedative effects and bed-time melatonin consumption could enhance the quality of sleep.[54] Melatonin has been identified in some kinds of foods. In animal foods, fish and eggs are rich sources of dietary melatonin, whereas in plant foods, cereals, germinated legumes, nuts, and mushrooms are also the highest melatonin-containing foods.[55] In our trial, based on 3-day food records, both groups are similar in terms of dietary intakes and various dietary patterns among participants cannot confound the results.

There are a few limitations in the current investigation. Due to budget limitations, we did not assess plasma or salivary melatonin levels and urinary 6-sulfatoxymelatonin. Also, we were unable to determine the impact of melatonin administration on other biomarkers of oxidative stress and inflammatory factors such as IL-6 and IL-1. During the baseline interview, PSQI questionnaire was completed for our subjects, though we did not analyze sleep behaviors of study participants according to PSQI criteria at baseline. At final data analysis, we found out that few participants had sleep problems. However, we also had asked the patients for taking sleep medications. And, since none of them were consuming sleep medications, we did not exclude anyone from the study, although those with sleep problem had been referred to specialist. This should be considered in the interpretation of our findings.

Conclusions

Overall, melatonin supplementation for 12 weeks by diabetic HD patients had favorable effects on BDI, BAI, PSQI index, FPG, insulin levels, HOMA-IR, HbA1c, QUICKI, hs-CRP, total nitrite, TAC, and MDA, and gene expression of IL-8, TNF-α, and PPAR-γ, but did not affect other metabolic profiles and gene expression of LDLR and TGF-β.

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