Effect of melatonin on C-reactive protein and lipid profile of hemodialysis patients

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Abstract

Introduction: Cardiovascular disease is one of the major causes of death in hemodialysis patients. Lipid metabolism abnormalities and increased inflammatory factors are related factors. In recent studies, melatonin inhibits dyslipidemia and reduces inflammatory factors by inhibiting LDL-C oxidation. The purpose of this study was to evaluate the effect of melatonin supplement on hyperlipidemia and C-reactive protein (CRP) level in hemodialysis patients.

Objectives: We aimed to determine the effect of melatonin on CRP and lipid profile of hemodialysis patients.

Patients and Methods: Among 200 hemodialysis patients, only 28 patients fulfilled the inclusion criteria to enroll in the study. Patients were treated with melatonin supplements 3 mg/d at bedtime for 12 weeks. Serum lipid profile and CRP levels were measured after 12 weeks. Five patients were excluded, 2 patients underwent kidney transplantation, and three patients did not cooperate. The Wilcoxon signed-rank test was used to compare the two groups according to the number of participants (less than 30) and study type (pre-test and post-test). \( P < 0.05 \) was considered as the level of significance.

Results: A total of 23 patients completed the treatment protocol. Participants was composed of 13 male and 10 female. The participants’ mean age was 30.6± 11.6 years. After treatment mean total cholesterol levels decreased from 139.95±35.49 mg/dL to 131.13 ± 34.96 mg/dL (\( P=0.194 \)) which was not statistically significant. However, the decrease in serum triglyceride level was statistically significant (\( P=0.004 \)). Plasma HDL-C increased significantly after treatment (\( P=0.032 \)). Serum CRP levels did not change.

Conclusion: Melatonin supplement improves serum triglyceride and HDL-C levels in hemodialysis patients but has no effect on total cholesterol and CRP in hemodialysis patients.

Trial Registration: This randomized controlled trial was registered by the Iranian Registry of Clinical Trials (identifier: IRCT20200308046724N1; https://en.irct.ir/trial/46407, ethical code; IR.SBMU.RETECH.REC.1397.687).

Implication for health policy/practice/research/medical education:
In a randomized controlled trial study on hemodialysis patients, we found melatonin supplementation improves triglyceride and HDL-C levels, but has no effect on total cholesterol and C-reactive protein (CRP) levels in these patients.

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Introduction

In recent decades, the number of patients with reduced kidney function and chronic kidney disease (CKD) has been increasing (1). Mortality and morbidity in end-stage renal disease patients is significantly higher than expected (2). The main cause of mortality of debilitating complications in CKD patients includes cardiovascular problems. Lipid metabolism abnormalities play a major role in the development of atherosclerosis. In addition, C-reactive protein (CRP) as an inflammatory factor, is a contributor to atherosclerosis progression. Furthermore, previous studies have shown a strong association between nutritional and systemic inflammation factors with dialysis complications (3,4). Recent studies have shown that the level of pro-inflammatory cytokines in hemodialysis patients is 8 to 10 times higher than in healthy controls.
(5) and CRP (5) and CRP is an appropriate indicator for the diagnosis of inflammation in patients; therefore, inflammation rate increases in these patients (3,6). Lipid metabolism abnormalities play a major role in the development of atherosclerosis (7). Melatonin (5-methoxy-N-acetyltryptamine) is a hormone produced by pineal gland, retina, digestive tract, and several other organs and secreted by pineal. In addition, the availability of nutritional factors such as tryptophan, folic acid, and B vitamin affects the rate of melatonin secretion (8). Previous researchers demonstrated that high melatonin concentrations inhibit low-density lipoprotein cholesterol (LDL-C) oxidation, thereby reducing the risk of atherosclerosis (7,9,10). Other studies suggest that this effect is more likely to be seen at higher doses, longer treatment periods as well as at higher cholesterol concentrations; however, it has no significant effect on LDL-C and HDL-C levels (11). The results of various studies have shown that melatonin is well-tolerated at prescribed doses (12,13). Recent studies have described other effects of melatonin consumption as follows; decreased metabolic effects of antipsychotic medications, schizophrenia, and bipolar disorder (14,15).

In various liver diseases, decreased plasma levels of anti-inflammatory cytokines, especially in patients with lipid metabolism disorder is associated with hypertriglyceridemia and high HDL cholesterol (16-18). Considering the possible role of melatonin in decreasing inflammatory factors on lipid profile of CKD patients, and since there have been few relevant studies in Iran so far, the aim of the present study was to determine the effect of melatonin on the inflammatory factors and lipid profile of dialysis patients.

**Objectives**
The present study aims at determining the effect of melatonin on CRP and lipid profile of maintenance hemodialysis patients.

**Patients and Methods**

**Study design**
This is a clinical trial study with pretest and posttest design. The data were collected through observations and simultaneous interviews. The target population of the study include the dialysis patients of Loghman hospital’s dialysis ward in 2018. The census sampling method was used for all eligible hemodialysis patients in the dialysis ward. Participants were selected based on inclusion criteria and enrolled in the study after obtaining their informed consent.

Inclusion criteria included patients over 18 years old who had been undergoing hemodialysis for at least three months and had high triglyceride levels (≥150 mg/dL), low HDL (40 mg/dL in men and 50 mg/dL in women), and CRP >5 mg/L. Exclusion criteria also included patients with a history of stroke and myocardial infarction, transient ischemic attack, peripheral vascular diseases, malignancy, liver cirrhosis, chronic obstructive pulmonary disease, active hepatitis, heart failure, autoimmune diseases, use of corticosteroid, non-steroidal anti-inflammatory drugs, statins, and positive viral, hepatitis and HIV markers. Prior to sampling, if the patient had unstable symptoms of infection, a history of hospitalizations and infection during the last evolved months, sampling was delayed for 1 month to minimize factors that may have come founding effect on the inflammatory factors.

The sample size was calculated 28 people considering 95% confidence interval, power of 80%, and effect size of 0.5 using one-sample Wilcoxon signed-rank test.

Patient information was extracted from the medical cases archived in the hemodialysis ward and the patients then participated in the study based on the inclusion and exclusion criteria after obtaining their informed consent. To prevent sample loss, patients’ addresses and telephone numbers were obtained and the researcher stayed in touch with patients throughout the study. Patients were first examined for inclusion and exclusion criteria at the baseline and at the end of the study. Finally, five participants were excluded, two participants underwent kidney transplantation and three participants did not cooperate. Patients received oral melatonin (3 mg/daily) for three months. Tests were performed at the baseline of melatonin administration and at the end of three months. Blood samples were analyzed for CRP and lipid profiles (triglyceride, total cholesterol and high-density lipoprotein cholesterol (HDL-C). Finally, data were entered the data collection form for each patient. In addition to CRP, lipid profiles (triglycerides, total cholesterol, and HDL-C), fast blood sugar (FBS), WBC, hemoglobin, platelet, and liver tests were also measured and compared in hemodialysis patients before and after melatonin administration.

**Data analysis**
Data analysis was then carried out using SPSS version 20. Quantitative data such as age was presented using mean and standard deviation, and qualitative data such as gender and complications were presented in frequency and percentage. The nonparametric Wilcoxon signed-rank test was used to compare the two groups according to the type of studied variable that had no normal distribution, sample size (less than 30), and the type of study (pre-test and post-test). P values less than 0.05 are considered significant.

**Results**
Of 23 eligible study participants, 13 were male and 10 were female. The mean ± SD of participants’ age was 60.16 ± 30.11 years. The age range of the participants was also 32 to 91 years (Table 1). The most comorbidities in the participants included hypertension and cardiovascular diseases (n = 18 cases) and diabetes mellitus (n = 14 cases). The most common medications used by participants were also vitamin D3 (n = 15 cases), vitamin B supplements
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(n= 14 cases), and atorvastatin and ASA (n = 7 cases). Tables 2 and 3 show the results of tests at the baseline and at the end of the three months for CRP and lipid profiles (triglycerides, total cholesterol and HDL-C), as well as the underlying factors, including FBS (fasting blood sugar), white blood cell, plasma hemoglobin, platelets, liver tests, and the vitamin D3 in dialysis patients before and after melatonin administrations and comparison of the mean changes in the above cases and their statistical significance. According to the above tables, mean FBS, white blood cell (WBC) and platelet counts decreased after melatonin treatment, since plasma hemoglobin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and vitamin D3 levels increased. However, these changes were statistically significant only with regard to vitamin D3 level. Moreover, mean total cholesterol and triglyceride levels decreased after melatonin treatment, and mean HDL-C levels increased after the trial. These changes were statistically significant in the case of triglyceride and HDL-C, but not for total cholesterol. The mean CRP level also increased after melatonin treatment; however, this increase was not statistically significant.

### Discussion

The aim of the present study was to determine the effect of melatonin and CRP and lipid profile in hemodialysis patients. Finally, the results revealed that the mean CRP level increased after melatonin treatment; however, this increase was not statistically significant. Therefore, three-month melatonin treatment (3 mg/d) did not have a positive effect on improving the conditions of patients and reducing the inflammatory factors; among which, CRP is an appropriate indicator for diagnosis of these factors. With regard to lipid profile, mean cholesterol and triglyceride levels decreased after melatonin treatment and mean HDL-C level also increased after such treatment. These changes are in favor of the effect of melatonin treatment on improving lipid profile in hemodialysis patients. However, these changes were only statistically significant in the case

**Table 1. Patients’ characteristics**

| Properties                          | Values             |
|-------------------------------------|--------------------|
| Age (year)                          | Mean ± standard deviation 60.30 ± 16.11 |
|                                     | Middle 61.00       |
|                                     | Domain (min-max) 59 (32-91) |
| Gender                              | Female 10 (43.5%)   |
|                                     | Male 13 (56.5%)     |
| Age of starting dialysis (year)     | Mean ± standard deviation 57.29 ± 17.19 |
|                                     | Middle 55.00        |
|                                     | Domain (min-max) 60 (29-89) |
| Etiology of ESRD                    | Diabetes mellitus 13 (56.5%) |
|                                     | Hypertension 5 (21.7%) |
|                                     | Others 5 (21.7%)     |

**Figure 1. The flow diagram of study.**
of triglyceride and HDL-C but not for total cholesterol. With regard to the underlying factors measured, only the vitamin D3 level was statistically increased after melatonin treatment compared to the pretreatment phase.

In a similar study, Goyal et al investigated the effect of melatonin administration on the treatment of metabolic syndrome. They revealed that metabolic syndrome was treated more commonly in melatonin-treated group as compared to the placebo group. In addition, melatonin was well tolerated and moderately improved the symptoms of such syndrome in patients as compared with the placebo (12). The present study showed that only three patients did not tolerate melatonin due to the effects its sleep-inducing effect, and triglyceride and HDL cholesterol levels increased after the treatment, which wasn’t statistically significant. Additionally, FBS level did not change significantly in the present study, which was consistent with our study.

Romo-Nav et al evaluated the metabolic complications of melatonin in patients treated with second-generation antipsychotics (SGAs). The results showed that melatonin is effective in reducing the metabolic effects of SGA, especially in bipolar disorder. Clinical findings indicated that melatonin can be an appropriate and cost-effective treatment option to reduce and prevent metallic effects of SGA (14). The present study also reported minimal side effects for melatonin treatment and lipid profile improved after the above treatment.

Cichoz-Lach et al studied the effects of melatonin and L-tryptophan on the biochemical blood paraeekets in patients with nonalcoholic steatohepatitis (NASH). They showed that melatonin and tryptophan had a significant effect on decreasing the plasma levels of anti-inflammatory cytokines and may be useful in the treatment of patients with NASH (16). In contrast to the above study, the present study showed that CRP increased slightly after melatonin administration; Although this increase was not statistically significant, overall, melatonin did not have a positive effect on the reduction of CRP levels.

Clinsky et al conducted a study to determine the effects of tryptophan and melatonin on selected biochemical parameters in patients with nonalcoholic fatty liver disease (NAFLD) and also in improving liver tissue in the above patients. The results showed that melatonin should be taken into consideration as a treatment option for NAFLD, especially in patients with lipid metabolism disorder along with hypertriglyceridemia and high LDL cholesterol (17). Our study showed that lipid profile improved after melatonin administration, but CRP level did not decrease. It should be noted that the present study was performed within 10 weeks but the above-mentioned study was conducted within 14 months and the melatonin doses was also 3 mg/d in our study. Since, the melatonin dose was 5×2 mg/d in the study by Clinsky et al, which could justify the difference in CRP results.

The toxic effects of melatonin were investigated by Seabra et al (13). According to the results, polysomnography analysis showed statistically significant decrease in stage one of sleep in melatonin-treated group. There was no other significant difference between the placebo and melatonin groups. The above study also showed that melatonin (10 mg/d) had no toxic effects on the patients throughout the study (13). The results of our study demonstrated that only three patients discontinued melatonin use due to sleep-related complications since no further complication was reported. It should be noted that

| Table 2. Mean, standard deviation and P value of triglyceride, total cholesterol, HDL-c and CRP before and after treatment with melatonin |
|---------------------------------|-----------------|-----------------|----------|
|                                | Before treatment with melatonin | After treatment with melatonin | P value |
| Triglyceride (mg/dL)           | 242.08 (±101.05) | 178.00 (±48.02) | 0.004    |
| Total cholesterol (mg/dL)      | 139.95 (±35.49)  | 131.13 (±34.96) | 0.194    |
| HDL-C (mg/dL)                  | 36.20 (±7.08)    | 40.16 (±5.35)   | 0.032    |
| CRP (mg/L)                     | 16.23 (±12.98)   | 25.43 (±20.92)  | 0.346    |

| Table 3. Mean, standard deviation and P-value of fasting blood sugar, WBC count, hemoglobin, platelets, liver tests and VIT D3 before and after treatment with melatonin |
|---------------------------------|-----------------|-----------------|----------|
|                                | Before treatment with melatonin | After treatment with melatonin | P value |
| FBS (mg/dL)                    | 122.43 (±77.11) | 105.74 (±41.98) | 0.475    |
| WBC (/µl)                      | 7743.5 (±2733.9)| 7221.7 (±2146.8)| 0.638    |
| Hemoglobin (g/dL)              | 10.62 (±1.72)   | 10.74 (±1.73)   | 0.416    |
| PLT (/µl)                      | 215217 (±66076) | 214636 (±61517) | 0.434    |
| ALT(IU/L)                      | 14.08 (±5.60)   | 14.26 (±5.86)   | 0.693    |
| AST (IU/L)                     | 14.43 (±5.67)   | 15.00 (±7.41)   | 0.762    |
| VIT D3 (ng/mL)                 | 32.88 (±22.91)  | 41.95 (±13.43)  | 0.046    |
melatonin was used at the dose of 3 mg/d for 10 weeks. In a study on the effect of long-term melatonin treatment on plasma liver enzymes and plasma lipids levels in patients with NASH, Gonciarz et al, showed that aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were significantly reduced only in melatonin-treated group. Plasma cholesterol, triglyceride, glucose concentrations, as well as plasma alkaline phosphatase remained in the normal range in the control and melatonin-treated groups during the long-term study. The results showed that the positive effects of melatonin on liver enzymes in patients with NASH (16). Nonetheless, in the present study, ALT and AST levels were not significantly different before and after melatonin treatment. The mean FBS decreased from 122 mg/dL to 105 mg/dL; however, this increase was not statistically significant. The difference in these results may be due to the different melatonin doses (5mg/twice a day in the above study and 3mg/daily in our study). Rindone and Achacoso investigated the effects of melatonin treatment on serum lipids in hypercholesterolemic patients. The results showed a decreasing trend in total cholesterol and LDL-C levels at melatonin dose of 3 mg/daily (10). In the present study, HDL cholesterol levels were increased after melatonin treatment, which was statistically significant. Previously, Chojnacki et al investigated the effects of melatonin on elevated liver enzymes during statin treatment. In the group that taking statin with melatonin, total cholesterol decreased statistically significant, after 6 months versus another group that taking statin with placebo. Furthermore, triglyceride levels were decreased significantly. In addition, no serious side effects were reported (15). Our study shows mean total cholesterol and triglyceride levels decreased after melatonin treatment, while mean HDL-C levels increased. These changes were statistically significant in the case of triglyceride and HDL-C, but not for total cholesterol. Concerning the drug complications, only three participants refused to take the drug due to its sleep-inducing effect. Recently, a systematic review and meta-analysis regarding the effect of melatonin on elevated liver enzymes during statin treatment. This study was presented as a poster at the ERA-EDTA Milan Italy June 6-9, 2020.

**Conclusion**
The present study concludes that three-month melatonin treatment (3 mg/d) has a positive effect on improving the patient’s lipid profile and leads to a decrease in triglyceride level and an increase in HDL-C levels; however, it has no effect in reducing the inflammatory factors such as CRP, which is a good indicator for detecting these factors. Considering the side effects, only three of the participants refused to take the drug due to its sleep-inducing effect, and other participants had no problems with the drug use.

**Limitations of the study**
To evaluate the inflammatory factors, in addition to CRP, IL-6 and IL-1 inflammatory factors should also measure

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**Authors’ contribution**
ZNS; preparation of research design and manuscript drifting. TA; manuscript reviewing. MP; data collection and statistical analysis. All authors read and signed the final paper.

**Conflicts of interest**
No conflicts of interest were declared by the researchers.

**Ethical issues**
The study was conducted based on the Declaration of Helsinki, and the patients filled out informed consent. Moreover, the Ethical Committee of Shahid Beheshhti University of Medical Sciences declared the approval of the research (IR.SBMU.RETECH.REC.1397.687). This study was extracted from the internal medicine residency thesis of Marjan Pouyamehr in the School of Medicine, Shahid Beheshti University of Medical Sciences. Besides, the study protocol was registered by the Iranian registry of clinical trials (identifier: IRCT20200308046724N1; https://en.irct.ir/trial/46407). Accordingly, ethical considerations (including plagiarism, data fabrication and double publication) have been completely observed by the authors.

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