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Melatonin Improves Lipid Profile and Ameliorates Lipid Peroxidation in Patients with Chronic Kidney Disease.

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Abstract:

Dyslipidemia and oxidative modifications of lipid are frequently associated in patients with chronic kidney diseases (CKD) and considered the most important risk factors for cardiovascular events. Melatonin is a well-known potent antioxidant and has beneficial effect on lipid metabolism. the study was designed to evaluate if Melatonin could improve lipid profile and ameliorates lipid peroxidation. This single blind placebo controlled clinical study carried out on 41 patients with CKD who were randomized into two groups, control groups (n=20) those who received placebo cap and melatonin group those who received 5mg melatonin (n=21). Lipid profile [total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C)] and parameters of lipid peroxidation [oxidized LDL (oxLDL) and malondialdehyde (MDA) were measured before and after 12 weeks of the treatment. After 12 weeks of treatment, melatonin significantly increased HDL-C and decreased LDL-C compared to the initial value. The elevation in HDL-C and reduction in LDL-C were significantly different from that in placebo group. Also, both oxLDL and MDA levels significantly lowered by melatonin compared to the baseline and to the placebo group. Collectively, the results of our study showed that melatonin has advantageous effect on lipid profile and inhibit lipid peroxidation in patients with CKD.

Key words: Melatonin, chronic kidney disease, Lipid profile, lipid peroxidation, .

الميلاتونين يحسن مستوى الدهون ويقلل الأكسدة في مرضى الذين يعانون من العجز الكلوي المزمن

حيدر جاسب

الخلاصة:

اضطرابات الدهون والتغييرات الحاصلة في اكسدة الدهون غالبا ما ترتبط مع المرضى الذين يعانون من أمراض الكلى المزمنة وتعتبرمن أهم عوامل الخطورة لأمراض القلب والأوعية الدموية. الميلاتونين وهومعروف جيدا كمضاد قوي للأكسدة وله تأثير مفيد على ايض الدهون ، لذلك، فإن الدراسة الحالية تهدف إلى تقييم ما إذا كان الميلاتونين يمكن أن يحسن من مستوى الدهون ويخفف اكسدتها. وهذه الدراسة هي تجربة سريرية مسيطرعليها،عمياء من جهة واحدة أجريت على 41 مريضا يعانون من أمراض الكلى المزمنة ،وزعوا عشوائيا في مجموعتين ،مجموعة السيطرة (ع= 20) أولئك الذين تلقوا علاج وهمي ومجموعة الميلاتونين (ع= 21)أولئك الذين تلقوا 5 ملغم من الميلاتونين. وقد تم قياس مستوى الدهون [الكوليسترول الكلي ، الدهون الثلاثية، البروتين الدهني عالي الكثافة، البروتين الدهني منخفض الكثافة] ومقاييس اكسدة الدهون [البروتين الدهني منخفض الكثافة المؤكسد و مالونديالدهيد] قبل وبعد 12 أسبوعا من بدأ العلاج. و بعد 12 أسبوعا من العلاج، سبب الميلاتونين زيادة معنوية في البروتين الدهني عالي الكثافة وانخفاض في البروتين الدهني منخفض الكثافة مقارنة مع القيمة الأولية. و قد كان الارتفاع في البروتين الدهني عالي الكثافة والانخفاض في البروتين الدهني منخفض الكثافة يختلف اختلافا معنويا عن ذلك في المجموعة الوهمية. و أيضا مستوى كل من البروتين الدهني منخفض الكثافة المؤكسد و مالونديالدهيد قد انخفض بشكل كبير من قبل الميلاتونين مقارنة مع المستوى الاولي ومع مجموعة الدواء الوهمي. بشكل ملخص، لقد أظهرت نتائج دراستنا أن الميلاتونين له تأثير مفيد على مستوى الدهون ويمنع اكسدة الدهون في المرضى الذين يعانون من أمراض الكلى المزمنة.

Introduction:

Cardiovascular disease (CVD) is a most common cause of morbidity and mortality in patients with chronic kidney disease (CKD) [1, 2]. Patients with CKD more

likely will die of CVD, mostly atherosclerotic coronary heart disease, before developing end stage renal disease (ESRD) [3]. American heart association has classified patients with chronic renal impairment in the highest risk for developing cardiovascular events [2]. Also, a report from the Atherosclerosis Risk in Communities Study showed that a declining in glomerular filtration rate (GFR) was independently associated with the occurrence of atherosclerotic CVD [4]. Dyslipidemia, Oxidative stress and accumulations of uremic toxin are the main factors that implicated in atherogenesis and CVD in patients with CKD. Dyslipidemia induced by CKD characterized by increasing triglyceride, accumulation of oxLDL and decreasing high density lipoprotein (HDL) levels [5, 6]. Hypertriglyceridemia in patients with CKD resulted from decreasing the activity of peripheral and hepatic lipoprotein lipase, thus delaying the catabolism of TG. On the other hand, hepatic production of triglyceride rich lipoprotein is increased [6, 7]. Diminishing in HDL level attributed to many mechanisms including decreased levels of HDL main component (apolipoproteins AI and AII) and impaired activity of lecithin–cholesterol acyltransferase which responsible for esterifying free cholesterol in HDL particles. In contrast, cholesterol ester transfer protein (CETP) is activated to transfer cholesterol esters from HDL to triglyceride-rich lipoproteins. Collectively, all these lead to reduced HDL level in patients with CKD [7, 8]. Additionally, the antioxidant and anti-inflammatory effects of HDL is impaired mainly due to inhibited activity of paraoxonase (PON) which is HDL associated enzymes that inhibit LDL oxidation [5]. Despite, LDL is not usually increased in patients with impaired kidney function, the form of LDL particles become smaller, denser and easily oxidized, therefore, more atherogenic [6, 8]. Increased oxidative stress and oxidative modifications of lipid and lipoproteins is well evident in chronic renal impairment. The pro-oxidative state detects almost at the beginning of CKD and excess generation of reactive oxygen species increases as GFR declines [9, 10]. Enhanced oxidative stress will induce lipid peroxidation which has a determent effect onset and progression of atherosclerotic process and putting the patients at high risk of CVD [10, 11].

Melatonin, N-acetyl-5-methoxytryptamine, is a pineal gland hormone. It has multiple bioactivities such as potent antioxidant, anti-inflammatory, immune modulating, tumor suppressing and cardiovascular protective effects as well as anti-diabetic and anti-obese activities [12]. Beneficial effects of melatonin on lipid metabolism have been reported in type 2 diabetes [13] and metabolic syndrome [14]. In addition to that, melatonin decreased lipid peroxide [14] and LDL susceptibility to oxidation [15]. Reduction in nocturnal melatonin secretion was associated with higher oxidized LDL levels in patients with acute myocardial infarction [16]. Interestingly; a defect in amplitude and rhythm of melatonin secretion have been observed in CKD [17]. Accordingly, administration of exogenous melatonin to the patients with CKD could improve lipid abnormalities and reduce lipid peroxidation. Therefore, this study was aimed to investigate the potential beneficial effect of melatonin on lipid profile and lipid peroxidation in those patients.

Patients and Methods

Study design

This study is prospective randomized, single blind placebo controlled clinical study. The study carried out at the Medical City of An Najaf health directorate-Iraq during the period of December 2016 till September 2017. The protocol of study was approved by the Scientific Committee of Faculty of Pharmacy Kufa University.

Patients

The potential participants of study were patients with stage 1-4 CKD who attend

the specialized Centre of kidney disease in the Medical City of An Najaf. Patients who were with hepatic diseases, pregnancy and breast feeding or CKD required dialysis, were excluded from the study. The eligible patients were 50 enrolled in the study. Nine patients could not complete the study because of noncompliance (4) and loss of follow up (5). The remaining 41 patients were divided into two groups. Group I: Placebo control group (N=20), the patients treated with placebo capsule for 12 weeks at bed time in addition to their standard medical treatments. Group II: Melatonin group (N=21); the patients of this group treated with 5mg melatonin at bed time for 12 weeks in addition to their standard medical treatments.

Biochemical measurements

After 12 hr. fasting, about 10 ml of venous blood sample were collected from all subject at zero time and after 12 weeks of treatment. Blood samples were allowed then cool centrifuged at 3000 rpm for 10 min to obtain serum for biochemical analysis. Urea level and Serum creatinine was measured by commercial available kit. Serum TC, TG and HDL-C levels were determined according to kit obtained from Bio Merieux, France. Plasma LDL-C was calculated using the formula: [LDL-C = Total cholesterol – (TG/5) – (HDL-C)] and the results were expressed in mg/dl. Oxidized LDL were determined by enzyme immunoassay technique according to ELISA kit (Cusabio biotech co. LTD) and the result expressed as mU/ml. MDA level were determined by ELISA kit (Cell Biolabs Inc., USA)

Statistical analysis

Paired Student's t test was used to compare values obtained before and after treatment within each group while independent sample t tests were used to compare between melatonin and control group. Data were presented as mean ± Standard error mean (SEM). $P<0.05$ was considered statistically significant using a two-tailed test. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 21.0 (SPSS, Chicago, IL).

Results

Baseline characteristics

Out of the total enrolled patients, 41 patients were completed the study and included in our statistical analysis as 20 patients in control group and 21 patients in melatonin group. The demographic and baseline clinical characteristics were not different between the two groups ($p>0.05$) (Table 1).

Table 1: Demographic and baseline biochemical patient characteristics

Parameters	Control (n=20)	Melatonin (n-21)	*P value*
Age (yr)	56.1 ± 2.4	58.2 ± 3.4	*0.6*
Male	13 (65%)	14 (67%)	*0.91*
Female	7(35%)	7 (34%)	
Body weight (kg)	78± 6.1	76± 5.4	*0.9*
S. Urea (mg، di)	93.10 ±2.42	96.60± 1.9	*0.43*
S. Creatinine	2.92 ±0.11	2.81± 0.13	0.2
TC	196.3±6.02	197.9±6.6	*0.33*
TG	211.7±12.5	207.4±14.4	*0.825*
HDL-C	38.3 ± 2.1	39.8±2.5	*0.653*
LDL-C	115.7±4.8	116.7±5.7	*0.898*

OXLDL	69.2± 5.1	71.9± 4.2	*0.683*
MDA	4.9±0.4	5.2±0.5	*0.630*

Data where expressed by mean±SEM

Effect of Melatonin on lipid profile

After 12 weeks of therapy, Melatonin significantly (P <0.05) elevated HDL-C level and significantly (P <0.05) lowered LDL-C level while it did not cause significant changes in the level of TC and TG compared to initial value (Table 2). There was no significant (P >0.05) change in lipid profile parameters of placebo group during the study period compared to the baseline. In comparing with placebo group, the elevation in HDL-C and the reductions in LDL-C were significantly (P <0.05) different in melatonin group Figure1(C)&(D) respectively but the changes in TC level and TG level were not significant (P >0.05) Figure1(A)&(B) respectively.

Table-2: Effect of 12 weeks melatonin treatment on lipid profile parameters.

Parameters	**Melatonin group**			**Placebo group**		
	Before	After	*P value*	Before	After	*P value*
TC	197.9±6.6	190.6±5.3	*0.201*	196.3±6.02	194.9± 4.2	*0.732*
TG	207.4±14.4	198.5±13.8	*.147*	211.7±12.5	206.6±11.2	*0.296*
HDL-C	39.8±2.5	50.6±2.8	***0.001***	38.3 ± 2.1	41.8±3.1	*0.056*
LDL-C	116.7±5.7	100.01±4.8	***0.01***	115.7±4.8	111.7±4.03	*0.239*

(Data expressed by mean ±SEM, analyzed by paired sample t test)

Effect of melatonin on lipid peroxidation

Melatonin treatment for 12 weeks significantly (P <0.05) decrease OXLDL level and MDA level compared to the baseline level while the decrease was not (P >0.05) significant in placebo group (table 3). Importantly, the reduction in the level of OXLDL and MDA were significantly (P <0.05) differt in melatonin group from that with the placebo group, Figure1 (E)&(F) respectively.

Table 3. Effect of 12 weeks melatonin treatment on lipid peroxidation parameters

Parameters	**Melatonin group**			**Placebo group**		
	Before	After	*P value*	Before	After	*P value*
OXLDL	71.9±4.2	52.9±4.1	***0.001***	69.2±5.1	66.1±4.7	*0.207*
MDA	5.2±0.5	2.9±0.3	***0.005***	4.9±0.4	4.3±0.5	*0.273*

Data where expressed by M±SEM

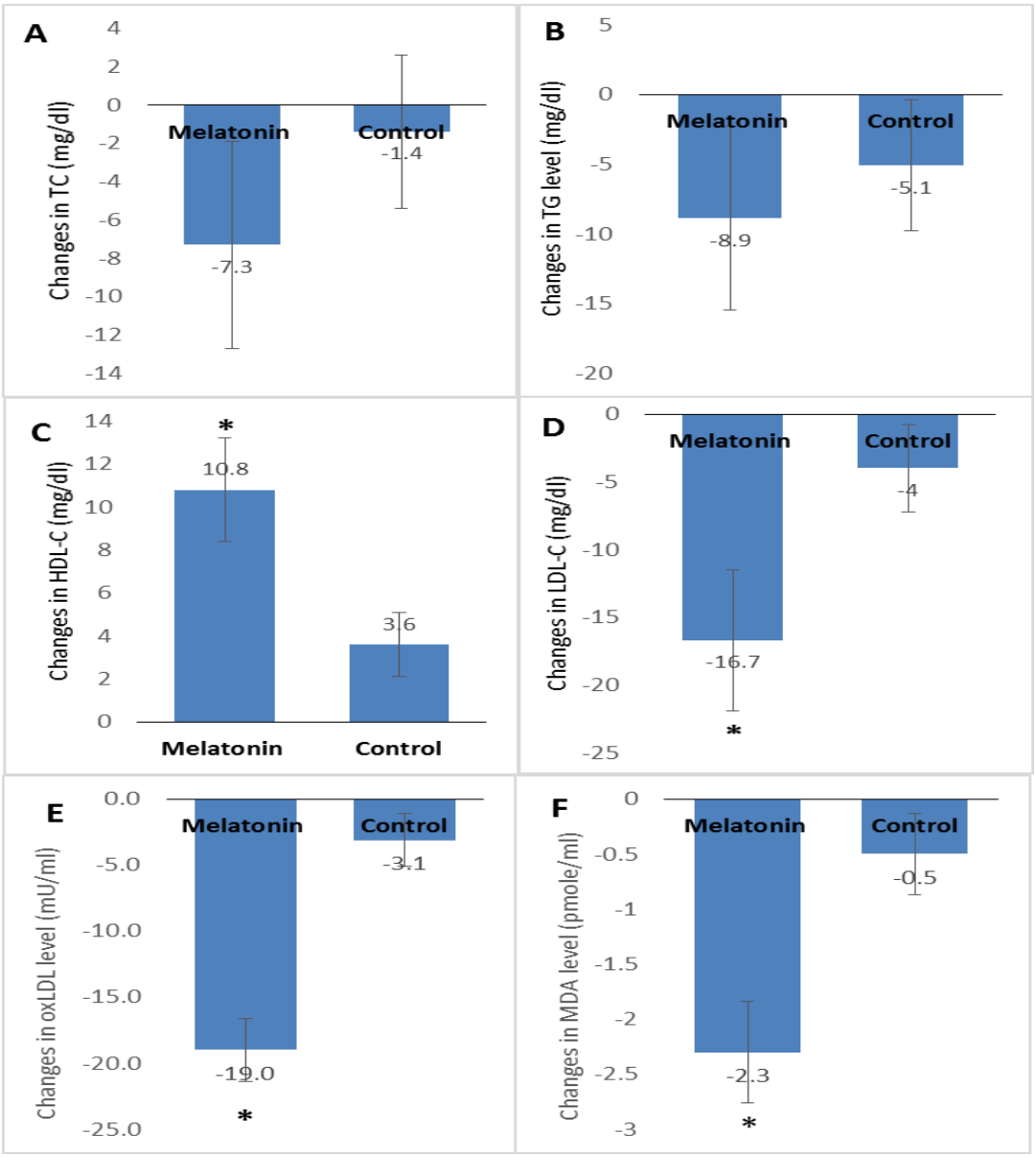

Figure 1. Changes in lipid profile with melatonin versus control group

The change value of each group obtained by subtracting the initial value from the value obtained of after 12 weeks of treatment. Graph illustrate changes (A) serum level of total cholesterol (TC), (B) serum triglyceride level (TG), (C) serum high density lipoprotein-cholesterol (HDL-C), (D) serum low density lipoprotein-cholesterol (LDL-C), (E) serum oxidized low density lipoprotein (oxLDL) and (F) serum malondialdehyde (MDA). The data is presented as mean ± SEM, statistically analyzed by independent sample t test. * P≤0.05 for melatonin versus control.

Discussion

Disturbance in lipid metabolism, mainly increase in TG and decrease in HDL-C, considers as the most important risk factors for high rate of cardiovascular morbidities and mortality in CKD patients [6] and therefore, it will be of high interest to find adjuvant therapeutic modalities that could improve these lipid abnormalities. Melatonin has remarkable pleiotropic effects. Recently, many researches have demonstrated beneficial and protective effect of melatonin on atherosclerotic CVD which might attributed to its antioxidant and anti-inflammatory activities as well as

regulation of lipid and glucose metabolism [18]. The present study demonstrated additional beneficial effects of melatonin on lipid profile as evident by significant improvement in HDL-C levels and reduction in LDL-C level after 12 weeks treatments in patients with CKD. To the best of our knowledge, this is the first study that was conducted to evaluate the effect of melatonin on lipid profile in patients with stage 1-4 CKD. However, Edalat-Nejad et al., demonstrated remarkable increase in HDL-C level after 6 weeks of 3mg melatonin treatment in hemodialysis patients while the decrease in LDL-C was not significant [19]. Meanwhile, significant lowering of LDL-C and no significant changes in HDL-C have been reported by Koziróg et al, after 8 weeks treatment of 5mg melatonin in metabolic syndrome [14]. Additionally, in patients with nonalcoholic fatty liver disease, treatment with 10mg melatonin per day for 14 months significantly lowered levels of TG and LDL-C }20{. The beneficial effect of melatonin on lipid parameters may be explained by decreasing intestinal absorption of cholesterol [21], inhibition of biosynthesis of cholesterol and LDL-C accumulation [22], or regulation of free fatty acid uptake via G-protein receptors [23].

In CKD, oxidative modifications of LDL-C to oxidized LDL is accelerated tremendously because of firstly, small and dense LDL molecule thus very susceptible for oxidation [24], secondly inhibited activity of paraoxonase (PON) which is HDL associated enzymes that inhibit LDL oxidation [8,24] and thirdly presence of enhanced oxidative stress state [9,10]. Therefore, about ten folds increase in oxLDL concentration have been reported in patients with CKD 25. The data of our study showed that 12 weeks of melatonin therapy notably attenuated lipid peroxidation as demonstrated by significant lowering of oxLDL and MDA level. We did not find previous study to evaluate the effect of melatonin on oxLDL level. However, there were several clinical studies that showed its antiperoxidative effects. In patients with ESRD, melatonin prevented oxidative stress by lowering plasma MDA and increasing RBC Glutathion level [26]. Additionally, it has been reported that melatonin reduced the level of thiobarbituric acid reactive substrates, a marker of byproducts of lipid peroxidation, in patients with metabolic syndrome [14]. Moreover, Melatonin administration caused significant decrease in lipid peroxidation byproducts reflected by lowering serum Hydroxynonenal level and the erythrocytic MDA level in obese subject [27]. Furthermore, decrease in MDA level and increase in the antioxidant enzyme by melatonin supplement have been reported in diabetes [28] and hypertension [29]. The anti-peroxidative effects of melatonin might be attributed to improve redox state via its direct free radical scavenging and activation of antioxidant enzyme and molecules [30]. Besides that, Melatonin inhibited LDL oxidation possibly by improving HDL level and hence restoring HDL associated antioxidant enzyme activity such as PON [31]. From the results of this study, one can conclude that melatonin administration remarkably improved lipid and inhibited lipid peroxidation in patients with CKD and hence may be clinically helpful to reduce atherosclerotic events in those patients when used as adjunct therapy.

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