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Melatonin Levels in Patients with Nonalcoholic Fatty Liver Disease Compared with Healthy Individuals according to Fibrosis Level

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ABSTRACT

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is one of the most common diseases worldwide. Studies have shown that melatonin, as a regulatory hormone, is effective in different cell protective pathways. We aimed to compare serum melatonin levels of patients with NAFLD with different stages of fibrosis with that of healthy individuals.

METHODS

In this cross-sectional study patients, aged >20 years with elevated serum liver enzymes and trance abdominal sonographic diagnosis of fatty liver who met the exclusion criteria for NAFLD were included. The participants were categorized into three groups as follows: 1) severe fibrosis (fibrosis > 9.1 kPa and steatosis > 285 dbm), 2) mild-moderate fibrosis (fibrosis: 6-9.0 kPa and steatosis 240-285), and 3) normal group with fibrosis < 5.8 kPa and steatosis < 240 dbm based on Fibroscan evaluation. Five ml of fasting venous blood was taken from each patient and the control group for laboratory assessment. A questionnaire including demographic, anthropometric, laboratories (serum ALT, AST, triglyceride, total cholesterol and melatonin level), and clinical data was completed for all participants.

RESULTS

97 people with a mean±SD age of 42.21 ± 11 years were enrolled. 59 (60.0%) patients were women. we observed that the melatonin levels were increased by advancing fibrosis. Based on control- attenuated parameter results the melatonin levels significantly differed between the healthy individuals and patients with severe steatosis. There was a direct association between increased melatonin levels and liver enzymes.

CONCLUSION

As a regulatory hormone, melatonin may directly be associated with liver cell injuries. Therefore, considered regulatory substances such as melatonin either diagnostic or therapeutic can improve the patients' outcome.

KEYWORDS:
Melatonin, Liver enzyme, Fibrosis, Non-Alcoholic Fatty Liver

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common diseases mainly resulting from the over-accumulation of fat in hepatocytes in the absence of alcohol usage and other liver diseases. The disease is highly influenced by obesity and being overweight. It has become an important health concern because of its morbidity and its association with metabolic syndrome factors. This condition can cover a broad spectrum of abnormalities from simple steatosis to frank fibrosis and consequently risk of hepatocellular carcinoma. However, the pathophysiology of NAFLD progression and its consequences are not clear. In fact insulin resistance, immunity and oxidative stress are considered as the key mechanisms in the development and progression of NAFLD.[1-4]

Melatonin, because of its characteristics, has been considered to be involved in NAFLD mechanism. Melatonin (N-acetyl-methoxytryptamine) is a neurohormone that originates from tryptophan and is secreted from the pineal gland. It is also produced by the liver and gastrointestinal tract. It seems that syntheses and secretion of this hormone is related to light intensity. Previous studies have shown that melatonin has multiple biological properties such as anti-oxidant and anti-inflammatory effects.[5-7] It also influences lipid metabolism, insulin sensitivity and glucose metabolism.[8,9] On the other hand, the liver is a place for many metabolic and detoxification processes that result in the generation of many toxic species with toxic effects on hepatocytes. Prevention of these toxic effects needs a complex antioxidant system, in which melatonin has an important place.[10,11] Studies have also shown that melatonin plays a protective role in cell injuries, through mitochondria protection, particularly because of NAFLD. Considering the inflammatory reactions in NAFLD, liver cells are injured and as a result, liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferases (AST) are released into the serum. These enzymes increase to 80% in advanced stages of the disease. Although this increase is not directly related to the disease severity, some studies have reported that melatonin can reduce serum liver enzymes. Melatonin inhibits the release of liver enzymes and the development of NAFLD via influencing inflammatory reactions.[10] Therefore, it can be hypothesized that serum melatonin level may be associated with different stages of NAFLD and its injuries. We aimed to assess this hypothesis and compare serum melatonin levels in patients with NAFLD compared with healthy individuals.

MATERIALS AND METHODS

Study design and sampling

This cross-sectional study was done on patients referred to the liver clinic in Firoozgar Hospital from June 2017 to January 2019, following trance abdominal sonographic diagnosis of fatty liver. The inclusion criteria were: age of more than 20 years with upper limit normal or elevated liver enzymes and presence of liver steatosis in transabdominal ultrasonography. Exclusion criteria were viral hepatitis, auto immune hepatitis, hepatic metabolic diseases, pos- treatment of hepatitis C infection, diabetes mellitus, bariatric surgery, taking medication with effects on liver status or melatonin level such as selemarin or oral antidiabetic medication. Also, men who consumed more than 30 g/day and women drinking more than 20 g/day of alcohol (for alcohol we do not use grams.) were excluded as well. NAFLD was approved by a master gastroenterologist.

The results of fibrosis were reported in kilopascals (kPa). Participants were categorized into the three following groups according to fibrosis: 1) Severe fibrosis with Steatohepatitis (NASH) suspicion defined as elevated serum liver enzymes, frank steatosis (>2 85 dbm) and fibrosis more than 9.1 kPa. 2) Mild to moderate fibrosis defined as fibrosis between 6-9.0 kPa and steatosis more than 240, and 3) Normal group: fibrosis less than 6 kPa and steatosis less than 285 dbm. Normal, mild to moderate, and sever steatosis were defined as CAP less than 240 dbm, 240-290 dbm, and more than 291 dbm, respectively.[12,13] The control group was selected among adults, aged more than 20 years of age, with no history of liver disease who were announced healthy by a gastroenterologist with respect to liver diseases. The normal (control) group should have had a normal serum liver enzymes level along with normal fibrosis (< 5.8 kPa) and steatosis (< 240 dbm) on Fibroscan evaluation. They were matched with the patients regarding age and sex.

Measurements

Five ml of fasting venous blood was taken from all

participated for laboratory assessment and a questionnaire including demographic information (age, sex), anthropometric data (high, weight, waist and hip circumference) was completed. These data were measured by a trained nurse. Serum ALT, AST, triglyceride, total cholesterol, and melatonin level and clinical examination and patients were recorded for each participants.

Ultrasonography

Fatty liver was defined as normal, mild, moderate, and severe on ultrasonography. Normal liver was considered when the consistency was homogeneous, displayed fine level echoes, and was minimally hyperechoic or even isoechoic in contrast to regular renal cortex. Mild steatosis was defined as the minor increase in liver echogenicity. In moderate steatosis, increased liver organ echogenicity was seen. Severe steatosis was considered as the marked increase in hepatic echogenicity, poor penetration of posterior segment from the right lobe of the liver, poor or any visual images from the hepatic vessels and diaphragm.[14,15]

Liver transient elastography

Transient elastography was performed for detecting liver fibrosis. This was performed by an expert in Firoozgar Hospital using Fibroscan (FibroScan; Echosens, Paris, France). The examination was performed according to the standard protocol with laying the patient in dorsal decubitus position with maximum abduction of the right arm. For each patient, at least ten successful shots were considered as a correct exam. According to the manufacturer's guideline, the median value of successful measurements was considered as liver stiffness. The minimum cut-off point for considerable fibrosis based on previous reports was 7.5 kPa. Control attenuated parameter (CAP) is a device that can evaluate the steatosis status of liver during Fibroscan evaluation with both probes M and XL. According to previous studies based on liver biopsy the CAP value can represent the grade of steatosis.[12,16]

Ethics

This study was approved by the Ethics Committee of Iran University of Medical Sciences according to Helsinki declaration (code:IR.IUMS1397.32992). A written informed consent was obtained from each patient before enrollment.

Laboratory methods

The blood samples were refferd to the central laboratory of our center. Plasma and peripheral blood mononuclear cells (PBMCs) were separated. After separating Buffy Coat with Phosphate Buffered Saline (PBS) as a buffer and eventually adding ficoll, the PBMCs were isolated. Serum AST, ALT, and melatonin were measured using ELISA kits (Qiagen, Germany).

Data analysis

Descriptive data are presented as mean ± SD. Analysis of variance (ANOVA) and post-hoc Turkey's tests were used to compare the results among the groups. The correlation between melatonin level and other parameters was also evaluated. Data were analyzed using SPSS software (IBM-SPSS, version 20.0 IL, USA). $p < 0.05$ was considered as statistically significant.

RESULTS

We enrolled 97 eligible patients with a mean ± SD age of 42.21 ± 11 years (60.0% were women). Table 1 shows the basic characteristics of the participants in the three groups of patients. Age, WC, LDL and HDL were not significantly different among the groups. serum melatonin level has increased in groups. In Post-hoc analysis regarding serum melatonin level we did not find a significant difference between groups 1 vs. 2, 1 vs. 3, and 2 vs. 3 (0.4 pg/mL, 0.06 pg/mL, and 0.60 pg/mL, respectively). Based on categorization of the patients according to CAP results, serum melatonin levels were significantly increased in patients with severe steatosis compared to the normal group. Moreover, regardless of fibrosis and steatosis groups, melatonin level was correlated with liver enzymes. Furthermore regarding anthropometric characteristics, we did not find a significant correlation between melatonin level and BMI, and chest, waist, hip, ankle, and mid-arm circumference.

DISCUSSION

Hepatocellular injury and eventually liver cirrhosis can occur via different ways including the oxidative pathway. While melatonin with its anti-oxidant and anti-inflammatory properties may be considered as an immune

Table 1: the basic characteristic of participants according to fibrosis

Variables	Total (N = 97)	Group 1 (N = 42)	Group 2 (N = 28)	Group 3 (N = 27)	*p* value
Age (yrs)	42.2 ± 11.3	37.1 ± 10.4	44.6 ± 11.2	47.5 ± 9.7	0.05
Sex (M/F)(N)	38/59	19/23	5/23	14/13	0.02
WC	18.0 ±3.9	17.2 ± 4.4	18.9 ±4.6	18.2 ± 1.7	0. 1
CHOL Total (mg/dl)	189.6 ± 52.1	169.0 ± 45.3	210.4 ± 64.1	205.4 ± 36	< 0.01
LDL (mg/dl)	120.2 ± 35.5	114.7 ± 31.2	130.0 ± 42.2	120.0 ± 25.1	0.23
HDL (mg/dl)	43.7 ± 8.9	44.2± 9.2	44.7 ± 9.4	41.9 ± 7.6	0.55
TG (mg/dl)	161.8 ± 89.7	124.1 ± 59.9	189.3 ± 97.1	198.4 ± 103.3	< 0.01
AST (U/L)	40.7 ± 31.1	21.4 ±7.3	53.6 ± 37.7	57.4 ± 30.6	< 0.01
ALT (U/L)	48.8 ± 41.2	21.0 ± 10.7	66.1 ± 46.2	74.0 ± 40	< 0.01
ALK.P (U/L)	193.2 ± 61.8	159.9 ± 53.8	215.5 ± 64.1	221 ± 45.5	< 0.01
FBS (mg/dL)	107.9 ± 34.8	92.7 ± 10.7	110.8 ± 23.9	137.6 ± 57.6	< 0.01

Groups:1 = Normal participants, 2 = mild to moderate fibrosis, 3 = sever fibrosis

Table 2: the Melatonin level according to Fibroscan results

Variable	Group1	Group2	Group3	
Melatonin Level (pg/ml)	Fibrosis (Fibrosis)			
	< 5.8kp	5.8-9.1kp	> 9.1 kp	*p*.value
	332.3 ± 459.7	494.3± 507.0	624.8 ± 483.0	0.05
	Steatosis (CAP result)			
	No steatosis (< 240)	Mild- Moderate steatosis (240-290)	Sever steatosis (> 290)	
	407.7 ± 496	330.3 ± 457	576.7 ± 516.3	0.03

Groups:1 = Normal participants, 2 = mild to moderate fibrosis, 3 = sever fibrosis

modulator.

We found that serum melatonin level increased by as fibrosis advance from almost healthy (group1) to steatohepatitis (group 3) although the difference was not statistically significant. This difference was not correlated with anthropometric indices or even FBS or lipid profile. In this setting, melatonin levels were significantly associated with increased liver enzyme levels. Furthermore, regarding steatosis, we revealed a significant association between increased the steatosis and serum melatonin level.

There are no specific and effective treatments for NAFLD, but in some studies melatonin was introduced as a treatment.[17] Melatonin has some specific properties that targets many molecular pathways including anti-inflammatory and antioxidant effects, that can consequently decrease fibrosis formation.[18] Previous studies revealed that melatonin treatment significantly decreased the severity of steatosis, immigration of inflammatory cells, hepatic cell damage and reduced serum and tissue inflammatory cytokine levels.[19-21] Moreover, melatonin induces T-cell proliferation and up-regulation of pro-inflammatory cytokines.[22, 23] Prior studies demonstrated that melatonin could be involved in liver protection in a high-fat diet, chemical component toxicity, biliary injuries, and eventually fibrosis.[24] As a result, melatonin could slow-down hepatocytes injury via various mechanisms. Furthermore, melatonin has a vasorelaxant and anti-fibrotic properties that may be involved in cell protection.[25] In this setting melatonin may protect liver injuries via decreased hepatic oxidative stress, increased expression of antioxidant factors, moderate the effect of hepatic endoplasmic reticulum (ER) stress, and sustain cellular calcium homeostasis .[9,17] In this regard, one study showed that melatonin may ameliorate insulin resistance.[26] Despite these facts the exact pathway of its protective effects on liver is not clear.

Regarding liver enzymes, former studies indicated that treatment with melatonin may decrease liver enzyme levels.[10,21,27] In recent study on 100 biopsy-proven

NAFLD cases, 12-week treatment with melatonin alleviates liver enzyme levels.[21] However,, these studies did not assess the association between melatonin level with steatosis or fibrosis. It can be hypothesized that along with cell injury advancement and liver enzyme increase, serum melatonin level increases for cell regulation effects but not in the treatment level.

It seems that serum melatonin level increases with fibrosis possibly as a regulatory and protective mechanism. But it should be considered that increased serum melatonin level is also significantly associated with steatosis level. Steatosis may be one of the preliminary steps of inflammation that can cause cell injuries.

In conclusion it can be hypothesized serum melatonin level increase is more sensitive to fat accumulation induced cell injury. Furthermore, almost all of former studies were performed on animal models and we have not enough human studies in this setting. Hence, our study may help perform new studies about regulatory hormones such as melatonin.

ETHICAL APPROVAL

There is nothing to be declared.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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