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THE EFFECTS OF LONG-TERM MELATONIN TREATMENT ON PLASMA LIVER ENZYMES LEVELS AND PLASMA CONCENTRATIONS OF LIPIDS AND MELATONIN IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS: A PILOT STUDY

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The present study represents the follow-up of our initial observations designed to investigate whether in patients with nonalcoholic steatohepatitis (NASH) the beneficial effect of 12-week course of melatonin (MT) on liver enzymes could be maintained with prolonged period of treatment and to analyze whether biochemical treatment responses could be sustainable after melatonin discontinuation. Forty two patients with histologically proven NASH (30 treated with melatonin 2×5 mg daily, 12 controls receiving placebo) enrolled to our previous 3-month study agreed to take part of subsequent 12 weeks treatment followed by 12-week follow-up period. Enrolled patients had biochemical determinations every six weeks during the melatonin treatment period and again after 12 weeks of follow-up. Significant reduction in median alanine aminotransferase (ALT) levels between baseline and week 18, week 24 and follow-up was observed in both MT-treated and control group: 43% and 31%, 42% and 33%, 32% and 31%. Aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) levels decrease significantly only in MT-treated group. In MT-treated group mean percentage change in AST level below baseline at week 18, at week 24 and at follow-up was 45%, 33% ($p<0.05$) and 8% (ns), respectively. The evolution of GGT levels was as follows: the mean percentage reduction in GGT below baseline level at week 18, 24 and follow-up was: 48%, 52% and 38% ($p<0.05$), respectively. In both MT-treated and control group plasma cholesterol, triglicerydes and glucose concentrations as well as plasma alkaline phosphatase persisted within normal values during the prolonged study period. Plasma concentration of melatonin (pg/ml) in MT-treated group averaged 7.5±3.5 at baseline and increased to 52.5±17.5 at 24th week. The results of our study demonstrating beneficial effect of melatonin on liver enzymes in patients with NASH would seem to encourage further controlled trials of melatonin given over a longer period of time with liver histology as end point.

Key words: *nonalcoholic fatty liver disease, lipids, liver enzymes, melatonin, nonalcoholic steatohepatitis, alanine aminotransferase, aspartate aminotransferase*

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disorders in Western countries. The reported prevalence of NAFLD varies largely because of different population studied and the diagnostic methods used. Recent studies indicate that in the general population of Western countries the prevalence of NAFLD is approximately 20%-30% (1). The spectrum of NAFLD encompasses simple hepatic steatosis with rather good prognosis and nonalcoholic steatohepatitis (NASH), that is often a precursor to the development of cirrhosis and in some cases, hepatocellular carcinoma (2-4). It is estimated that NASH occurs in ~5% of adult Americans and in ~1% of Western population (3). Accumulation of fat in hepatocytes may be accompanied by insulin resistance, obesity and hypertension thus it is considered the hepatic manifestation of metabolic syndrome (5, 6). The currently available noninvasive tests are not sufficient for diagnosis of NASH, this can only be confirmed by examination of hepatic tissue (7). Multiple factors such as insulin resistance, alteration of plasma adipocytokines, cytokines and oxidative stress have been proposed to explain the transformation of NAFLD from simple steatosis to NASH (8). The mechanism by which these factors participate in the pathogenesis of NASH is an area of intense investigations. Interaction of free radicals with cellular constituents may result in their peroxidative deterioration leading to an impaired of cellular functions (9).

Currently, there is no effective pharmacologic treatment of patients with NASH. Since oxidative stress seems to play an essential role in the development and consequences of NASH, the effectiveness of several antioxidant agents such as vitamin E, vitamin C and betaine have been evaluated (6, 10-12), however, the evidences of the efficacy of such treatments have been equivocal. We have previously reported the preliminary pilot results of a three

months of combined therapy with lifestyle intervention and antioxidant agent - melatonin in patients with histologically proven NASH (13). Because we demonstrated a significant improvement in mean alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) levels among subjects receiving melatonin, the present study was designed as follow-up to our initial observations to (1) investigate whether in patients with NASH the beneficial effect of short course of melatonin treatment on liver enzymes could be maintained with prolongation of this therapy, and (2) to analyze whether biochemical treatment responses could be sustainable after melatonin discontinuation. Because of uncertainty on potential benefit of such treatment, the prolonged study period was limited to 12 weeks of melatonin and 12 weeks of follow-up.

MATERIAL AND METHODS

Patients

Forty two patients with NASH participated in the study were included in an earlier report (13) describing the initial response to a 12 week-programme consisting of appropriate diet, moderate exercise and melatonin. All patients agreed to take part in subsequent 12 weeks treatment followed by a 12-week follow-up period. Inclusion and exclusion criteria were described in detail previously (13). Briefly, only patients with histologically (liver biopsy) proven NASH, elevated plasma aminotransferases and no evidence of another form of liver disease were enrolled. Excluded were patients who had taken supplements containing antioxidants such as vitamin E, vitamin C and herbals or agents that boost antioxidative reactions *e.g.* selenium and zinc. All patients were included in a lifestyle program consisting of walking for 30–60 min/day and a diet tailored on the individual requirement. They were asked to maintain this regimen during the whole period of the study including 3 months of follow-up. Participants who fulfild the inclusion criteria were randomly assigned to melatonin treatment group or control group receiving placebo instead of melatonin, in a 2:1 ratio. Three patients from the control group dropped out their study. The treatment group consisted of 30 patients who ingested melatonin (LEK-AM, Zakroczyn, Poland) twice a day: 5 mg at 09:00 h plus 5 mg at 21:00 h for 24 weeks and the control group finally consisted of 12 patients. Enrolled patients had physical examination and aminotransferases (ALT, AST), gamma-glutamyl

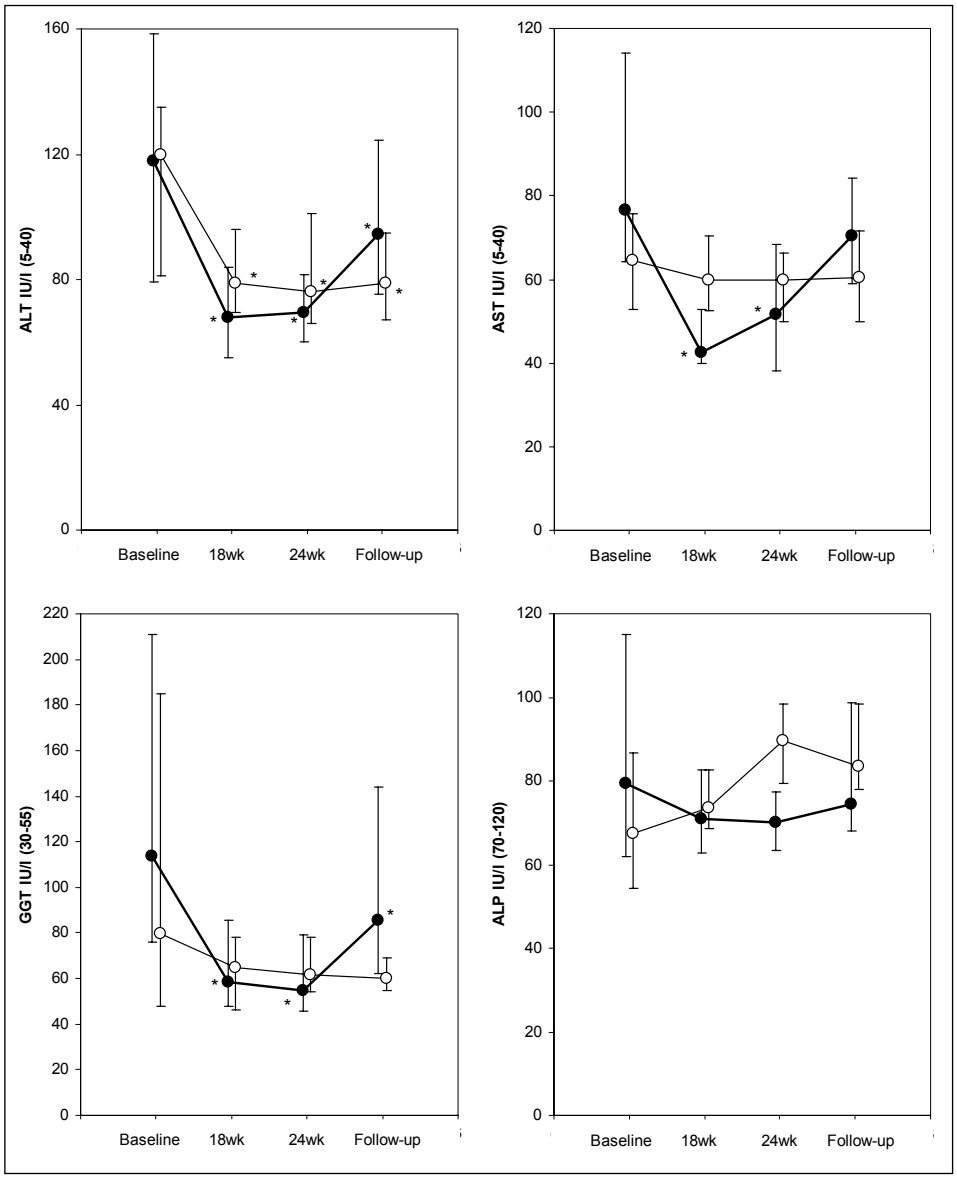

Fig. 1. Evolution of plasma liver enzymes ALT, AST, GGT and ALP at 18th and 24th weeks and follow-up in melatonin-treated (closed circles) NASH patients and vehicle controls (open circles). Asterisks indicate significant ($p<0.05$) difference compared to controls. In this and subsequent figures data are expressed as median values with the upper and lower quartiles.

transpeptidase (GGT), alkaline phosphatase (ALP), total cholesterol, triglycerides (TG) and glucose assays at 6-week intervals between 12th and 24th week of treatment and again after 12 weeks of follow-up. For routine laboratory assays standard automated techniques were used while plasma melatonin concentration was determined using RIA-technique (13).

Statistical analysis

All parameters were checked for normality using the Shapiro-Wilk test. Student's t test for unpaired samples was used to compare demographic characteristics between groups and data were expressed as the mean ±S.D. Because of non-Gaussian distributed variables, for the comparison of basal-treatment differences between each of the liver enzymes and the other laboratory parameters Wilcoxon's rank sum test was used. Results are expressed as median and upper and lower quartiles for all parameters. The Mann-Whitney test was used to perform the comparison between groups. Statistical significance was established at $p \leq 0.05$.

RESULTS

During the prolonged treatment period no patient complained somnolence and no significant side-effects were observed. Patients compliance with recommended diet and exercise program was declared as good, however, no significant changes in BMI were noted, thus, these results have not been included. *Fig. 1* presents evolution of plasma liver enzymes over time, *Fig. 2* represents the mean percentage change of initial plasma liver enzymes levels at each time point of the study and *Fig. 3* presents evolution of plasma concentration of glucose, cholesterol and triglycerides over time. Significant difference in median ALT levels between baseline and week 12 in both control and MT-treated group reported previously (13) was also seen at week 18, at week 24 and at follow-up ($p<0.05$). In MT-treated group median ALT(IU/L) was 118.0(79.2–158.5) at baseline, 68.0(55.0–83.8) at week 18th , 69.5(60.3–81.5) at week 24th and 94.3 (75.3–124.5) at follow-up. In control group median ALT (IU/L) was 120.0(81.3–135.0) at baseline, 79.0(69.5–96.0) at week 18th , 76.0(66.0–101.0) at week 24th and 79.0(67.3–94.8) at follow-up.

The mean percentage reduction in ALT below the pretreatment level was: at week 18–43% in MT-group, and 31% in control group, at week 24–42% in MT-group and 33% in control group and at the end of follow-up 32% in MT-group and 31% in control group. Four patients of MT-group had normal ALT values at week 18, three patients at week 24 and no patient showed this effect at follow-up. In control group there was no patient who normalized ALT level during the whole study period. The decrease in median plasma ALT level in MT-group at week 18 and week 24 was significantly more intense ($p<0.5$)

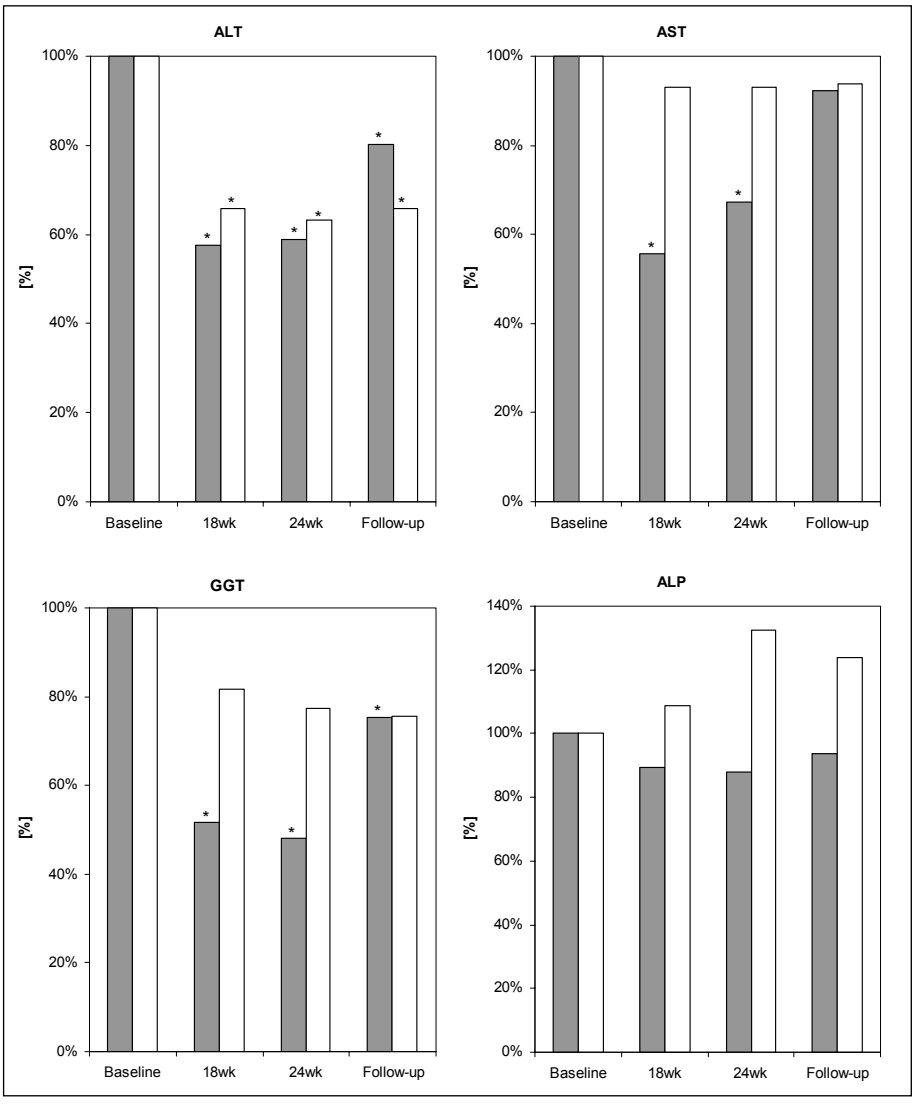

Fig. 2. Mean percentage changes in plasma levels of ALT, AST, GGT and ALP in melatonin-treated patients (on the left of each column) and in vehicle-treated controls (on the right of each column). Asterisks indicate significant ($p<0.05$) difference compared to controls.

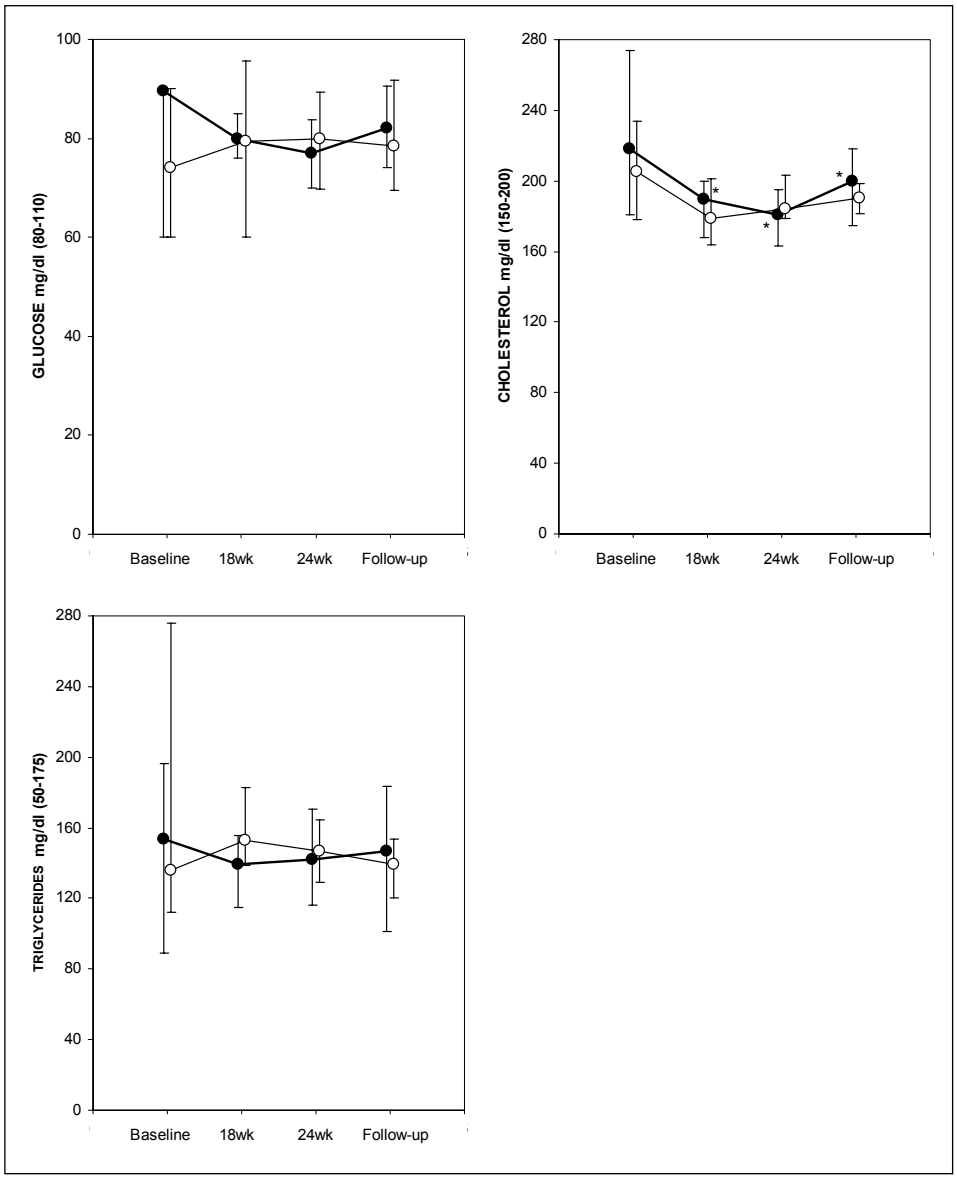

Fig. 3. Evolution of plasma concentrations of glucose, cholesterol and triglycerides over time in melatonin treated patients (closed circles) and vehicle-treated controls (open circles). Asterisk indicates significant difference ($p<0.05$) compared to controls.

Table 1. Demographic characteristics of patients.

	melatonin group n=30	control group n=12
Sex		
Male	18 (60%)	8 (40%)
Female	12 (40%)	4 (33%)
Age (years)		
Mean	41.5	40.8
±S.D.	4.0	3.6
Weight (kg)		
Mean	82.5	81.1
±S.D.	3.4	3.7

No statistically significant differences were found between the groups for sex, age and weight.

than that observed in control group, however at follow-up the difference between the two groups was not significant.

In MT-treated group median AST level (IU/L) was 76.5(64.2–114.2) at baseline, 42.5(40.0–52.8) at week 18th, 51.5(38.3–68.5) at week 24th and 70.5(59.0–84.3) at follow-up. In control group median AST level (IU/L) was 64.5(54.0–70.7) at baseline, 60.0(52.5–70.5) at week 18th, 60.0(50.0–66.3) at week 24th and 60.5(50.0–71.5) at follow-up. In MT-treated group mean percentage change in AST level below baseline at week 18th at week 24th and at follow-up was 45%, 33% ($p<0.05$) and 8% (ns) respectively. Six patients showed normal AST level at week 24 and patients none of at follow-up. In control group mean percentage decrease in AST below pretreatment value was below 10% at each time point of the study and the differences were not significant.

The evolution of GGT levels in MT-treated group was similar to that relating AST. In MT-treated group median GGT (IU/L) was 113.0(75.8–210.8) at baseline, 58.5(48.0–85.8) at week 18th, 54.5(45.5–79.0) at week 24th and 85.5(62.3–143.8) at follow-up. In control group median GGT (IU/L) was 79.0(47.8–185.0) at baseline, 65.0(46.0–78.3) at week 18th, 61.5(54.3–78.3) at week 24th and 60.0(54.5–69.0) at follow up.

The mean percentage reduction in GGT below baseline level was: at week 18th - 48%, at week 24th - 52% and at follow-up - 38% ($p<0.05$, respectively). Thirteen patients showed normal GGT level at week 18th and week 24th and 8 patients at follow-up. In control group at each time point of the study the reduction

of GGT as compared to baseline was not significant. One patient showed normal value of GGT at follow-up.

The median values of plasma cholesterol concentrations (mg/dL) in both MT-treated and control group were slightly elevated at baseline: 218.0 (181.0–273.7) and 205.0(178.2–234.0) respectively, and reached the normal range at the next time points. Median levels of ALP, triglycerides and glucose in both MT-treated and control group were within normal range at baseline and did not change significantly during the whole study period.

Plasma concentration of melatonin (pg/ml) in MT-treated group averaged 7.5±1.5 at baseline and significantly rose at 18 to reach 52.5±17.5 at week 24th.

DISCUSSION

In this study we evaluated the effects of 24 weeks of lifestyle intervention combined with 10 mg daily melatonin treatment on plasma liver enzymes levels and concentrations of lipids, glucose and melatonin in patients with NASH. One of the major difficulties in evaluation of the efficacy of any drug in NASH is the lack of adequate biochemical surrogate markers that would allow assessing disease severity. However, an increase in liver enzyme level is sensitive indicator of liver cell damage in any form of liver disease and despite of the lack of strict correlation with histologic liver lesions may suggest a liver disease that warrants treatment (7).

The present study revealed that significant improvement in mean plasma ALT, AST and GGT levels among patients with 12-week course of melatonin (3) were sustained throughout the next 12 weeks in which the patients were receiving treatment. Although AST level returned practically to the baseline value after discontinuation of melatonin, the associated GGT decrease was not reversed. However, the mean percentage reduction in GGT below baseline was only 38% at follow-up *vs*. 52% at the end of medication. The higher ALT, AST and GGT levels shown at follow-up in comparison with that found at 18th and 24th week of treatment reflected the high efficacy of melatonin, linked closely to the period of medicine administration.

In the past few years many reports have suggested the potential of weight reduction in improvement of liver enzymes in overweight or obese patients with NAFLD/NASH (14, 15). Pomrat *et al*. (15) reported that substantial decrease in plasma ALT level could be obtained from basic education about diet and exercise even when there was no accompanying weight loss; however, greater improvement in ALT they observed in patients who achieved weight reduction. In our patients there was no significant weight loss during the study period in both control and MT-treated group, while in both groups the decrease in ALT was significant at each time point. Thus, our findings are in accordance with that of Pomrat *et al*. (15) and confirm the findings obtained from previous 12-week pilot data showing that declared patient's compliance with recommendation of diet and exercise lead to significant decrease in plasma ALT level. It was of note that this decrease found in MT-treated group was significantly more intense than that observed in control group.

Oxidative stress is considered one of the most important pathogenic factors in the development of NASH (16, 17). Several studies in NASH gave promising results with antioxidants such as Vitamin C, Vitamin E and betaine (6, 12, 14). Foster *et al*. (18) examined the combined effect of atrovastin with vitamin E and vitamin C on NAFLD assessed by computed tomography. The results of this study suggested that such combined therapy improved liver steatosis. Recent data from large, double blind, randomized trial which examined the effects of pioglitazone and vitamin E as antioxidant on liver histology showed that improvement was achieved in 43% of the patients assigned to vitamin E (12). Although the prevailing view is that vitamin E acts as antioxidant, there are no confirmations that vitamin E effect *in vivo* depends exclusively on its antioxidative action (19). The encouraging antioxidant effect of melatonin in several experimental and clinical studies, prompted our investigation to determine whether this agent could improve liver enzymes in patients with NASH. Melatonin, produced in the pineal gland and to the greater extent in the gastrointestinal tract (20), plays an important role in immuno-regulation, inflammatory responses and oxidative stress (21, 22). It is well established that melatonin functions as free-radical scavenger and broad-spectrum antioxidant (16). The emerging ROS are successfully neutralized by a cell through both antioxidant enzyme systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and low molecular compounds such as glutathione (GSH), melatonin and others. Melatonin is the most powerful endogenous antioxidant which can be regenerated after radical quenching and its metabolites still present antioxidant activity (23). Melatonin also promotes *de novo* synthesis of GSH and stimulates GPx, thus melatonin efficiently protects the cell from oxidative damage by a variety of mechanisms (23, 24). The main intracellular source of reactive oxygen species are mitochondria. Melatonin as a lipophylic molecule easily crosses all cell membranes and reaches mitochondria where accumulates in high concentration (22). Several studies showed beneficial role of melatonin in experimentally induced liver and inflammatory bowel diseases (24-27). Recent experimental study of Zaouali *et al*. (28) has demonstrated that the addition of melatonin to preservative conventional cold storage solutions protected steatotic and nonsteatotic liver grafts against reperfusion injuries. Results of our study and the above mentioned ones makes possibility of melatonin use before organ donation to prevent liver injury and make it possible to use the graft that would otherwise fail if transplanted. In clinical trials melatonin has been used in extensive range from 3 mg/daily to 70 mg/daily for long time (29-33). The melatonin dosage used in the current study arbitrary was set on 10 mg/daily. At time of our study we did not know what dosage of melatonin would be optimal in patients with NASH. In patients enrolled to our study plasma basal levels of melatonin in NASH patients were within the expected values which were reported in healthy subjects (31, 32). Whereas pharmacological levels were showed during the first 12 weeks of medication (13) and these were maintained within the whole 24-week treatment period.

The strength of our study is that diagnosis of NASH was based on liver biopsy, and that circulating levels of melatonin at baseline and during the treatment were evaluated. However, the main limitation of this study are the small sample size and the lack of histological assessment after treatment. Thus we are not certain whether and to what extent the melatonin treatment improves liver histology in patients with NASH.

In summary, our study demonstrates that 24-week melatonin therapy for NASH results in significant improvement of plasma liver enzymes and that tolerability of melatonin ingested in 10 mg daily dose was excellent. The results of this study would seem to encourage further controlled trials of melatonin given over a longer period of time in patients with NASH with liver histology as end point.

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Conflict of interest: None declared.

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