INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is recently recognized as a major liver disease throughout the world (1, 2). The available data indicate the NASH affects around 1% of Western population and the related cirrhosis occurs in up to 20% of these patients over a 10-year period (1). Compared to the general population, NAFLD is associated with a significantly higher overall and liver-related mortality (3, 4). The spectrum of NAFLD encompasses liver diseases ranging from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH). While simple steatosis has a good prognosis, since complete reversibility can be achieved, NASH may progress into liver fibrosis and cirrhosis.

The pathogenesis of NASH is multifactorial. Several studies of patients with NAFLD have demonstrated that accumulation of fat in hepatocytes, especially TG and fatty acids is the hepatic manifestation of metabolic syndrome, also known as the insulin resistance syndrome (5). The most prevailing theory which has been proposed to explain the natural history of NAFLD from steatosis to NASH is the “two hits” hypothesis (6). The first hit is steatosis and the second one is chronic oxidative stress which increases the production of free radicals with lipid peroxidation and induction of inflammatory cytokines. These make the hepatocytes vulnerable to apoptosis and necrosis, leading to NASH (7, 8). Reactive oxygen species can be generated in liver mitochondria, where free fatty acids undergo beta-oxidation through peroxisomal and microsomal pathways (9).

THE PILOT STUDY OF 3-MONTH COURSE OF MELATONIN TREATMENT OF PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS: EFFECT ON PLASMA LEVELS OF LIVER ENZYMES, LIPIDS AND MELATONIN

The mechanism by which nonalcoholic fatty liver disease (NAFLD) progresses into nonalcoholic steatohepatitis (NASH) is unknown, however, the major process is oxidative stress with increased production of reactive oxygen species and excessive inflammatory cytokine generation. To date, there are no effective treatments for NASH and the published data with treatment using antioxidants are not satisfactory. Melatonin (MT), the potent endogenous antioxidant secreted in circadian rhythm by pinealocytes and in large amounts in the digestive system, was reported to improve oxidative status and to exert beneficial effects in NASH pathology in experimental animals, but no study attempted to determine the possible effectiveness of MT in humans with NASH. In this study, 42 patients (12 placebo controls and 30 MT-treated) with histological evidence (liver biopsy) of NASH and no history of alcohol abuse, were included. The treatment group took melatonin (2x5 mg/daily orally), while controls were treated with placebo. At baseline no significant differences between the groups were found for age, body mass index (BMI) as well as for plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and concentrations of cholesterol, triglycerides (TG), glucose and MT. During the study period plasma ALT level and cholesterol concentration decreased significantly in both MT-treated and control groups, however AST and GGT levels decreased significantly only in MT-treated groups. Median value of AST level at baseline was 76.5 (64.2-114.2) IU/L and its percentage decrease at 4, 8 and 12 week was 20, 36 and 38%, resp. Baseline GGT median level was 113 (75.7-210.7) IU/L and its mean percentage decrease at week 4, 8 and 12 was 46, 48 and 47%, resp. Plasma ALP levels did not change significantly during MT treatment. Median value of plasma concentrations of MT (pg/mL) in MT-treated group rose from 7.5 (5.0-14.25) at baseline to 35.5(18.8-110.0), 43.5(17.0-102.5) and 49.5(18.0-99.5) at the end of 4, 8 and 12 week of treatment, respectively. Plasma levels of TG and glucose as well as BMI in controls and MT-treated patients were not significantly different from baseline. This study demonstrates for the first time in humans that three months treatment with MT significantly improves plasma liver enzymes in patients with NASH without causing any side-effect. Plasma MT levels during the whole period of MT treatment persisted above that at baseline. Our findings show that treatment with MT significantly improves plasma liver enzymes in NASH patients, but larger cohort trials and longer treatment with MT are required before this indole could be included into the spectrum of the NASH treatment.

Key words: nonalcoholic fatty liver disease, antioxidants, liver enzymes, melatonin, nonalcoholic steatohepatitis, triglycerides, cholesterol
Non-invasive methods including liver biochemical tests and imaging procedures can not accurately distinguish NASH from other forms of NAFLD, but this is possible by liver biopsy and histological examination, which is generally accepted as the gold-standard for the diagnosis (10).

To date, there are no effective treatments for NASH. Usual recommendations include weight reduction and physical exercise, which promote the increase of insulin sensitivity. Pharmacological therapy is generally directed to treatment of risk factors. Insulin sensitizers, hepatoprotective, hypolipidemic agents, angiotensin receptor blockers and antioxidants have also been used (11). Published data on the efficacy of treatment of patients with NAFLD/NASH with antioxidants such as vitamin E, vitamin C and betaine (which generates glutathione as a major hepatic antioxidant) are promising but sparse and univocal (11-13). Recently Sanyal et al. (14) published a controlled study that enrolled 84 patients with NASH who received vitamin E 800 IU daily for 96 weeks. This treatment lowered significantly serum aminotransferases and improved hepatic steatosis and lobular inflammation, however without improvement in fibrosis. Other studies using vitamin E in several small pediatric and adult studies showed that this treatment was relatively well-tolerated and resulted in modest improvement in serum aminotransferase levels, and ultrasonographic appearance, but randomized controlled studies with liver biopsy and histological inclusion criteria are needed to determine whether vitamin E leads to histological improvement in NASH (15-18). Thus, it seems desirable that new agents with antioxidant action should be tested.

A number of studies have demonstrated a potent antioxidant properties of major derivate of tryptophan - melatonin (MT), which acts as a hydroxyl and peroxyl radical scavenger and as immunomodulatory agent (19, 20). MT is produced in diurnal rhythm by pinealocytes and in also in large amounts by entero-endocrine cells distributed throughout the gastrointestinal tract and liver (21). Several experimental studies showed therapeutic effects of MT on liver injury through its antioxidant action (22, 23). Pan et al. (24), have demonstrated that MT exerts a protective effect against fatty liver induced in rats by high-fat diet and accompanied by decreased plasma ALT and AST activity. Recently, we demonstrated that four week treatment with L-tryptophan, a precursor of MT, in patients with NASH resulted in statistically significant reduction in plasma GGT and proinflammatory cytokine levels (25).

The aim of the present study was to evaluate the effects of MT on plasma liver enzymes and lipids in patients with NASH and to assess plasma MT concentration during the treatment period with exogenous MT.

MATERIAL AND METHODS

Forty two patients with NASH were enrolled in this pilot study. They were cases diagnosed in the Department of Gastroenterology, St Barbara's Main District Hospital, Sosnowiec, Poland, between 2008 and 2010. The study was approved by the Ethics Committee at the Jagiellonian University Medical College, Cracow, Poland. Written informed consent was obtained from each patient. The diagnostic criteria of NASH were established based on liver biopsy, alcohol consumption <20 g of alcohol per day and elevated plasma activity of aminotransferases. Liver needle biopsies were obtained within 6 months before patients were enrolled to the study group. Histologic minimal criteria of NASH were the presence of steatosis, lobular inflammation and hepatocyte ballooning degeneration. Mallory bodies were not used as histological evidence of NASH. All patients had undergone upper gastrointestinal endoscopy and those with esophageal varices or portal gastropathy did not enter the study. The patients were sero-negative for HbsAg, anti-HCV antibodies, anti-mitochondrial antibodies, anti-nuclear antibodies and anti-smooth muscle antibodies. Serum concentrations of ceruloplasmin and ferritin in all patients were within normal limits. Patients on regular supplements containing antioxidants such as vitamin E or vitamin C, and herbs such as silymarin or agents that boost antioxidative reactions e.g. selenium and zinc were excluded. Body mass index (BMI) was calculated from weight (kg)/height (m²). Enrolled patients returned for study visit on a monthly basis. Physical examination was conducted at each visit and venous blood was drawn following a 12-h overnight fast. The following routine biochemical parameters using standard automated techniques were assessed in plasma: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total cholesterol, triglycerides (TG) and glucose.

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Statistical analysis

Results are expressed as median and upper and lower quartiles for all parameters. The Wilcoxon signed rank test was used to compare groups at baseline. The non-parametric Friedman test was used to assess the evolution of analytical parameters over time in each group and the Mann-Whitney test to perform the comparison between groups. P values less than 0.05 or 0.001 were considered statistically significant.

RESULTS

All patients completed 12 weeks of MT treatment without significant side effects. However, 12 patients complained slight somnolence within the first 4 weeks of MT treatment. Subjective quality of sleep was positive and no patient reported insomnia. Analytical data are presented as median values with upper and lower quartile in parenthesis. Changes in plasma aminotransferases activities in MT and placebo-control groups are presented in Fig. 1. In MT-treated group median ALT (IU/L) was 118.0 (79.2-158.5) at baseline and showed significantly lower values during the treatment period: at week 4 - 92.0 (68.2-120.0) (p<0.05), at week 8 - 71.5 (61.0-93.7), (p<0.05), reaching a nadir of 66.0 (55.0-79.5) at week 12 (p<0.001) (Fig. 1). The mean percentage changes for ALT at week 4, 8 and 12 was 22, 40 and 44, respectively, below the pretreatment level (see Fig. 2). Among 30 patients of MT-treated group, four (13%) showed ALT
normalization: two cases at week 8 and two cases 2 at week 12. The evolution of ALT levels in control group was similar to that of MT-treated group. Median ALT (IU/L) was 120.0 (81.3-135.0) at baseline and significantly decreased to 93.0 (75.5-120.3) at week 4 (p<0.05), 87.0 (75.0-103.5) at week 8 (p<0.05) and 83.5 (70.7-100.0) at week 12 (p<0.05), (see Fig. 1). The mean percentage changes in control group at week 4, 8, and 12 were 23, 28 and 30% below baseline level, respectively (see Fig. 2). No patient normalized ALT level during the 12 week period of observation.

In MT-treated group median AST level (IU/L) was 76.5 (64.2-114.2) at baseline and decreased significantly at week 4 to 61.0 (44.0-80.0), at week 8 to 49.0 (38.0-71.0) and at week 12 to 47.0 (38.2-58.7). The differences in AST levels between basal and those recorded at week 4, 8, 12 of MT treatment were significant (p<0.001), (see Fig. 1). Eight patients (26%) normalized AST level: one case at week 4, six cases at week 8 and one case at week 12. The mean percentage change in AST values in MT-treated group at week 4, 8, and 12 was 20, 36 and 38%, respectively, below baseline level (see Fig. 2) and had similar tendency as in the case of ALT. In control group median AST level (IU/L) was 64.5 (54.0-70.7) at baseline and 59.5 (54.0-70.7), 57.5 (45.0-70.5) and 57.0 (40.0-72.5) at week 4, 8 and 12 resp. (see Fig. 1). The differences between AST levels at week 4, 8, 12 and baseline were not significant. One patient showed normalization AST level - at week 4. The mean percentage change for AST at week 4 and 8 was 6 and 19, respectively, below baseline level and 5 above baseline at week 12 (see Fig. 2). No significant differences between baseline levels and those recorded during the 12 weeks of observation were found. The mean percentage change for GGT at week 4 and 8 was 6 and 19, respectively, below baseline level and 5 above baseline at week 12 (see Fig. 2). Among 8 patients with abnormal GGT levels at baseline one case showed normalization - at week 4. Median levels of ALP in both MT-treated and control groups were within normal range at baseline and did not change significantly during the 12 week study periods (see Fig. 1).

In MT-treated group, median GGT (IU/L) was 113 (75.8-210.8) at baseline and significantly decreased at week 4 to 73 (56.0-130.0), at week 8 to 59.5 (47.0-92.0) and at week 12 to 60 (47.3-85.3), (see Fig. 1). Among 26 patients with elevated GGT levels at baseline 13 (50%) showed GGT normalization: six cases at week 4, five cases at week 8 and two cases at week 12. The mean percentage reduction in GGT below baseline level at week 4, 8 and 12 was 38, 48 and 47, respectively (see Fig. 2).

In control group GGT levels (IU/L) averaged 79 (47.8-185.0) at baseline and 75.5 (54.8-167.0) and 83 (51.5-148.3) at week 4, 8 and 12, respectively (see Fig. 1). No significant differences between baseline levels and those recorded during the 12 weeks of observation were found. The mean percentage change for GGT at week 4 and 8 was 6 and 19, respectively, below baseline level and 5 above baseline at week 12 (see Fig. 2). Among 8 patients with abnormal GGT levels at baseline one case showed normalization - at week 4. Median levels of ALP in both MT-treated and control groups were within normal range at baseline and did not change significantly during the 12 week study periods (see Fig. 1).

The mean value of cholesterol concentration (mg/dL) at baseline - 218.0 (181.0-273.7) in MT-treated group was above upper limit of normal (200 mg/dL) and decreased significantly (p<0.05) to normal values: at week 8 to 190.0 (178.5-199.5) and at week 12 to 190.0 (170.0-200.0), (p<0.05), (Fig. 3). In control group plasma concentration of cholesterol at baseline was 205.0 (178.2-234.0) and significant decrease to normal value was recorded at week 8 to 190.0 (168.7-196.0) and at week 12 - 183.5 (166.5-190.5), (p<0.05), (see Fig. 3). The mean values of plasma TG, and glucose in patients included in our study were within normal range at baseline and no significant changes were observed after the week 4, week 8, and week 12 - in both MT-treated and control group (see Fig. 3).

Plasma concentration of melatonin (pg/ml) in MT-treated group rose significantly (p<0.001) from median value 7.5 (5.0-14.25) at baseline to 35.5 (18.0-110.0), 43.5 (17.0-102.5) and 49.5 (18.0-99.5) at the end of 4, 8 and 12 week, respectively (see Fig. 3). In control group plasma concentration of melatonin (pg/ml) averaged 8.5 (5.0-12.5) at baseline, and 8.5 (8.0-12.5), 9.0 (7.75-11.25), and 9.0 (6.75-9.25) at week 4, week 8 and week 12, respectively.

BMI in both control and MT-treated group slightly decreased during the 12 week period, however the differences
from baseline were not significant and these results have not been included.

DISCUSSION

Generally, NAFLD/NASH is considered as the most common cause of a long-term increase in aminotransferases, however 10-20% of patients show normal values (1). Usually, ALT and AST values are at under 250 IU/L, and modest elevations of ALT and GGT have been reported (27). The results of our study showed that patient's compliance with appropriate diet and moderate physical exercise (control group) was sufficient to achieve significant decrease of ALT level during the 12 week observation period. It has been widely accepted that the weight reduction and the increased physical activity improves liver enzymes in overweight patients with NAFLD. Ueno et al. (28) showed that restricted diet and exercise therapy in obese patients with NAFLD significantly improved both ALT and AST levels as well as the degree of steatosis in liver biopsy after the 3 months of therapy. Suzuki et al. (29) have demonstrated that reducing weight by at least 5% and regular exercise significantly decreased ALT level in patients with NAFLD. Our results are in accordance with these studies, however, we have not observed decreasing of AST level as it was reported by Ueno et al. (28). This may be explained, at least in part, by no improvement in patients' BMI in our study. All patients included in our study have biopsy proven diagnosis of NASH, while the above mentioned authors included patients not homogeneous with respect to histopathological findings at initial liver biopsy. Furthermore the diagnosis of NAFLD made by Suzuki et al. (29) was not based on liver histology. Thus, it is likely that not all patients included in those studies had NASH.

The main findings in our study is that NASH treatment with natural antioxidant agent such as melatonin in combination with diet and exercising resulted in better biochemical response than diet restriction and exercise alone. Decrease in ALT level in MT-treated group at each time point was significantly more intense than that observed in control group. Additionally significant decrease in AST and GGT levels was seen in patients of MT-treated group only. There are several studies indicating that surrogate markers of
NAFLD/NASH such as GGT and ALT are positively associated with cardiovascular events. Beyond association with baseline level of GGT, an association between longitudinal increases in GGT and incident cardiovascular disease was reported (30). Our patients were treated with MT for 3 months only and it remains unclear whether a longer treatment period beyond 3 months could result in further improvement in plasma liver enzymes.

Studies on the beneficial effect of antioxidants including liver histology are scare. Nobili et al. (31) demonstrated that in children with NAFLD the addition of alpha-tocoferol and ascorbic acid as antioxidants to lifestyle intervention did not result in an extra benefit to that achieved by lifestyle changes alone. Betaine, another antioxidant agent has been tested in a small pilot study with a promising results - seven patients completed the 1-year betaine treatment and in six of them the follow-up of liver biopsy showed improvement in steatosis, inflammation and fibrosis (32).

Melatonin as an antioxidant agent exerts its effects directly via free-radical scavenging and inhibiting their generation as well as indirectly by activating the enzymes which enhance the endogenous antioxidant defense capacity of organism (19, 20). Thus, melatonin seems to be promising agent in treating liver diseases in which oxidative stress plays an important pathogenic role. As far as we know, our report is one of the first study indicating a relationship between melatonin treatment and improvement in liver enzymes in patients with NASH. Other abnormal laboratory biochemical finding in patients with NASH is hypertiglyceridemia presented in about 25-45% of patients (30, 33). The mean values of plasma TG in patients included in our study were within normal range at baseline, however, a slight decrease in TG and cholesterol concentrations was seen at week 12 compared to baseline in both groups. Several studies have demonstrated that plasma concentration of melatonin in patients with liver cirrhosis is elevated due to its impaired metabolism in cirrhotic liver (26, 34). Patients included in our study had no signs of decompensated liver disease and in all of them portal hypertension, assessed by upper gastrointestinal endoscopic features, was excluded.

Plasma pharmacokinetic of MT has been poorly investigated. In healthy subjects the peak serum MT after 2 mg melatonin administered orally occurred within 1-2 hours (35) and after the dose of 80 mg this concentration rose from 350 to 1000 times over the concentration showed at night-time (36). In our study plasma concentration of MT after administering the preparation containing 5 mg of melatonin twice daily tested at week 4, 8 and 12 after 12 hours of overnight fast was about twenty times higher than that at baseline. Because there are no studies on plasma melatonin pharmacokinetics in healthy subjects after prolonged administration of melatonin it is not possible to conclude that this high plasma concentration of melatonin in our patients with NASH was caused or not by liver pathology.

In summary, our study demonstrates that 3 months of combined therapy with lifestyle intervention and antioxidant agent, melatonin 5 mg twice a day, improves significantly plasma liver enzymes in patients with NASH. Plasma melatonin levels during the 3 month period of treatment was persistently increased above that at baseline. Our preliminary findings suggest the need for further large scale trials and longer treatment with melatonin before this indole could be included the basic spectrum of pharmacologic treatment of NASH.

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Conflict of interests: None declared.

REFERENCES


