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# Comparison of the Therapeutic Effects of Melatonin, Metformin and Vitamin E on Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial

**Ali Akbar Hajiagha Mohammadi[1], Sina Khajeh Jahromi[1*], Somayeh Ahmadi Gooraji[2], Ali Bastani[1]**

1. *Metabolic Diseases Research Center, Velayat Hospital, Qazvin University of Medical Sciences, Qazvin, Iran*
2. *Dept. of Biostatistics, School of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

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**Corresponding Information:**
**Dr. Sina Khajeh Jahromi,**
Metabolic Diseases Research Center, Velayat Hospital, Qazvin University of Medical Sciences, Qazvin, Iran
**E-Mail:**
sina.khajehjahromi@gmail.com

**ABSTRACT**

**Background & Objective:** Currently, no therapeutic or surgical treatment for non-alcoholic fatty liver disease (NAFLD) has been officially approved. This is a serious void in the medical field given the increasing prevalence of NAFLD cases in developing countries. Our study compared the therapeutic effect of metformin, melatonin and vitamin E on serum parameters that are associated with NAFLD pathogenesis.

**Materials & Methods:** This randomized clinical trial was performed on 140 patients with NAFLD who were referred to the central hospital of Qazvin. Patients were assigned randomly into four groups. The first group received metformin (500mg daily) with two placebos, the second group received melatonin (10mg daily) with two placebos, the third group received daily 800 IU vitamin E with two placebos and the fourth group received three placebos daily. All four groups were placed on the same diet regimen and had the same lifestyle changes to increase their daily activity time. Ultrasonography was used for the evaluation of the appearance of liver. Weight, BMI, AST, ALT, lipid profile and FBS were measured at baseline, 3 months and 6 months later.

**Results:** All of the therapeutic agents caused a decrease in aminotransferases but metformin was also more potent in improving lipid profiles. A significant difference in LDL was obtained by melatonin (mean change in the control vs. melatonin 15.9; P=0.032) and AST (mean change in control vs. melatonin -18.3; P<0.001). Metformin improved liver appearance in ultrasonography imaging better than the other treatments (P=0.043).

**Conclusion:** Metformin can be considered an effective medication but melatonin may accelerate the healing process in NAFLD patients.

**Keywords:** Non-alcoholic Fatty Liver Disease, Melatonin, Metformin, Vitamin E



## Introduction

The pathogenesis of non-alcoholic fatty liver disease is associated with insulin resistance and sedimentation of triglycerides (TG) in hepatocytes. The "two- hit hypothesis" is commonly used to describe the pathogenesis of NAFLD. The "first hit" is steatosis, the abnormal infiltration of lipids in a liver cell, which can lead to inflammation and steatohepatitis. The "second hit" is oxidative stress and mitochondrial injury, leading to further inflammation and cellular injuries (1). There are no definitive and approved medical or surgical treatments for NAFLD although changes in life style, weight loss and medical treatments, including oral hypoglycemic agents, statins and vitamin E, have been suggested (2).

Since insulin resistance and oxidative stress play an essential role in the pathogenesis of NAFLD, medications that improve these conditions have been the subject of previous research. Biguanides (such as metformin) and thiazolidinediones are two groups of insulin sensitizers that have been frequently studied (3, 4).

Some anti-oxidative agents have also been mentioned for the treatment of this disease. Vitamin E is an anti-oxidant that acts as a peroxyl radical scavenger, disabling the production of damaging free radicals in tissues. Studies, such as one by Sanyal, demonstrated that vitamin E was more effective than a placebo in the treatment of patients with NAFLD but found no difference between vitamin E and pioglitazone (4).

Melatonin (N-acetyl-5-methoxy tryptamine) is an indoleamine produced during the darkness phase of the pineal gland in mammals. It has been shown that melatonin had an anti-inflammatory effect and reduced

cell damage. It is a potential anti-oxidant which protects against free radicals and oxidative stress, as demonstrated in laboratory settings and in vivo **(5-8)**. This indoleamine molecule can also play a role in metabolic diseases **(9)**; melatonin can improve the lipid metabolism in diabetic rats, suggesting that this function may be due to a reduction or an improvement in insulin resistance **(10)**. Melatonin can decrease the resistance of cells to insulin in patients with obesity **(11)**. The c-jun N-terminal kinase (JNK) pathway that contributes to insulin resistance, obesity and hepatotoxicity due to fatty acid, oxidative stress and hepatocyte apoptosis can be influenced by melatonin, reducing phosphorylation of JNK by one half **(12, 13)**. In this setting, researchers proposed that melatonin could be effective in the treatment of NAFLD.

Our study compared the effect of melatonin with two other medications, metformin and vitamin E, in the treatment of patients with NAFLD who also underwent a dietary regimen and changes in their lifestyle, comparing the therapeutic effect of the medications with a placebo-control group.

## Materials and Methods

### Study design and sample:

This double-blinded, randomized clinical trial was performed on patients with NAFLD who were referred to the gastroenterology clinic of Velayat Hospital, Qazvin, Iran, from January 2018 to Sep 2019. (Figure 1)

### Inclusion and exclusion criteria:

The patients were the adults between the ages of 18 to 75 years with NAFLD, diagnosed clinically by ultrasonography, elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and after ruling out other diagnoses. Viral, autoimmune, toxic and drug- induced hepatitis, hemochromatosis, Wilson disease, any thyroid disorders and any kind of biliary disease (primary sclerosing cirrhosis or primary biliary cirrhosis) were ruled out in each case. Patients who used weight loss agents in the previous 6 months were excluded from the study. The Lifetime Drinking History (LDH), a structured interview to collect clients' patterns of alcohol consumption, was used to obtain a complete history of each patient. The exclusion criteria were alcohol consumption of more than 20 gr per day for women and 30 gr per day for men for at least 3 consecutive months during the last 5 years. Patients who had autoimmune hepatitis, hepatitis B, hepatitis C or other chronic liver diseases, or heart failure (New York Heart Association Class II to IV), and those who were consuming medications known to cause steatohepatitis such as fibrates, statins, NSAID, amiodarone, and tamoxifen were excluded from the study. Patients with diabetes mellitus and patients who received vitamin C, zinc, selenium or antioxidant agents during the last three months were also excluded from the study.

**Ethics statement:** This study was approved by the ethical committee of Qazvin University of Medical Sciences (ID: IR.QUMS.REC.1394.218), and recorded in IRCT with ID, "IRCT2016040318124N3".

### Randomization and study groups:

All 140 patients (72 females and 68 males) with NAFLD who satisfied the eligibility criteria completed a written informed consent form prior to entering the study. Patients were randomly assigned into four groups by sealed envelopes method. (Block Randomization list from an online source: website: www.sealedenvelope.com.) Neither the patients nor the physicians were aware of the actual medication distribution. All patients were placed on the same diet regimen and lifestyle changes were made by increasing their daily activity time. Having been visited and examined by the doctors, patients were referred to trained personnel who had no role in the analysis of the collected data. Patients were given drug packages based on their assigned group. The first group received melatonin (Natural Made, USA) at a dose of 10 mg once daily with two placebos at night (after dinner and 30 minutes before going to bed), the second group received 500 mg of metformin (Abidi, Iran) daily with two placebos at noon and the third group received vitamin E 800 IU, (Nature Made, USA) with two placebos in the morning. Patients in the matched control group received three placebos daily. All four groups were followed up for 24 weeks.

### First assessment:

Participating patients were assessed for weight, height and BMI. A lipid profile (high density lipoprotein [HDL], total cholesterol, triglyceride [TG], low density lipoprotein [LDL]) and fasting blood sugar (FBS) following 12 hours of fasting were obtained. Additional tests for liver cell injury (ALT, AST) and a liver and biliary tract ultrasonography were performed. Ultrasonography was conducted by an operator who had no knowledge of the test groups.

### Follow-up visits:

After randomization, patients were followed according to a predetermined schedule for the assessment of the safety and tolerability of the study drugs. The patients were asked to follow their diet and medication plan carefully and given standardized and functional recommendations about lifestyle changes and diet. Patients were monitored by phone call and questioned in four areas:

(1) Was the patient using the medication correctly?

(2) Were there any side effects for the medication? (Drug intolerance, drowsiness, sleeping more than usual, gastrointestinal upset)

(3) Were there any missing samples?

(4) Was the patient willing to continue the treatment?

**Primary outcomes:** AST and ALT were measured at baseline, 3 and 6 months later.

**Secondary outcomes:** Weight, BMI, lipid profile and FBS were measured at baseline, 3 and 6 months later.

### Statistical analysis:

By using G-power software (version 3.0), and given a power of 80%, type one error of 0.05, and expected reduction in primary outcomes of about 30% between the treatment group and placebo, the required sample size was estimated to be 128 cases totally. Given the attrition rate of 5%, total sample size was estimated at 136 cases (34 cases in each group). The data was analyzed using SPSS software version 20. Kolmogorov – Smirnov, Shapiro-Wilk and normal Q-Q plot tests were used to check normality for the study. The Results were expressed as mean and standard deviation (SD) for quantitative data and frequency with percent for qualitative data. Paired sample T-test was used for comparison within the groups. Repeated measurements using ANOVA (RM- ANOVA) and Bonferroni test (post hoc-test) were used for between and within group comparison. Chi-square test was used for qualitative factors and Pearson correlation test was used for quantitative factors to test the relationship. Ancova test was used for adjusting baseline effect of continues variables.

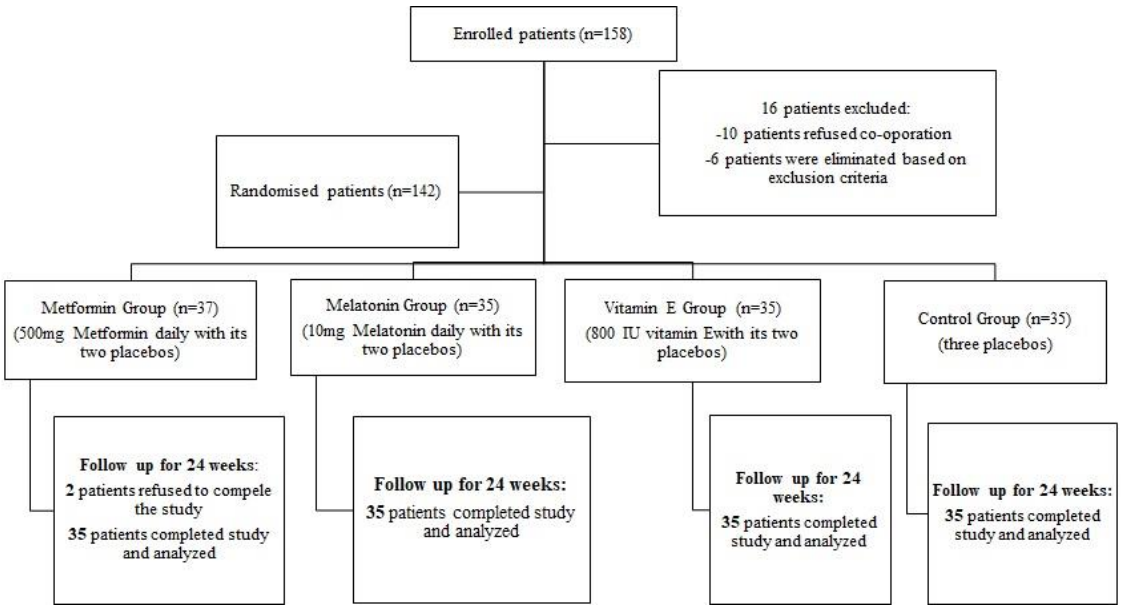


**Fig1. Study groups design**

## Results

### Demographic results

The data from 140 patients in the four groups was analyzed; metformin (35 cases), melatonin (35 cases), vitamin E (35 patients), and the control group (n=35). The age range of patients was 18-74 years with a mean age of 42.6 ± 13.06 years. There was no statistically significant difference between the groups in age (P = 0.055) and gender (P=0.069). The results are summarized in Table 1. Based on the results of analysis of variance, weight at baseline was significantly different among the groups, so this could affect the results after intervention, however, analysis of covariance revealed that weight change was not significant between the four groups at the 2nd and 3rd follow- ups after adjusting for weight at baseline. A within-group comparison of weight did obtain significant results with the metformin group (baseline vs. 6-months; P=0.000) and the vitamin E group (baseline vs. 6-months; P=0.003). In the melatonin group, significant results were only obtained between baseline and 3-month follow up after intervention (P=0.000). All results were significant at the level of 0.02 based on the Bonferroni adjustment. Patients' BMI was normal in all groups.

**Table 1. Descriptive and analytical statistics of patients' demographic data by therapeutic groups**

| Variables | Level | Therapeutic groups | | | | P-value |
|---|---|---|---|---|---|---|
| | | Metformin | Melatonin | Vitamin E | Control | |
| Gender, N (%) | Female | 24(68.6) | 19(54.3) | 18 (51.4) | 13 (37.1) | NS* |
| | Male | 11 (31.4) | 16(45.7) | 17 (48.6) | 22(62.9) | |
| Age, Mean ± SD | | 45.03 ± 12.44 | 47.5 ± 13.02 | 43.3 ± 10.8 | 44.4±2.07 | NS |
| Weight(kg), Mean±SD | Baseline | 75.73± 12.74 | 74.89 ± 11.88 | 81.09± 13.04 | 89.44±11.23 | <0.001 |

| Variables | Level | Therapeutic groups | | | | P-value |
|---|---|---|---|---|---|---|
| | | Metformin | Melatonin | Vitamin E | Control | |
| | 6-months | 74.4±11.6 | 73.4±12.2 | 77.7±12.2 | 84.4±12.5 | 0.977 ** |
| **Height (cm), Mean ± SD** | | 163.09 ±11.3 | 164.4 ± 9.4 | 165.5± 9.25 | 162±11.7 | NS |
| **BMI (kg/m$^2$), Mean±SD** | Baseline | 28.5 ±4.4 | 27.6 ± 3.8 | 29.1± 2.8 | 28.05±4.27 | NS |

*** NS: Not Significant; ** obtained by Ancova (comparison adjusted for weight at baseline)**

### Serological & Biochemical results

The results of our comparison showing the mean changes in serum parameters within groups are indicated in Table 2. For the melatonin group, changes in parameters of TG, Cholesterol, FBS, AST and ALT free pre- to post-treatment were significant (P<0.001). However, the parameters of TG, AST, and ALT were significantly decreased within all groups that received medication. For the metformin group, TG, Cholesterol, LDL, AST and ALT had significant differences between pre- and post-assessment but only TG and ALT showed significant difference at each comparison interval. Finally, Vitamin E showed significant differences in TG, AST and ALT and these three parameters had significantly different results when compared pair by pair at each assessment time.

**Table 2. A comparison of clinical factors changes within groups (metformin, vitamin E and melatonin, and control group) adjusted for weight (at baseline). (n=140)**

| Variables | Time | Metformin therapy (Mean ± SD) | Vitamin E therapy (Mean ± SD) | Melatonin therapy (Mean ± SD) | Control group (Mean ± SD) |
|---|---|---|---|---|---|
| **Triglyceride (mg/dl)** | Before | 226.9 ±53.8 $^{ab**}$ | 260.8 ±105.6 $^{ab**}$ | 242.3 ±62.2 $^{ab**}$ | 225.9 ±51.3 $^{b**}$ |
| | 3-months | 182.1 ±63.03 $^{c**}$ | 243.3 ±70.8 $^{c**}$ | 207.03 ±58.4 | - |
| | 6-months | 172.7 ±4.74 | 216.03 ±6.19 | 179.8 ±4.07 | 214.5 ±4.47 |
| | **P-value** | <0.001$^{*}$ | <0.001$^{*}$ | <0.001$^{*}$ | 0.007$^{*}$ |
| **Cholesterol (mg/dl)** | Before | 226.6 ±33.7 $^{a**}$ | 207.2 ±29.7 | 203.8 ±39.3 $^{ab**}$ | 211 ±28 $^{b**}$ |
| | 3-months | 201.2 ±46.2 | 200.6 ±25.3 | 184.4 ±32.1 | - |
| | 6-months | 201.8 ±32.4 | 201.4 ±22.3 | 186.2 ±30.6 | 197.9 ±24.5 |
| | **P-value** | 0.001$^{*}$ | 0.136 | 0.026$^{*}$ | 0.001$^{*}$ |
| **HDL (mg/dl)** | Before | 43.7 ±8.3 $^{a**}$ | 43.7 ±8.05 | 44.7 ±8.5 $^{a**}$ | 41.4 ±9.9 |
| | 3-months | 48.3 ±6.8 | 41.5 ±5.7 | 47.5 ±8.02 | - |
| | 6-months | 47.2 ±13.8 | 41.4 ±8.2 | 46.8 ±9.4 | 40.7 ±6.5 |
| | **P-value** | 0.200 | 0.828 | 0.249 | 0.499 |
| **LDL (mg/d)** | Before | 137.4 ±30.4 $^{a**}$ | 111.3 ±22.4 | 110.6 ± 33.1 | 127.6 ±26.3 |
| | 3-months | 116.4 ±39.8 | 101.7 ±41.9 | 95.4 ±27.7 | - |
| | 6-months | 120.1±32.5 | 114.7±21.3 | 103.4±30.1 | 120.9±13.8 |
| | **P-value** | 0.017$^{*}$ | 0.333 | 0.326 | 0.247 |
| **FBS (mg/dl)** | Before | 93.6 ±14.2 $^{ab**}$ | 92.4 ±8.2 | 98.2±9.2 | 93.7 ±10.5 $^{b**}$ |

| Variables | Time | Metformin therapy (Mean ± SD) | Vitamin E therapy (Mean ± SD) | Melatonin therapy (Mean ± SD) | Control group (Mean ± SD) |
|---|---|---|---|---|---|
| | 3-months | 94.7 ±13.1 $^{c**}$ | 91.8 ±6.3 | 89.4 ±7.2 | - |
| | 6-months | 87.5 ±8.5 | 87.4 ±9.9 | 80.6 ±12.7 | 91.6 ±8.8 |
| | **P-value** | 0.053 | 0.027$^{*}$ | <0.001$^{*}$ | 0.012$^{*}$ |
| **AST (U/L)** | Before | 48.1 ±25.1 $^{ab**}$ | 56.1 ±21.4 $^{ab**}$ | 59.8 ±14.9 $^{ab**}$ | 46.5 ±15.4 $^{b**}$ |
| | 3-months | 37.9 ±20.14 | 48.5 ±14.9 $^{c**}$ | 49.5 ±16.4 | - |
| | 6-months | 34.05 ±14.3 | 41.05 ±13.05 | 49.7 ±16.5 | 41.8 ±10.5 |
| | **P-value** | 0.001$^{*}$ | <0.001$^{*}$ | 0.003$^{*}$ | 0.003$^{*}$ |
| **ALT (U/L)** | Before | 56.8 ±31.6 $^{ab**}$ | 68.09 ±27.4 $^{ab**}$ | 77.4 ±17.9 $^{b**}$ | 63.2 ±20.8 |
| | 3-months | 48.5 ±30.04 $^{c**}$ | 57.9 ±20.5 $^{c**}$ | 65.2 ±15.8 $^{c**}$ | - |
| | 6-months | 40.4 ±20.5 | 45.6 ±14.02 | 48.9 ±15.4 | 59.7 ±15.8 |
| | **P-value** | <0.001$^{*}$ | <0.001$^{*}$ | <0.001$^{*}$ | 0.127 |

Another finding in this study showed that losing weight independently could improve the biochemical tests as demonstrated by the Pearson correlation coefficient (Correlation for AST post: 0.27, P<0.001; for ALT post: 0.34, P=0.003).

The results of our comparison showing the mean changes in serum parameters among the four groups obtained by repeated measurement ANOVA test are indicated in Table 3. Based on these results, mean AST (P<0.001), ALT (P=0.004), cholesterol (P=0.018), and LDL (P=0.001) were significantly different among the four groups after adjusting for weight loss. These results were significant between the metformin and melatonin groups with mean changes of AST (M.D: -13.5, P<0.001) and ALT (M.D: -15.8, P=0.001) in the metformin group being lower in the melatonin group and the opposite was found for LDL (M.D: 21.5, P=0.001) and cholesterol (MD: 18.12, P=0.013). We found no significant difference between the vitamin E and melatonin groups in mean changes of parameters but by comparing the mean changes between metformin and vitamin E we found vitamin E decreased TG more than metformin but metformin decreased LDL more than vitamin E. Finally, there was only a significant mean change difference between the control and melatonin groups in LDL and AST. Results of our comparison of melatonin versus the control group showed that mean changes of AST (M.D: 18.3, P<0.001) and ALT (M.D: 10.1, P=0.163) in the melatonin group were greater than in the control group; however, the ALT change was not significant.

**Table 3. A comparison of mean changes among groups (metformin, vitamin E and melatonin and control) adjusted for weight (at baseline) using RM- ANOVA and Bonferroni test (post hoc-test) (n=140)**

| Clinical factors | P-value $^{a}$ | M.D $^{b}$ (P-value) | M.D $^{c}$ (P-value) | M.D $^{d}$ (P-value) | M.D $^{e}$ (P-value) | M.D $^{f}$ (P-value) | M.D $^{g}$ (P-value) |
|---|---|---|---|---|---|---|---|
| **Cholesterol (mg/dl)** | **0.018$^{*}$** | 18.12 (0.013) | 10.38 (0.346) | 7.74 (0.711) | 5.13 (0.988) | -13.8 (0.24) | -2.5 (0.988) |
| **TG** | 0.07 | -17.2 (0.598) | 21.2 (0.378) | -38.4 (0.018) | -11.3 (0.988) | 1.08 (0.988) | -29.7 (0.184) |
| **LDL (mg/dl)** | **<0.001$^{**}$** | 21.5 **(0.001)$^{**}$** | 6.28 (0.811) | 15.3 (0.024) | 15.9 (0.032) | -5.8 (0.988) | 9.9 (0.394) |
| **HDL** | 0.346 | 0.045 (0.988) | -3.43 (0.096) | 3.48 (0.087) | -3.45 (0.607) | -3.2 (0.767) | -1.65 (0.988) |
| **FBS** | 0.796 | 2.5 (0.281) | 0.543 (0.988) | 1.95 (0.601) | 0.764 (0.988) | -0.309 (0.988) | 1.34 (0.988) |
| **AST (U/L)** | **<0.001$^{**}$** | -13.5 **(<0.001)** | -7.7 (0.067) | -5.8 (0.244) | -18.3 **(<0.001)** | -4.2 (0.988) | -8.6 (0.056) |
| **ALT(U/L)** | **0.004$^{*}$** | -15.8 **(0.001)$^{**}$** | -10.2 (0.069) | 5.6 (0.613) | -10.11 (0.163) | 4.9 (0.988) | -0.051 (0.988) |

**a Comparison of clinical factors among four groups; * Significant at level of 0.05; ** significant at level of 0.01**
**b Mean difference for Metformin versus Melatonin; c Mean difference for vitamin E versus Melatonin;**
**d Mean difference for Metformin versus vitamin E; e Mean difference control group for Melatonin versus;**
**f Mean difference control group for Metformin versus; g Mean difference for control group versus vitamin E.**

### Ultrasound results

Ultrasound results showed an improvement in all samples after six months (P=0.043). However, based on ultrasonography, normal echo, which was not detected at the baseline, was detected after 6 months of treatment, most often in the metformin group (91.4%). This is summarized in Table 4.

**Table 4. Comparing the Ultra Sound results of improvement in liver appearance among groups**

| Baseline | Groups | | | | $\chi^{2*}$ | P-value |
|---|---|---|---|---|---|---|
| | Metformin | Vitamin E | Melatonin | Control | | |
| **Stage 1** | 28 (80%) | 21 (60%) | 23 (65.7%) | 23 (65.7%) | 4.94 | 0.551 |
| **Stage 2** | 5 (14.3%) | 12 (34.3%) | 11 (31.4%) | 11 (31.4%) | | |
| **Stage 3** | 2 (5.7%) | 2 (5.7%) | 1 (2.9%) | 1 (2.9%) | | |
| | | **After 6-months** | | | | |
| **Normal Echo** | **32 (91.4%)** | 27 (77.1%) | 29 (82.9%) | 18 (56.2%) | | |
| **Stage 1** | **3 (8.6%)** | 7 (20%) | 5 (14.3%) | 12 (37.5%) | 12.98 | 0.043** |
| **Stage 2** | - | 1 (2.9%) | 1 (2.9%) | 2 (6.2%) | | |

*** Chi-square test; **significant at level of 0.05**

## Discussion

No therapeutic or surgical treatment for non-alcoholic fatty liver disease (NAFLD) has been officially approved to date which is a serious void in the medical field given the increasing prevalence of NAFLD in industrialized and developing countries. Proper treatment could prevent or minimize the consequences of NAFLD, hence, there is a consistent effort by researchers in the world to identify and introduce the most appropriate drugs for the treatment of this disease.

A systematic review by Li et al., reported that metformin reduced ATL and AST significantly in NAFLD patients but indicated that metformin was not able to induce histological improvement of hepatocytes **(14)**. Haukeland and colleagues investigated metformin in NAFLD patients and showed statically significant reduction of lipid levels, such as total cholesterol and LDL **(15)**. Our study indicated improvements in the pre-and-post treatment for all liver and lipid factors except HDL and FBS during the 6-month test period, but these changes were not statistically significant when compared to the control group. This difference in our results and other studies could be due to the methods used in these studies, which differed in the dosage of the drugs, the duration of the treatment and the ethnicity of the patients.

Treatment with vitamin E had beneficial effects on liver enzymes and triglyceride in our study. Sanyal and colleagues compared the effect of vitamin E and pioglitazone on non-diabetic patients with NAFLD and reported that vitamin E could significantly reduce aminotransferases **(4)**, a conclusion consistent with our study; however, Lavine's study compared vitamin E and metformin with a placebo and concluded that neither vitamin E nor metformin significantly reduced the level of aminotransferases compared with the placebo **(16)**. In our study, vitamin E did not make a significant difference in accessing parameters when compared with our control group.

It is important to consider that vitamin E can increase the total mortality rate **(17)**, risk of prostate cancer and hemorrhagic stroke **(18, 19)**. With these documented complications and the results reported by various studies, it seems that vitamin E should not be considered as the first choice of medical therapy although further investigation is needed to determine its benefits and adverse effect thoroughly.

In 2010, Gonciarz and colleagues reported that three months of treatment with melatonin (10mg/day) in patients with steatosis hepatitis reduced their liver enzymes, cholesterol and gamma glutamyl transpeptidase (GGT) with no observed side effects **(20)**. In another study in which the patients were treated for more than 6 months, Gonciarz observed that melatonin significantly reduced hepatic factors such as aminotransferases and GGT and also decreased lipid factors and glucose levels, and this was statistically significant in comparison with their placebo group **(21)**. These findings were similar to our study which showed that melatonin reduced serum

aminotransferases, triglyceride, cholesterol and fasting glucose when our data was compared before and after medication with melatonin but when we compared the result of melatonin with our control group, only LDL and AST had significant changes. Unexpectedly, LDL was reduced more in our control group than in the melatonin group.

Celinski et al., treated the patients with melatonin (10 mg/day) and tryptophan (1000 mg/day) for 14 months and reported that neither melatonin nor tryptophan was able to significantly reduce the level of aminotransferases but GGT, a marker for NAFLD, was significantly reduced and melatonin also significantly reduced cholesterol, triglycerides and LDL levels **(22)**. Celinski et al., suggested that this difference with the results of Gonciarz's study was due to the fact that the Gonciarz study was 24 weeks in length and their study was 14 months **(22)**. They suggest that melatonin may, over a longer treatment period, be able to improve lipid metabolism in patients with NAFLD and therefore be a good drug for the treatment of this disorder.

We observed that melatonin during 6 months of treatment in NAFLD patients caused significant changes in triglyceride and cholesterol but not in HDL and LDL levels. This drug also made improvements in AST and ALT, two liver enzymes. Our results showed that the control group patients who had weight loss due to changes in their dietary regimen and an increase in their daily activities made a significant positive change in their TG, cholesterol, FBS and AST levels. Previously, several studies and systematic reviews had suggested that weight loss of about 5 percent or more could improve NAFLD without any medical treatment **(23-25)**.

Based on improvements shown by ultrasonography, when we compare the results of our three NAFLD medical- therapy groups (melatonin, metformin and vitamin E) with our control group, who only experienced weight loss, the greatest improvement was demonstrated by metformin (P=0.043). It should be noted that our control group had approximately 5 percent weight loss and this may be the reason why weight loss was not more effective as other studies showed greater improvement by ultrasonography when more than 10 percent of the weight was lost **(23-25)**.

## Conclusion

Melatonin improved hepatic biochemical factors and lipid metabolism but it seems that metformin, with greater improvement in ultrasonography features and aminotransferases and lipid profiles in NAFLD, is a better choice for the treatment of these patients. We suggest that melatonin be considered an effective treatment of NAFLD as this drug made vast improvement in different aspects of NAFLD injuries. Our findings suggest that a large-scale trial with these drugs, a longer period of treatment, more accurate diagnostic testing and the inclusion of histopathologic studies will be most valuable at this time.

## Acknowledgments

None.

## Conflict of Interest

There is no conflict of interest.

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