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Mental and Muscle Relaxation in Water Decreases Liver Enzymes and Inflammatory Markers in Non - Alcoholic Fatty Liver Disease

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) has increased over the past years. NAFLD occurs when liver cells begin to collect fat droplets. Liver enzymes are the best indicator for assessing liver status. Incidence of this disease in women is higher than men, although opposite results have been reported. NAFLD patients have unhealthy lifestyle in both diet and physical activity. In the present study, the effect of mental and muscle relaxation in water was investigated to evaluate changes in liver enzymes and inflammatory markers (IL-6 and C-reactive protein (CRP) of NAFLD patients.

Methods: The present study was a randomized clinical trial with pretest-posttest and follow-up with control group. Liver enzymes and inflammatory markers include IL-6 and CRP were measured before and after mental and muscle relaxation in water. Finally, the data analysis was made with independent samples t-test for baseline variables in two groups, paired t-test and two-way mixed ANOVA (repeated measures) used for measuring indices in pretest and posttest of within group and between groups respectively. Also, the correlation test was used to evaluate the correlation between age and FBS with liver enzymes.

Results: We found significant reductions in measured indices (FBS, AST, ALT, ALP, IL-6, and CRP), within the treatment group in comparison with the control group ($P < 0.05$). Also, there were significant differences in means of AST ($P < 0.001$), ALT ($P = 0.002$), ALP ($P=0.021$), IL-6 ($P < 0.001$), and CRP ($P = 0.019$) between two groups after mental and muscle relaxation in water.

Conclusion: our results demonstrated that mental and muscle relaxation in the water had a significant role in improving and reducing the symptoms of NAFLD patients.

Keywords: Nonalcoholic Fatty Liver Disease, Liver Enzymes, Interleukin 6, C Reactive Protein, hydrotherapy

Introduction

Non-alcoholic fatty liver disease (NAFLD) has increased over the past years. NAFLD occurs when liver cells begin to collect fat droplets (mainly triglycerides). Accumulation of fat in the liver promotes the development of inflammation and progression of fibrosis, which can ultimately lead to patient death. Pathologically, NAFLD is associated with obesity and overweight, type 2 diabetes, hypertension, insulin resistance, and metabolic syndrome [1].

Studies have shown that liver enzymes are the best indicator for assessing liver status [2]. Elevations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) may be due to NAFLD. Serum levels of ALT enzyme are higher than AST in NAFLD patients [3].And among the enzymes in the liver, the ALT enzyme has been associated with inflammation from fat accumulation in the liver more than others [4]. NAFLD is influenced by age, gender, ethnicity, and endocrine dysfunctions (hypothyroidism, hypopituitarism, hypogonadism, and polycystic ovary syndrome) [5, 6]. NAFLD has a direct relationship with obesity. 80% of obese people suffer from NAFLD [7, 8]. The incidence of this disease in women is higher than of men, although opposite results have been reported [9].NAFLD patients have an unhealthy lifestyle in both diet and physical activity [10]. Regarding the pathology of non-alcoholic liver disease, treatment has not yet been completely and specifically developed for this disease [11]. Many previous studies have shown on human specimens that regular exercise and diet play an important role in improving insulin resistance and other important factors involved in the NAFLD pathogens, such as hypertriglyceridemia, hyperglycemia, syndrome metabolism, obesity, and the reduction of AST and ALT enzymes [12]. Studies have also been done on the role of vitamin D in improving the symptoms of fatty liver disease. In their opinion, the Vitamin D supplements might improve NAFLD by inflammation reduction [13]. The accumulation of fat -both in the adipose tissue and in liver steatosis- that leads to increased CRP (C-reactive protein) levels among obese patients. Thus, CRP may be a marker of NAFLD, in obese patients. CRP was reported to increase in metabolic syndrome and type-2 diabetes [14]. C-reactive protein (CRP) is made in the liver. This protein is known as acute phase protein. Its level rises when there is inflammation in the body. Some studies have shown that CRP increases in NAFLD [15]. Cytokines play a pivotal active role in NAFLD and are considered as potential therapeutic targets. Among cytokines, Interleukin 6 promotes the synthesis of acute phase proteins such as CRP. It can be said that IL-6 plays a role in the pathogenesis of NAFLD. Serum levels of interleukin6 are high in people with high fatty liver [16]. IL-6 mediates the synthesis of acute phase proteins such as C-reactive protein [17]. Serum levels of IL-6 are higher in patients with NAFLD [18, 19]. Due to these characteristics, the role of IL-6 in the pathogenesis of fatty liver patients cannot be ignored. Perhaps this role is indirect. Using water as a therapy is one of the oldest treatments in natural medicine, which is called water therapy or hydrotherapy [20]. Studies have shown the effect of hydrotherapy on various systems of the body, such as management of pain, chronic obstructive pulmonary diseases, anorectal disorders, fatigue, anxiety, obesity, hypercholesterolemia, and hyperthermia. In the present study, the effect of mental and muscle relaxation in water was investigated with the aim to evaluate changes in liver enzymes and inflammatory markers (such as IL-6 and CRP) in NAFLD patients.

Methods

Study design

The present study was a randomized clinical trial with pretest-posttest and follow-up with control group. The independent variable of this study was water therapy. The dependent variables of this study were: ALT, AST and ALP liver enzymes, also

inflammatory markers (such as IL-6 and CRP). In this study, clinical trials have been conducted at Dr. Zahedi's lab in Larestan city. This study was conducted in 2018(May to November). Ethical consent was taken from individuals to participate in this research.

Inclusion criteria

Inclusion criteria were NAFLD disease with grade I, obesity and overweight, gender female, age 15 to 65 years, Body mass index (BMI) 25 to 30 or higher and mental and physical health for learning and exercising.

Exclusion criteria

Patients with any of the following conditions were excluded:Smoking and drinking, Opioid drug addiction, Consumption industrial psychosocial drugs, Cardiovascular disease, viral hepatitis B and C disease, Respiratory diseases that could lead to shortness of breath in the water, Sensitivity and skin ulcers, Inability to learn swimming skills (at elementary level), Fear of water, Physical disability and the presence of symptoms of mental disorders.

Method of treatment protocols

In order to determine the level of liver enzymes (ALT, AST and ALP) also IL-6 and CRP, 10 cc of the fasting blood sample were taken from the anterior venous of the patients. There were two groups. Non - NAFLD patients (n= 10) and NAFLD patients (n=10). To determine liver enzymes used enzyme kinetic method and levels of ALT, AST and ALP enzymes were measured. First, mixed substrates and coenzymes were placed in bain marie for 10 to 15 minutes, then by adding serum, enzymes were measured and recorded in 4 minutes using a photometer. Serum levels of IL-6 and CRP were measured with ELISA kit.

Mental and Muscle relaxation test in water

In this study, 4 types of walking in the water were performed in every other day, and each session was performed for 60 minutes, in 8 weeks, with the training intensity being 55-60% of the Vo2max. Four types of hydrotherapy exercises included: moving marsh, walk on the heel of the foot, walk on toe, and butterfly swimming. Immediately after the completion of therapeutic courses, 10cc of fasting blood samples were taken from the patients and the parameters were measured and recorded as post-test. Weight and body mass index were also measured and recorded in the post-test stage.

Data analysis

The data were analyzed using SPSS (version 25). Kolmogorov-Smirnov and Shapiro–Wilk test was used for the evaluation of the normal distribution of data. Non normal distribution normalized by the logarithmic conversion. Comparison of means was made with paired t-test and two-way mixed ANOVA (repeated measures) for FBS, ALT, AST, ALP, CRP and IL-6 before and after mental and muscle relaxation in water for each group (within-group and between-group). Independent samples t-test was used to evaluate the difference between baseline characteristics of study participants in two groups. Also, the correlation test was used to evaluate the correlation between age and FBS with liver enzymes. $P < 0.05$ were considered statistically significant.

Results

Twenty of 40 volunteers completed the study. 20 participants discontinued the study for some reasons. 17 participants (eight in the treatment group and nine in the control group) withdrew during the study period for personal reasons. Three participate (two in treatment group and one in control group) not following up. Thus, the main analyses were conducted with 20 participants (treatment group, n = 10; control group, n = 10) [Figure 1].

Baseline characteristics of the patients

The median age of the patients was 40.9 ±12.3 years. The mean fasting blood glucose (FBS), liver enzymes (ALT and AST), IL-6 and CRP were higher than the standard laboratory scale. The characteristics of the patients showed no significant differences between groups [Table 1].

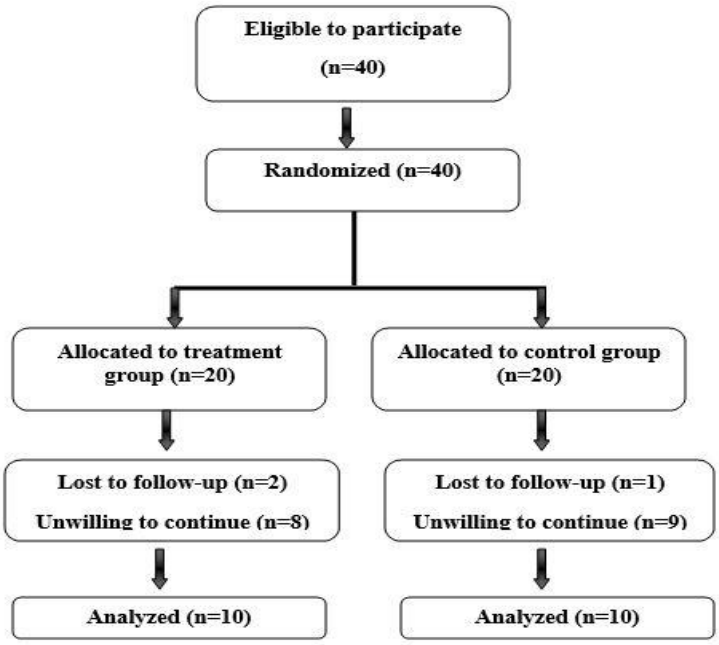

Figure 1. Trial CONSORT flow diagram

Table1. Baseline variables in participants of study groups

Variable	Treatment	Control	P^*
Female (%)	10 (100%)	10 (100%)	0.837
Age (years)	40.90±12.306	40.20±11.114	0.895
BMI (kg/m^2)	29.336±4.806	27.57±5.808	0.47
FBS (mg/dl)	107.3±18.571.11	115.7±23.118.57	0.383
AST (IU/L)	56.80±4.366	69.60±13.69	0.077
ALT (IU/L)	72.90±8.279	79.60±26.26	0.458
ALP(IU/L)	239.20±49.95	260.50±118.47	0.61
IL-6 (pg/ml)	84.40±6.203	89.90±6.367	0.066
CRP (mg/dl)	8.69±0.344	8.15±0.485	0.117

All values are mean ±SD. *: Resulted from independent samples t-test. BMI= Body Mass Index, FBS=Fasting blood sugar, AST= Aspartate transaminase, ALT= Alanine transaminase, ALP=Alkaline phosphatase, IL-6= Interleukin 6, CRP=C-reactive protein. SD=Standard deviation.

Level of liver enzymes increased with increasing age

There was a positive and significant correlation between the AST and ALP liver enzymes with age. So that this relationship was confirmed at a significant level of 95% ($P \leq 0.05$). Also, there was a statistically significant correlation between IL-6 and CRP with subjects' age.

Table 2. Mean and standard deviation of FBS, liver enzymes and Inflammatory factors in study groups

Variable		Group		Mean ± SD	$P^{\bullet}$	F	$P^{\#}$	effect size
FBS		Treatment	Before	107.3 ± 18.571	0.001	2.373	0.141	0.116
			After	99.5 ± 6.67				
		Control	Before	115.7 ± 23.118	0.780			
			After	116.4 ± 23.575				
Liver Enzymes	ALT	Treatment	Before	72.9 ± 8.279	0.007	13.117	0.002	0.422
			After	48.9 ± 6.118				
		Control	Before	79.6 ± 26.268	0.484			
			After	81.4 ± 14.615				
	AST	Treatment	Before	56.8 ± 4.366	0.030	50.401	0.000	0.77
			After	45.6 ± 3.687				
		Control	Before	69.6 ± 13.696	0.344			
			After	63.2 ± 11.66				
	ALP	Treatment	Before	239.2 ± 49.95	0.013	6.374	0.021	0.262
			After	177.8 ± 24.498				
		Control	Before	260.5 ± 118.475	0.783			
			After	269.7 ± 86.71				
Inflammatory factors	IL-6	Treatment	Before	84.4 ± 6.203	0.000	24.225	0.000	0.574
			After	79.8 ± 3.994				
		Control	Before	89.9 ± 6.367	0.206			
			After	93.9 ± 4.383				
	CRP	Treatment	Before	8.15 ± 0.485	0.000	6.644	0.019	0.673
			After	7.24 ± 0.432				
		Control	Before	8.69 ± 0.344	0.048			
			After	8.49 ± 0.519				

All values are mean ±SD. *Resulted from paired sample t test; comparing before and after of each variable in each group. # Resulted from two way mixes ANOVA (Repeated measures); comparing the studied outcomes between two groups. FBS=Fasting blood sugar, AST= Aspartate transaminase, ALT= Alanine transaminase, ALP=Alkaline phosphatase, IL-6= Interleukin6, CRP=C-reactive protein. SD=Standard deviation

FBS can be involved in liver enzyme disorder in NAFLD

Our Results showed that fasting blood glucose (FBS) can predict 38.2% of ALT variance, 38.5% of AST liver enzyme variability and 35.9% of ALP variability in liver enzymes. Furthermore, the results of this study indicated that whenever the FBS increased, the levels of ALT, AST and ALP enzymes increased in blood plasma. This means that the FBS variable can be involved in liver enzyme disorder of NAFLD disease. Analysis results showed there was a significant positive correlation between age and FBS with ALT, AST. There was no significant correlation between FBS and ALP.

Mean and standard deviation of FBS, liver enzymes levels and inflammatory markers before and after mental and muscle relaxation was reported in Table 2. This table presents within-group and between-group comparisons of treatment and control group before and after 8 weeks mental and muscle relaxation in water. We found significant reductions in measured indices (FBS, AST, ALT, ALP, IL-6, and CRP), within the treatment group in comparison with the control group ($P < 0.05$). Also, there were significant differences in means of AST ($P < 0.001$), ALT ($P = 0.002$), ALP ($P=0.021$), IL-6 ($P < 0.001$), and CRP ($P = 0.019$) between two groups before and after mental and muscle relaxation in water.

Discussion

The purpose of this study was to determine the effect of mental and muscle relaxation in water on liver enzymes and inflammatory markers (such as CRP and IL-6) which their value is high in obese inactive women with non-alcoholic fatty liver disease. In this study, the required data were collected using clinical trials. and parameters included: levels of liver enzymes (ALT, AST, and ALP) also CRP and IL-6 were measured and recorded in two stages of pre-test and post-test and control group. Finally, the collected data were analyzed by descriptive statistical tests.

The findings of this study indicated that the mean FBS, ALT, AST, CRP and IL-6 in research group were higher than standard and optimal levels. As has been reported by others studies [21, 22, 23]. Also, the relationship between non-alcoholic fatty liver and fasting blood glucose (FBS), has been confirmed in studies. People with high blood sugar usually have a fatty liver [1]. Our results also confirmed this. Liver enzymes show different aspects of the normal functioning of the liver, for example, the normal levels of the ALT or AST enzymes represent the integrity of the liver cells and the normal levels of the ALP enzyme, indicating the production and secretion of sufficient albumin to make the protein [24]. The results of this study indicated that ALT and AST increased with increasing age. Some studies have shown that age has a significant effect on liver enzymes [25]. Also, in other research, the results of muscle therapy showed that muscle therapy methods has an effective role on reducing stress and headache in women with migraine headaches [26].

Our results of paired sample t-test and two-way mixed ANOVA (repeated measures) showed that there was a significant difference in reduced levels of ALT, AST, ALP, CRP and IL-6 after 8 weeks of treatment, which is confirmed at 95% level. Exercise reduced plasma free fatty acids, apoptosis and advanced fibrosis [10]. Script Nick and et al in a study aimed at examining the effect of endurance and strength training on biochemical parameters of liver function in women with abdominal obesity, showed that conducting a course of exercise could decrease the level of serum ALP in patients with NAFLD [27]. Also, the results of other research in 2004 showed that ALT and AST levels decreased after the end of the six-week exercise program [28].

The effect of exercise training on inflammatory factors has been done. Studies have shown that muscle movement has anti-inflammatory properties [29]. In a research, the effect of aerobic and resisted

exercise training on the reduction of inflammatory factors in NAFLD was investigated. They observed that significantly decreased the amount of inflammatory factors [30].

Recent data showed that regular exercise was effective in reducing liver fat content and improving the diagnostic profile of patients with non-alcoholic fatty liver [31].

Therefore, due to the fact that increased fat stores play an important role in the development of NAFLD and its underlying metabolic diseases, including increased cholesterol and triglycerides may be aerobic exercise activities Such as water therapy can have a significant effect on weight loss, BMI, body fat loss, de-aggregation and decrease in triglyceride accumulation in the liver, decrease in sedimentation and total cholesterol and LDL accumulation in the liver.

According to the studies, it can be said that exercise reduces fat stores, diseases cholesterol and triglycerides, diabetes and metabolic syndrome, thereby reducing the risk of developing NAFLD. On the other hand, to this day, weight loss is the only definitive treatment for non-alcoholic liver, and lifestyle interventions are only part of its management. Therefore, exercise activities can potentially be effective in reducing fatty liver.

Conclusion

mental and muscle relaxation in water (aqua yoga) is one of the gentlest yet effective forms of water sports that takes advantage of buoyancy and water suspension as well as in-depth stretching and relaxation. Increases respiratory capacity without strain, so they can have relaxation and stretch at the same time. The novelty of our work relates to the use of water to help patients achieve muscle and mental relaxation in water, which has changed the parameters of our study.

The results of this research indicates that mental and muscle relaxation in water and sport activity due to increase daily energy intake, improved muscle oxidation, decreased free fatty acid transfusion into the liver, reduced fatty sedimentation in the liver. And as a result, muscle relaxation in water (aqua yoga) plays an important role in improving the NAFLD.

Declarations

Ethical consent to participate

Not Applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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