Geleneksel ve Tamamlayıcı Tıp Dergisi
Journal of Traditional Medical Complementary Therapies
J Tradit Complem Med. 2022;5(2):96-105

ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/jtracom.2021-87383

Effects of Acupuncture on Cholesterol, Antioxidant and Oxidant Parameters in Obese Women: Clinical Trials

Akupunkturun, Obez Kadınlardaki Kolesterol, Antioksidan ve Oksidan Parametreler Üzerine Etkileri: Klinik Deneyler

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ABSTRACT Objective: Obesity has a profound adverse impact on health care systems. Acupuncture has been shown to have beneficial effects on obesity. The aim of this study is to investigate the effects of acupuncture on serum oxidative stress parameters in obese women. **Material and Methods:** During the study, 20 obese patients had acupuncture treatment, and as a control group, 20 obese patients had sham acupuncture treatment. Serum samples were taken from each patient before the first session and at the end of the 10th session and serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, malondialdehyde (MDA), advanced oxidation protein products (AOPP), the serum activity of superoxide dismutase enzyme (SOD) and glutathione peroxidase enzyme (GPx) were measured. **Results:** Changes in the serum levels of TG, HDL, MDA, AOPP and activity of SOD after the treatment compared to before the treatment in both acupuncture and sham acupuncture treated groups were found insignificant ($p>0.05$). On the other hand, the TC and LDL levels were observed to decrease significantly in comparison to the before-treatment values in both the acupuncture and sham acupuncture groups ($p<0.05$). In addition to this, the GPx activity significantly increased only as a result of the acupuncture treatment ($p<0.05$). **Conclusion:** The results of our study indicated that acupuncture treatment may be effective in reducing cholesterol levels and increasing the activity of antioxidant enzymes.

Keywords: Acupuncture; obesity; cholesterol; oxidative stress; antioxidants

ÖZET Amaç: Obezite, bireyin sağlığı üzerinde olumsuz etkiye sahiptir. Akupunktur tedavisinin obezite üzerinde olumlu etkileri olduğu gösterilmiştir. Bu çalışmanın amacı, obez kadınlarda akupunkturun serum oksidatif stres parametreleri üzerindeki etkilerini araştırmaktır. **Gereç ve Yöntemler:** Çalışma süresince 20 obez bireye akupunktur tedavisi, kontrol grubu olarak 20 obez bireye sham akupunktur tedavisi uygulanmıştır. Her hastadan ilk seans öncesi ve 10. seans sonunda serum örnekleri alınarak toplam kolesterol [total cholesterol (TC)], trigliseridler (TG), yüksek yoğunluklu lipoprotein [high-density lipoprotein (HDL)] kolesterol ve düşük yoğunluklu lipoprotein [low-density lipoprotein (LDL)] kolesterol serum seviyeleri alınmıştır. Alınan serum örnekleri üzerinden kolesterol, malondialdehit (MDA), ileri oksidasyon protein ürünleri [advanced oxidation protein products (AOPP)], süperoksit dismutaz enzimi (SOD) ve glutatyon peroksidaz enzimi (GPx) serum aktivitesi ölçülmüştür. **Bulgular:** Hem akupunktur hem de sham akupunktur uygulanan gruplarda tedavi öncesi ile karşılaştırıldığında, tedavi sonrası serum TG, HDL, MDA, AOPP ve SOD aktivitesindeki farkın anlamlılığı bulunamamıştır ($p>0,05$). Buna rağmen hem akupunktur hem de sham akupunktur gruplarında TC ve LDL düzeylerinin tedavi öncesi değerlere göre anlamlı derecede düştüğü gözlenmiştir ($p<0,05$). Ek olarak, GPx aktivitesi sadece akupunktur tedavisi sonucunda anlamlı olarak artmıştır ($p<0,05$). **Sonuç:** Çalışmamızın sonuçları, akupunktur tedavisinin kolesterol düzeylerini düşürmede ve antioksidan enzimlerin aktivitesini artırmada etkili olabileceğini göstermiştir.

Anahtar Kelimeler: Akupunktur; obezite; kolesterol; oksidatif stres; antioksidanlar

Since 1975, the number of those who suffer from obesity worldwide has increased by almost 3 times. In 2016, 1.9 billion adult people were overweight, 650 million of these had the disease obesity, and this constituted 13% of the population.[1]

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Peer review under responsibility of Journal of Traditional Medical Complementary Therapies.

Received: 03 Dec 2021 ***Received in revised form:*** 04 Apr 2022 ***Accepted:*** 05 May 2022 ***Available online:*** 17 May 2022

Oxidative stress is of great importance as a pathogenic mechanism in obesity-related metabolic syndrome. Arteriosclerosis, hyperglycemia, dyslipidemia and hypertension may be seen together with metabolic syndrome and oxidative stress which has been indicated to play an important role in the pathogenesis of many diseases such as cardiovascular diseases, diabetes mellitus or cancer.[2]

Oxidative stress develops when free radical production is increased or when the antioxidant mechanism is not sufficient. Protein, lipid, nucleic acid and enzyme structures and functions are affected as a result of oxidative stress.[3] Obesity is the chronic exposure of body to oxidative stress.[4]

Lipid peroxidation is a non-enzymatic process triggered by the increase of free radicals. Serum malondialdehyde (MDA) is the end product formed as a result of the oxidation of polyunsaturated fatty acids and is a frequently used parameter as an oxidative stress marker.[5] Protein oxidation is a factor associated with a variety of diseases such as Type 2 diabetes, aging, neurodegenerative diseases, cardiovascular diseases and hypertension.[6] Advanced oxidation protein products (AOPP) is a marker indicating protein oxidation, defined as cross-linked proteins containing dityrosine.[7]

Antioxidant non-enzymatic glutathione and antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) protect the cell from oxidative stress by scavenging free radicals.[8]

Metabolic changes lead to obesity by making weight loss difficult. The treatment of obesity includes diet programs, increasing exercise, medication and some surgical interventions. Recently, acupuncture treatment which is considered effective methods for weight loss has been increasing in popularity. Understanding the role of acupuncture therapy in the regulation of metabolism is of great importance.[9]

Acupuncture treatment imposes significant effects over the cholesterol metabolism in the liver by stimulating the modulation of the biological pathway (transcription regulation, stress-related gene expressions, cell cycle, cell adhesion and immunity).[10]

We aim to examine the weight loss ratio of obese women after acupuncture treatment and evaluate the effects of acupuncture on the serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, SOD, GPx, MDA and AOPP in these patients.

MATERIAL AND METHODS

This study was conducted at Gazi University Hospital, Division of Acupuncture and Complementary Medicine. Before and after treatment, blood taken from the participants was analyzed. Our research is in thorough compliance with the Declaration of Helsinki. Ethics approval was received from Gazi University Medicine Ethics Committee with the number of 122/2009. This study was funded by Gazi University Scientific Research Projects Unit with the project number of 01/2009-16.

The sample size was measured using the G-Power 3.1 (Heinrich Heine University, Germany) program. In the study conducted by Cabioğlu et al., the effect size was calculated as 1.29.[11] The values of α=0.05 and 1-β=0.95 were selected. The minimum sample size was calculated as 11 for the control group and 19 for the sham and acupuncture groups.

In this power analyze result, 40 female patients who visited the acupuncture outpatient clinic were selected for this study. Those who were clinically diagnosed with obesity and pre-menopausal adult women over the age of 19 were selected for this study. The body mass index values of these patients were between 30 and 40. None of the patients had hypertension or diabetes mellitus, and they were also not using any medication. Patient consent forms were signed by all patients included in our study. Participants received acupuncture treatment (n=20) or sham acupuncture treatment (n=20) twice a week for a total of 10 sessions.

Acupuncture and sham acupuncture treatments were applied to the Hegu (LI 4), Quchi (LI11), Shenmen (HT7), Zusanli (ST36), Neiting (ST 44), Feng Long (ST40), San Yin Jiao (SP6) points as 2 sessions a week and 10 sessions in total (Figure 1). Each ses-

sion lasted for 20 minutes. Stainless steel needles (0.25x25 mm, Kingli, China) were used for acupuncture therapy, while plasters were applied for sham acupuncture.

Serum samples were taken from each patient before the first session and at the end of the 10th session. The serum samples were placed at -80 °C until measurement of the MDA, AOPP, SOD and GPx levels.

The serum levels of MDA were analyzed as described by the method of Ohkawa et al.[12] Measurement of products such as MDA, diene conjugates, lipid peroxides, and alkanes is an indicator of lipid peroxidation in the membrane. MDA can be measured with thiobarbituric acid (TBA). Peroxide membranes produce reactive substances that react with TBA in color. The color was measured with a spectrophotometer at 532 nm. The result of MDA was expressed as nmol/mL (Figure 2).

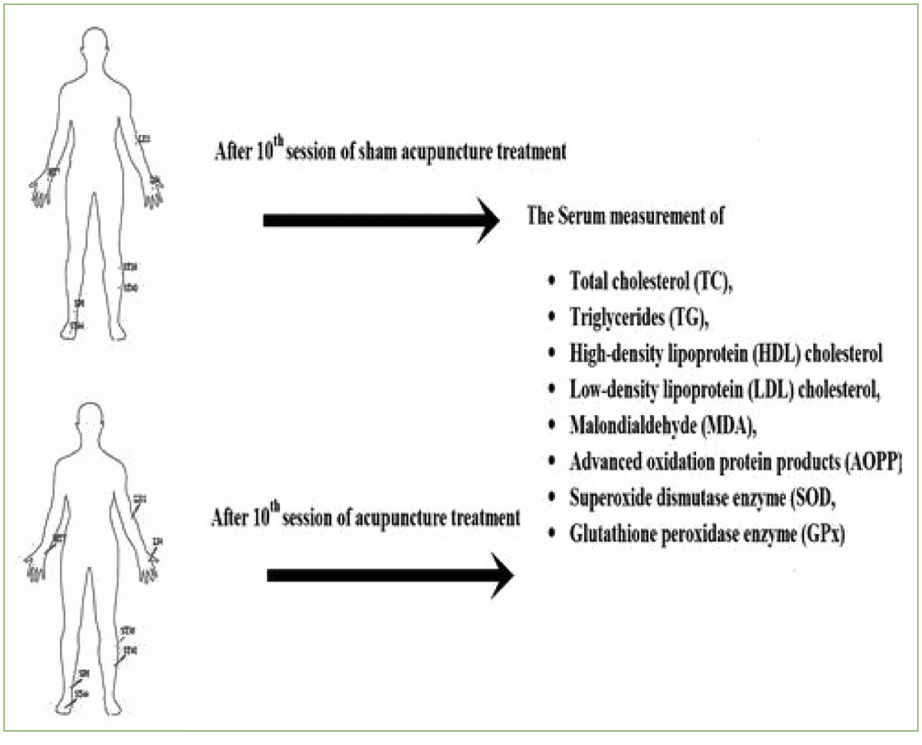

FIGURE 1: The point of sham acupuncture and acupuncture points and measurement of eight parameters before and after treatment.

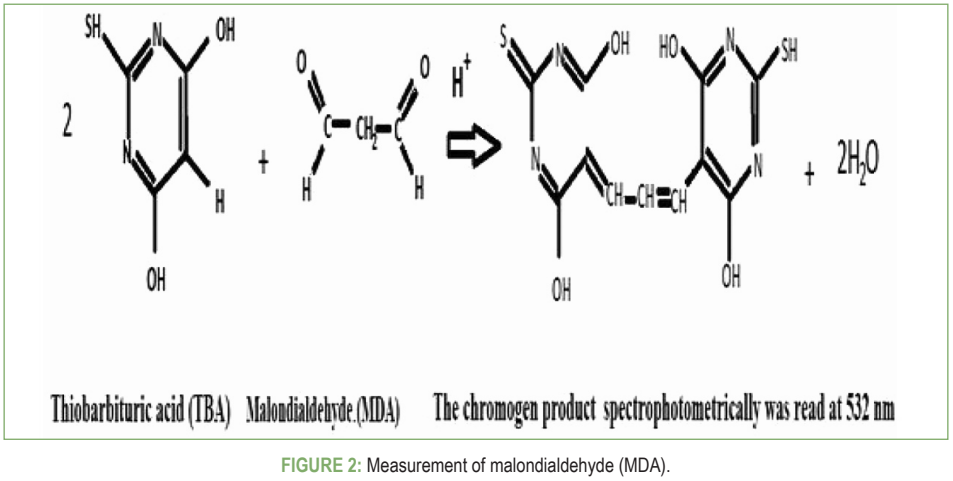

FIGURE 2: Measurement of malondialdehyde (MDA).

Protein damage products include AOPP and protein carbonyl. For measurement of AOPP, the method of Witko-Sarsat et al.[13] In this method, the serum was diluted by using phosphate buffered saline. Chloramine-T solutions (0-100 mmol/mL) were used as a standard. The AOPP levels of serum and chloramine-T solutions were induced with acetic acid and potassium iodide (KI). Iodide was oxidized and turn I- to I_3.[7] The yellow-brown color of I3- was spectrophotometrically measured at 340 nm. AOPP concentrations are expressed as mmol/mL chloramine-T equivalent (Figure 3).

SOD (EC 1.15.1.1) activity was analyzed by the method of Sun et al.[14] Super oxide ($O_2^{\cdot-}$) production was provided by adding xanthine oxidase enzyme into the samples. In the next step, pale yellow nitroblue tetrazolium (NBT^{2+}) was added and insoluble NBT diformazan which is a dark blue compound was formed reaction with $O_2^{\cdot-}$. Spectrophotometric measurement of the $O_2^{\cdot-}$ reduction of the SOD activity in samples and the corresponding reduction of the colored complex was obtained within minutes. SOD activity was measured as 50% inhibition in the NBT reduction rate and was expressed as units per liter serum volume (Figure 4).

GPx activity was determined by the Paglia and Valentine method.[15] According to the method, GPx catalyzes the conversion of hydroperoxide to water by oxidizing reduced glutathione (GSH). GSH reductase converts oxidized glutathione (GSSG) into its reduced form (GSH), nicotinamide adenine dinucleotide phosphate (NADPH) converts into NADP+ simultaneously. The decrease of NADPH absorption was measured at 340 nm. GPx activities were expressed in IU/mg (Figure 5).

The TC, TG, LDL and HDL levels were studied with a Roche Diagnostics brand Cobas E411 (Roche Holding AG, Switzerland) model autoanalyzer. The TC, TG, LDL and HDL levels were expressed as mg/dL.

The amount of TC was determined as follows in accordance with the principle of the device. Cholesterol esters generate cholesterol and fatty acids by catalyzing an enzyme cholesterol ester hydrolase. Subsequently, hydrogen peroxide and cholestenone are formed by the catalysis of cholesterol oxidase enzyme. In the next step, peroxidase catalyzes the re-

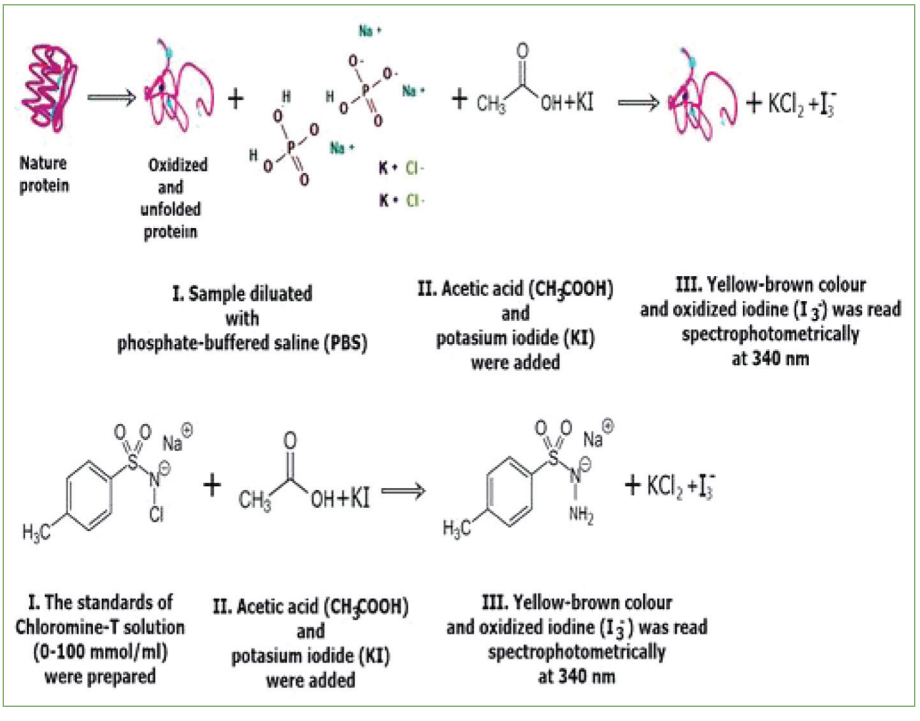

FIGURE 3: Measurement of advanced oxidation protein products (AOPP).

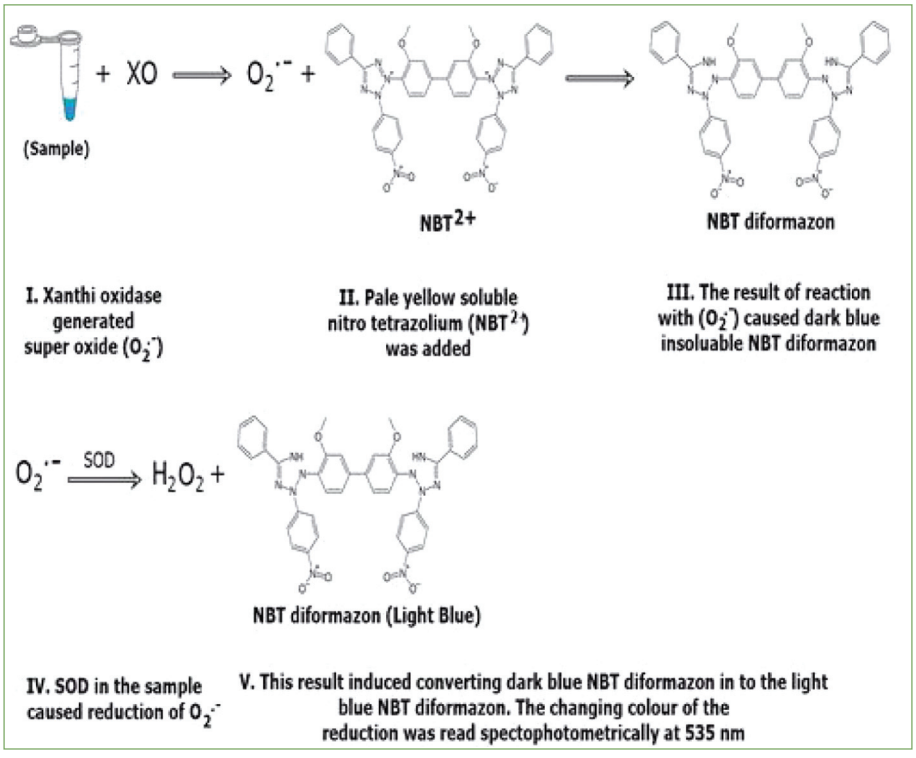

FIGURE 4: Measurement of superoxide dismutase enzyme (SOD).

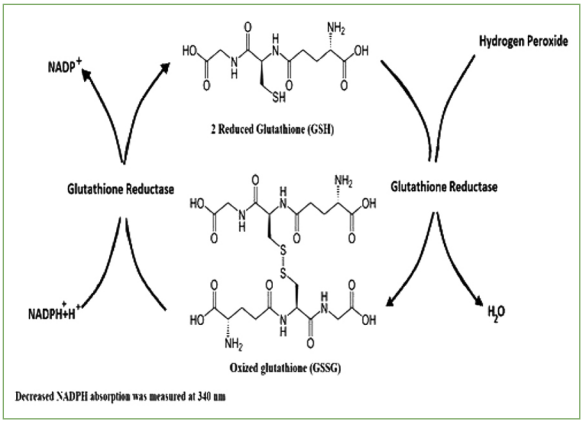

FIGURE 5: Measurement of glutathione peroxidase (GPx).

action of hydrogen both prooxide 4-aminophenazone and phenol and a colored product 4-(p-benzoquinone-monoimino)-phenazone and H_2O are formed.

The amount of cholesterol is measured by quantitating this colored product spectrophotometrically at 500 nm.

The amount of TG was measured as follows in accordance with the principle of the device. TG is broken down into glycerol and fatty acids by the enzyme lipase, and then glycerol is phosphated by the enzyme glycerolkinase to form glycerol 3 phosphate. Glycerol phosphate is oxidized by the glycerol oxidase enzyme to form dihydroxyacetone phosphate and hydrogen peroxide. In the next step, peroxidase catalyzes the reaction of hydrogen with both prooxide 4-aminophenazone and chlorophenol and a colored product 4-(p-benzoquinone-monoimino)-phenazone and H_2O are formed. The amount of TG is measured by quantitating this colored product spectrophotometrically at 500 nm.

The amount of HDL was determined as follows in accordance with the principle of the device. The marked HDL cholesterol esters are converted to HDL cholesterol by breaking the fatty acids with the enzyme polyethylene glycol-coupled (PEG) cholesteryl esterase. Subsequently, this enzyme (PEG cholesteryl esterase) is converted to cholestonone, where it is also formed in hydrogen peroxide as a result of the reaction. The colored qunoneimine is formed by the reaction of hydrogen peroxide, 4-aminophenazone and N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine and the peroxidase enzyme catalyzes reaction. In this way, HDL cholesterol is measured as

mg/dL by measuring this color spectrophotometrically.

LDL-cholesterol is calculated from the measured values of total cholesterol, triglycerides and HDL-cholesterol according to this formula: [LDL-cholesterol]=[TC]-[HDL-cholesterol]-[TG]/5.

For the statistical analyses, Statistical Package for Social Sciences (SPSS) version 25 [International Business Machines (IBM), USA] was preferred. In order to analyze whether patient and control groups showed parametric test conditions, Shapiro-Wilk test was utilized. The data did not display a normal distribution. As a result, the non-parametric Wilcoxon signed-rank test was used to make comparisons between the 2 dependent groups for the pre-treatment and post-treatment values. To compare the post-treatment values of the sham acupuncture and real acupuncture treatments between the 2 independent groups, the non-parametric Mann-Whitney U test was preferred. $p<0.05$ was accepted as statistically significant.

RESULTS

The mean age of the patients was 34.6±6.3 years in the sham acupuncture group and 36.8±7.8 years in the acupuncture group. While the mean body weight in the sham acupuncture group was 84.1 before the treatment, it was also 84.1 after the treatment. In the acupuncture group, the mean body weight of 82.6 reduced to 79.7 (by 4%) after the treatment.

No significant difference could be found in the parameters when the acupuncture and sham acupuncture groups were compared based on the post-treatment results ($p>0.05$).

Changes in serum TG, HDL, MDA, AOPP and SOD levels after the treatment in comparison to before treatment in both the acupuncture and sham acupuncture groups were found statistically insignificant ($p>0.05$) (Table 1, Table 2).

The TC and LDL levels in both the sham acupuncture and acupuncture groups were found to significantly decrease after the treatment in comparison to the pre-treatment values ($p<0.05$) (Table 1, Table 2). However, the GPx levels significantly increased only in the acupuncture group after the treatment (Table 2).

DISCUSSION

Obesity is defined as excessive accumulation of fat in the body that severely degrades health and increases mortality. Serious complications occur as a result of obesity. These include Type 2 diabetes, hypertension, coronary heart disease, paralysis and some types of cancer.[16]

Many studies have stated significant differences in energy balance and metabolism comparisons between men and women. It is emphasized that the necessity of developing different diagnosis and treatment methods in diabetes, metabolic syndrome and obesity complications that develop as a result of

TABLE 1: List of median (interquartile range) levels of parameters and p-values before and after sham acupuncture treatment.

	Sham acupuncture group (n=20)		
Parameters	**Pre**	**After**	**p value**
TC (mg/dL)	166.5 (149-200)	157 (144-196)	0.004
TG (mg/dL)	108 (73-127)	100.5 (69-125)	0.245
LDL (mg/dL)	117.7 (79-148)	99.2 (76-143)	0.02
HDL (mg/dL)	47.5 (41-57)	44.5 (39-54.5)	0.123
GPx (IU/mg)	61.1 (53-79)	84 (57-97)	0.61
AOPP (nmol/mL)	509 (418-811)	482 (399-959)	0.968
MDA (nmol/mL)	13.7 (13.2-15)	14 (13.3-14.6)	0.421
SOD (U/mg)	11.5 (10.6-21.2)	13.5 (9.8-15.5)	0.76

TC: Total cholesterol; TG: Triglycerides; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; GPx: Glutathione peroxidase; AOPP: Advanced oxidation protein products; MDA: Malondialdehyde; SOD: Superoxide dismutase.

TABLE 2: List of median (interquartile range) levels of parameters and p-values before and after sham acupuncture treatment.

	Acupuncture group (n=20)		
Parameters	**Pre**	**After**	**p value**
TC (mg/dL)	169 (167-205)	166.5 (152-196)	0.016
TG (mg/dL)	93.5 (68.5-125)	95.5 (65-109.8)	0.75
LDL (mg/dL)	103.2 (99-121)	97.5 (91-113)	0.03
HDL (mg/dL)	54 (43.5-59)	52.5 (37.8-58.3)	0.339
GPx (IU/mg)	44.6 (10-58)	57.3 (46-66)	0.009
AOPP (nmol/mL)	451.4 (304-585)	470.5 (387-637)	0.126
MDA (nmol/mL)	13.2 (12.7-14.6)	13 (12.4-14.6)	0.943
SOD (U/mg)	14.3 (11.3-18.4)	13.8 (12-20.4)	0.983

TC: Total cholesterol; TG: Triglycerides; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; GPx: Glutathione peroxidase; AOPP: Advanced oxidation protein products; MDA: Malondialdehyde; SOD: Superoxide dismutase.

these changes.[17] Cabioğlu et al. analyzed the reduction of weight loss and improvement of lipoprotein profile with acupuncture treatment in women.[13] In addition, studies have shown that acupuncture treatment has curative effects after 6-10 session in the treatment of diseases such as obesity, insulin resistance and diabetes.[18] Due to this reason, we designed to have 10 sessions of acupuncture treatment over the curative effect on the antioxidant and lipid profile of women.

Acupuncture treatments are effective in treatment of obesity by decreasing insulin resistance and increasing insulin sensitivity.[19,20] He et al. indicated that acupuncture treatment alone is beneficial in reducing overweight.[21] In a previous study of ours, we observed a mean decrease of 2.9 kg (4%) in body weight after acupuncture treatment. In addition, our result indicated that this was effective in decreasing the insulin and leptin hormones.[22]

Chen and et al. indicated that acupuncture significantly decreases the body weight and improves serum lipid parameters.[23] A similar study was conducted by Kim et al. and demonstrated that manual acupuncture plus electroacupuncture treatment led to weight loss in obese participants and the HDL cholesterol levels significantly increased.[24]

Our new findings in this study were in agreement with previous studies in terms of the reduction effect of acupuncture treatment on TC and LDL cholesterol levels.

Oxidative stress creates a complementary damage mechanism in several types of diseases. An imbalance occurs with this change in terms of reactive oxygen species production, the fast detoxification ability of biological systems from reactive byproducts or the easy repair of the damage that has occurred. As a result of oxidative stress, DNA, lipid, and protein damage occurs by the methylene group removing hydrogen atoms from the fatty acid chain.[25]

Lipid peroxidation is an autocatalytic chemical reaction triggered by free radicals and causes oxidation of unsaturated lipids in the membrane structure. Tissue damage caused by lipid peroxidation may be detected by measuring the end product of lipid peroxidation. The most toxic one among these products is MDA.[26] Amino acid side chains of proteins are subjected to oxidation under oxidative stress. The protein products of advanced oxidation that is an indicator of protein oxidation are defined as cross-linked protein products. AOPP is a proinflammatory indicator showing the inflammation step of protein oxidation.[27] SOD, GPx and CAT are important antioxidant defenses that remove free radicals. Oxidative stress occurs when super radicals cannot be compensated by antioxidants. Under the cell oxidative stress, it severely damages lipids, proteins and nucleic acids.[28]

Oxidative stress that increases due to fat accumulation is one of the significant pathological mechanisms that are found in obesity. NADPH oxidases

increase the production of reactive oxygen species in the adipose tissue of obese individuals by expression of oxidant enzymes and reduce the expression of antioxidant enzymes such as SOD, CAT and GPx.[29]

There are studies showing that acupuncture used in obesity treatment may affect oxidative stress. Increased free radicals in obesity cause destruction of the structure and function of the cells. Manna et al. indicated that after acupuncture treatment of hyperglycemic rats, lipid peroxidation levels decreased and SOD activity increased.[30] Thus, increasing antioxidants and reducing oxidative stress in obese patients may be a useful way in the treatment process.[31]

The endogenous antioxidant mechanism is insufficient in obesity which may cause many complications related to obesity. Current drug treatments for obesity can be largely inadequate and there is a need to identify more reliable and more effective treatment methods.[32]

Wang et al. conducted a study with obese rats and reported that acupuncture treatment increased SOD and GPx enzyme activity and reduced MDA levels.[33] Similar results were revealed by Öztaş et al. and it was stated that thiol disulfide balance was developed in obese patients.[34]

In our study, neither sham acupuncture nor acupuncture treatment revealed a significant change in the MDA, AOPP and SOD levels ($p>0.05$). However, we determined that the acupuncture treatment significantly increased the GPx activity. The findings in our study showed that acupuncture treatment may be effective in increasing antioxidant enzymes in humans.

Mazidi and et al. applied a low-calorie diet and 12 weeks of electroacupuncture treatment on 196 obese individuals and observed a significant reduction in the prooxidant-antioxidant balance in both the sham and acupuncture groups after treatment.[35]

In our study, we could not standardize the patients in terms of their calorie restrictions. We believe that these parameters should be reassessed by increasing the number of patients, increasing treatment time and applying calorie restrictions by diet.

Furthermore, we showed for the first time in our study that acupuncture treatment increased GPx activity, and we revealed its effectiveness in reducing the TC and LDL cholesterol levels. In our further studies, we are planning to reorganize our study with different parameters.

CONCLUSION

We evaluated the effects of acupuncture on oxidative stress in obese women. The MDA, AOPP and SOD levels were not found to be significantly different between the acupuncture and sham acupuncture treatment groups. The GPx levels were increased in the acupuncture treatment group after the treatment in comparison to the pre-treatment values.

Our findings showed that acupuncture treatment may be effective in terms of reducing cholesterol and increasing the activities of antioxidant enzymes. In order to be able to more clearly explain these effects, we believe that studies need to be organized on both experimental animals and humans by increasing the numbers of patients, applying diets and considering different treatment durations.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Şebnem Setenay Mit, Cemal Çevik;* ***Design:*** *Şebnem Setenay Mit, Funda Güçel;* ***Control/Supervision:*** *Cemal Çevik, Şebnem Setenay Mit;* ***Data Collection and/or Processing:*** *Şebnem Setenay Mit, Ersel Geçioğlu, Ayşe Banu Çaycı Sivri;* ***Analysis and/or Interpretation:*** *Çınar Severcan;* ***Literature Review:*** *Çınar Severcan, Şebnem Setenay Mit;* ***Writing the Article:*** *Çınar Severcan, Şebnem Setenay Mit;* ***Critical Review:*** *Çınar Severcan, Şebnem Setenay Mit;* ***References and Fundings:*** *Cemal Çevik, Şebnem Setenay Mit;* ***Materials:*** *Şebnem Setenay Mit, Cemal Çevik, Ersel Geçioğlu.*

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