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Effects of Acupuncture on the Glutathione System in Overweight and Obese Individuals

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ABSTRACT

Introduction: Obesity is the fifth leading cause of death worldwide. Several approaches are used for the treatment of obesity, and acupuncture has attracted attention globally in this regard. However, the therapeutic effects of acupuncture at a molecular level remain unclear. Obesity is reported to cause oxidative stress through various mechanisms. The glutathione system is one of the main antioxidant defense mechanism. The present study aimed to evaluate the effects of acupuncture on the glutathione system as the most abundant intracellular antioxidant mechanism in overweight and obese individuals.

Methods: This study was conducted on 40 obese and overweight individuals with the BMI of ≥24.9 kg/m^2 who were selected randomly. The participants received authentic acupuncture (case) or sham acupuncture (control) for six weeks combined with a low-calorie diet. Before and after the intervention, the activity of glutathione peroxidase, glutathione reductase, and reduced/oxidized glutathione levels were measured.

Results: Higher glutathione peroxidase activity was observed in both groups after the treatment, while the increase was more significant in the case group compared to the control group (P=0.005). In addition, reduced glutathione levels were observed to increase in both groups after the treatment. Similarly, the rate of increase in the case group was more significant than the control group (P=0.02). A significant increase was also denoted in the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio in the case group compared to the control group (P=0.02).

Conclusion: According to the results, the combination of acupuncture with a standard obesity diet could increase antioxidant activity in the overweight individuals. Furthermore, the approach could further prevent oxidative damage through increasing reduced glutathione and improving the GSH/GSSG ratio.

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Introduction

Obesity is a major health issue in the modern era, which is associated with numerous diseases, including cancer, asthma, renal dysfunction, infertility, hepatic dysfunction, sleep disorders, diabetes, and cardiovascular diseases (1, 2). Body mass index (BMI) is an internationally accepted criterion for assessing the severity of obesity. According to the World Health Organization (WHO), BMI of 25-29.9 kg/m^2 is defined as overweight, and BMI of ≥30 kg/m^2 is defined as obese (11, 15). In 2016, more than 1.9 billion adults were overweight worldwide, 650 million of which were obese. In Iran, the prevalence of obesity in the population aged more than 18 years has been reported to be 21.7% (3). In addition, models predict the prevalence of obesity to reach 33% by 2030 (4). Several therapeutic approaches are applied clinically for obesity management, including lifestyle modification/diets, pharmacotherapy, surgery, and complementary medicine. Recently, complementary therapies have been increasingly used as a promising approach to obesity

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management, and acupuncture has attracted special attention in this regard (5, 6).

Oxidative stress refers to the imbalance between oxidants and antioxidants. Physiologically, several factors are involved in maintaining the balance between produced reactive oxygen species (ROS) and antioxidant defense components. Oxidative stress occurs in obesity and contributes to outcomes such as metabolic syndrome (7). The biochemical mechanisms that cause oxidative stress in obesity include free fatty acid accumulation in plasma (8, 9), oxidative phosphorylation, hyperglycemia, and high-fat diets (7, 10-13). In addition, the increased consumption of cellular oxygen due to mitochondrial dysfunction in obesity may be associated with increased ROS production (14).

According to the literature, acupuncture combined with dietary regimen could decrease the serum pro-oxidant/antioxidant balance (PAB) values in overweight and obese individuals (15). Glutathione is the most abundant antioxidant within cells (16), and the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio is considered to be the main determinant of oxidative stress (17).

The present study aimed to investigate the effects of acupuncture on the glutathione system in obesity, as well as the concentration of glutathione-dependent enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), and blood glutathione (reduced: GSH, oxidized: GSSG) as well as GSH/GSSG ratio.

Materials and Methods

Study Design

This study was performed on the remaining disposable serum samples of a recorded randomized clinical trial (RCT code: IRCT201706107265N9), which was conducted on participants with the BMI of ≥24.9 kg/m^2 (unpublished under review data). Briefly, the participants of the mentioned trial were selected from the obese and overweight volunteers referring to an acupuncture clinic affiliated to Mashhad University of Medical Sciences.

The present study was conducted on a case group, which received acupuncture with a standard diet treatment as prescribed by a nutritionist (n=20), and a control group, which received sham acupuncture with the same standard regimen (n=20). In the original RCT, all the patients received this treatment and had restricted physical activity, the data of which was recorded and monitored weekly during the study period.

The case and control groups in our study were age-, gender- and BMI-matched. The patients, statistical analyst, and treatment team (except the acupuncturist) were blinded to the research procedure. Serum samples were collected before and six weeks after the intervention. The sera were kept frozen until the measurement assays.

Ethical Considerations

This study was performed on the remaining disposable serum samples of a recorded randomized clinical trial (RCT code: IRCT201706107265N9). The study aimed to assess completely distinct factors associated with oxidative stress. The study protocol on the remaining sera was reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences (ethics code: IR.MUMS.fm.REC.1397.133). In addition, written informed consent was obtained from the subjects prior to enrollment. All the procedures were in accordance with the human research ethics of the Declaration of Helsinki.

Electroacupuncture and Sham Electroacupuncture

The electroacupuncture process has been explained in detail in the original RCT. Accordingly, all the subjects participated in 18 sessions of acupuncture therapy based on standard procedures for six weeks. In the intervention, six bilaterally acupuncture points were defined on the abdomen, including ST21, ST25, SP15, CV4, CV6, and CV12 as well as three bilateral points on the lower extremities including ST44, ST37, and LR3. In the case group, the abdominal acupoints were at the depth of 15-30 millimeters, and the needles in the lower extremities were punctured bilaterally until the participant stated the sensation of de-qi (soreness, numbness, distention or heaviness). Following that, the inserted abdominal needles were connected to three electrode pairs. The electroacupuncture process was performed with sterile, disposable needles (0.25x40 mm; Suzhou, China) using the Great Wall multi-purpose health electroacupuncture device (KWD 808, China) at the frequency of 2 Hz and intensity of 1-5 mA for 30 minutes.

To implement the sham acupuncture approach in the control group, sterile, disposable needles (0.25x25 mm; Suzhou, China) were used. The

needles were inserted through the skin to the defined depths considering the de-qi sensation. In this protocol, no electrical current was applied. The acupuncture process was performed by an experienced acupuncturist in accordance with the revised STRICTA guidelines (18).

Measurement of GSH/GSSG Ratio, GPX, and GR

In this study, the kits used for determining the antioxidant markers were purchased from the ZellBio GmbH Company (Germany).

In reaction with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), GSH generated 2-nitro 5-thiobenzoic acid, which was yellow. The absorbance of the yellow product was measured at 412 nanometers, and the GSH concentration was calculated based on the kit manual. The difference between total glutathione and GSH indicated the amount of GSSG.

GR activity was assessed using NADPH as the substrate. The decreased absorbance at 340 nanometers indicated NADPH oxidation, which was proportional to the reduction of GSSG to GSH. GR activity was calculated in units per liter (U/L). GPX activity was determined on the basis of a colorimetric assay at 412 nanometers. GPX has a selenocysteine at its active site, which directly contributed to the reduction of the peroxide substrate. The enzyme also regenerated the reduced form of selenocysteine by adding excessive GSH as the ultimate reductant. Afterwards, GSH was converted into GSSG by the GPX enzyme, and the remaining GSH reacted with DTNB to produce a yellow product, which was measured calorimetrically at 412 nanometers. The production of the yellow color indirectly indicated the activity of GPX.

$$2\ GSH + H_2O_2 \longrightarrow GS\text{–}SG + 2\ H_2O$$

Statistical Analysis

Data analysis was performed in SPSS version 22 (Chicago, IBM, USA). Quantitative data were assessed in terms of normal distribution using the Kolmogorov-Smirnov test. Wilcoxon test was applied to compare the quantitative variables in each group before and after the intervention. Moreover, the Mann-Whitney test was used to compare the variables between the case and control groups. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Table 1. Demographic and clinical characteristics of participants.

Group	N	Gender		Age (year)	BMI (kg/m^2)
		Male	Female	(mean ± standard error)	(mean ± standard error)
Case	20	5	15	38.75 ± 9.7	32.6 ± 3.6
Control	20	1	19	38.33 ± 11.6	32.2 ± 4.2
p-value			0.06	0.9	0.7

BMI= body mass index

Table 2. Oxidative stress markers before and after 6 weeks of intervention in two groups as case (treatment) and control (sham group).

	Case (N=20) Median (IQR)	Control (N=20) Median (IQR)	Mann Whitney test
GR (U/ml)			
Before	56.2 (24-96.1)	88.4 (72.3-140.6)	$p = 0.02$
After	112.5 (50.2-136.6)	96.4 (72.3-120.5)	$p = 0.5$
Wilcoxon test	$p = 0.3$	$p = 0.7$	
GPX (U/ml)			
Before	160 (115-267)	-8.9 ((-160)-240)	$p = 0.6$
After	329 (189-509)	485 (262-770)	$p = 0.1$
Wilcoxon test	$p = 0.001$	$p = 0.13$	
GSH (nmol/L)			
Before	0.14 (0.13-0.16)	0.15 (0.14-0.17)	$p = 0.3$
After	0.18 (0.17-0.19)	0.17 (0.16-0.18)	$p = 0.1$
Wilcoxon test	$p = 0.001$	$p < 0.001$	
GSSG (nmol/L)			
Before	0.58 (0.55-0.65)	0.58 (0.56-0.62)	$p = 0.9$
After	0.59 (0.57-0.63)	0.59 (0.57-0.65)	$p = 0.7$
Wilcoxon test	$p = 0.62$	$p = 0.2$	
GSH/GSSG (ratio)			
Before	0.24 (0.2-0.27)	0.26 (0.24-0.28)	$p = 0.2$
After	0.29 (0.27-0.31)	0.28 (0.26-0.3)	$p = 0.4$
Wilcoxon test	$p = 0.001$	$p = 0.004$	

Results

In total, 40 overweight and obese subjects were enrolled in the study. The characteristics of the participants are presented in Table 1. According to the information in Table 2, GR activity and GSSG concentration had no significant differences before and after the intervention in the case group. However, GPX activity and GSH concentration increased significantly after the intervention. In the control group (sham acupuncture), a significant increase was observed in GSH concentration (P<0.001).

According to the obtained results, the GSH/GSSG ratio had a significant difference during the course of the treatment (P=0.02) and increased in both groups after the treatment, which was considered more significant in the case group compared to the control group (P=0.001). After six weeks of treatment, the difference in GPX before and after the intervention (Δ GPX) was more significant in the control group compared to the case group (P=0.005). Furthermore, GSH concentration increased after the treatment in both groups, which was more significant in the case group compared to the control group (P=0.001). No significant differences were observed in the other variables before and after the treatment between the case and control groups.

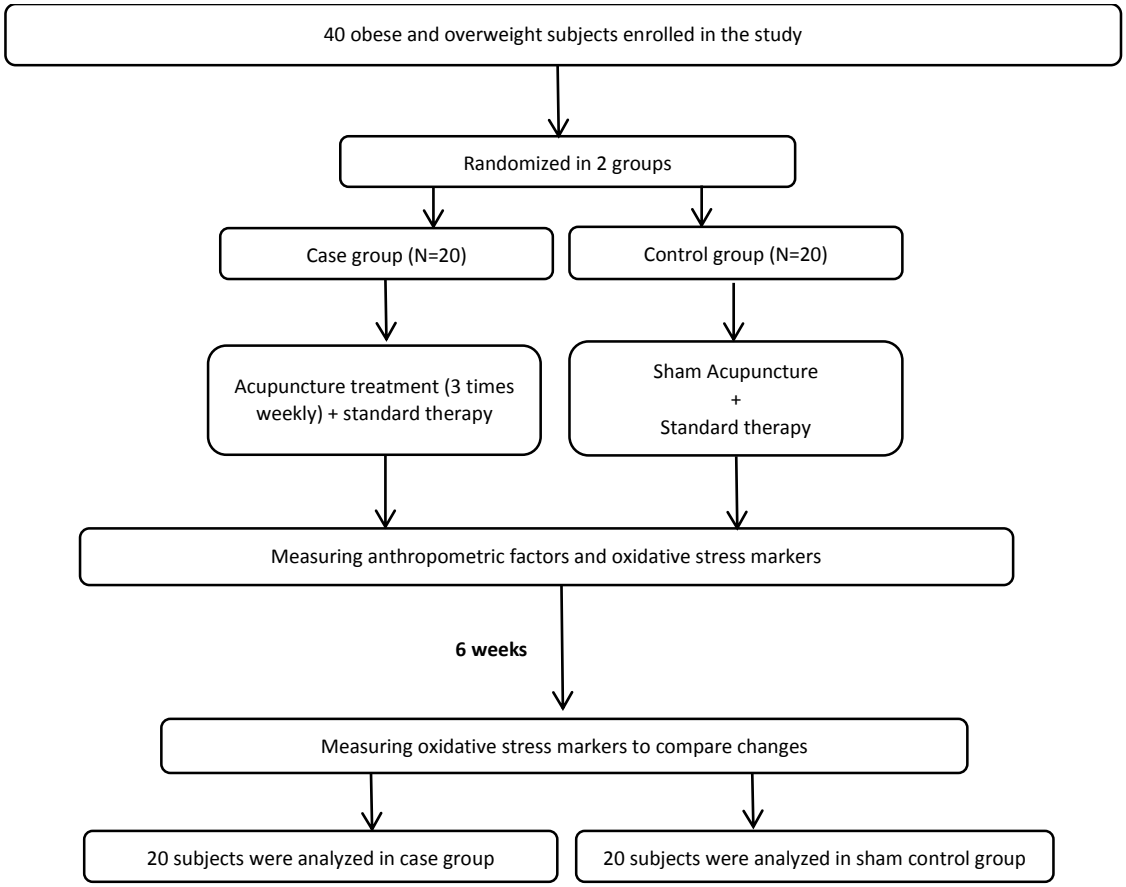

Figure 1. Flow diagram of the study selection procedure.

Discussion

Data are scarce regarding the mechanism of action of acupuncture against oxidative stress, especially in humans. Animal studies have proposed contradictory results in this regard, and the subject remains controversial. The present study aimed to investigate the possible changes in oxidative stress induced by acupuncture. To the best of our knowledge, this

was the first English report on the human glutathione system.

According to our findings, acupuncture increased GPX enzyme activity and GSH concentration in the obese and overweight subjects, which is consistent with the previous studies, indicating that acupuncture could reduce the PAB values in overweight and obese individuals (19). However, previous studies have evaluated the balance in general, while we extended the study to explore the glutathione system as a major component of this balance.

Several animal studies have demonstrated the increased activity of other antioxidant enzymes, such as GPX, catalase (CAT), and super oxide dismutase (SOD) through the acupuncture procedure (20-24). To justify, it could be stated that acupuncture signals are transferred to the central nervous system (CNS) and alter the antioxidant defense system. The effects of psychological conditions or CNS signals on oxidative stress have been previously elucidated. For instance, Jinata et al. evaluated the role of acupuncture stimulation in the regulation of oxidative stress in cerebral ischemic rats, concluding that acupuncture could significantly decrease malondialdehyde (MDA), while elevating CAT, GPX, and SOD activity in the animal model (20).

In another research, Zhang et al. examined rats with multi-infarct dementia, reporting that acupuncture could regulate the GSH/GSSG ratio, increase the activity of SOD, and decrease serum MDA, superoxide anion, and subsequently oxidative stress in the mitochondria. The authors of mentioned study attributed these effects to increased glucose and oxygen sources following the improvement of the cerebral blood flow (24). Similarly, Liu et al. observed that acupuncture could enhance antioxidant effects by increasing the activity of SOD and GPX in the hippocampus of rats with multi-infarction, thereby maintaining the oxidant-antioxidant balance properly (21).

Other studies have proposed inconsistent results in this regard. The findings of Wang et al. indicated that acupuncture exerted antioxidant effects on a mouse model of Parkinson's disease by increasing glutathione concentration and SOD activity, while GPX activity was observed to decrease, which is inconsistent with the results of the present study (25). The discrepancy could be due to the difference in the study conditions (animal/human) and various interfering factors. As such, summative data are required to reach a definite conclusion. Due to increasing application of acupuncture, it seems that more human studies must be performed in this regard in order to bridge the knowledge gap.

Some of the limitations of the present study were the small sample size and remaining confounders (e.g., socioeconomic data). This is one of the few reports to explore the effects of acupuncture on oxidative stress markers in humans. It is recommended that studies on other acupuncture sites, in addition of vast list of other molecules that are involved in the oxidative stress network, be performed to obtain more accurate results regarding the association of acupuncture and oxidative stress.

Conclusion

According to the results, GPX activity significantly increased in the case group, which suggested that acupuncture may enhance the antioxidant defense system. Furthermore, acupuncture could regulate the GSH/GSSG ratio by increasing GSH concentration. Therefore, it seems that acupuncture is a viable option for the complementary treatment of obesity and overweight, while it could also decrease oxidative damage in these individuals.

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