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Combined Radiofrequency and Ultrasonic Cavitation Therapy Does not Have Adverse Effects on Hematological and Liver Markers in Overweight Women

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ABSTRACT

Background: Although dietary management is the principle intervention for treating obesity, noninvasive body-contouring methods are also gaining currency.
We aimed to explore the effects of combined radiofrequency (RF) and ultrasound (US) body contouring on anthropometric indices, hematological markers, and anti-HSP27 levels in overweight females.
Methods: In this randomized control clinical trial, fifty overweight females were enrolled and divided into two groups. Each participant was prescribed a diet with a daily calorie deficit of 500 kcal. RF and US were each used once a week for 5 weeks in the intervention group. Anthropometric and hematological markers were measured in all subjects before and after the intervention.
Results: Abdominal circumference (AC), waist circumference (WC), body mass index, and body fat mass were reduced significantly in both groups ($p < 0.001$). However, the mean reduction in AC ($p < 0.05$) and WC ($p < 0.001$) were significantly greater in the intervention group compared with the control group. Moreover, levels of WBC, HCT, Hb, MCV, and MCH were significantly reduced after the RF-US intervention, although all the changes were in the normal range, suggesting that this therapy did not have adverse effects on hematological parameters. Also, the level of anti-HSP27 did not show any significant change.
Conclusion: Our study suggests that body-contouring devices based on RF and US cavitation reduce measures of adiposity and do not have adverse effects on hematological factors, liver function markers, and HSP27 level in overweight women. Further investigations are required to explore the value of this method in a larger multicenter setting.

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Introduction

Obesity is a major concern associated with several health problems such as cardiovascular diseases, dyslipoproteinemia, cancer, and insulin resistance [1]. Recent studies indicated that more than 1.1 billion adults worldwide are overweight, with 312 million being obese [1]. In 2005, 54% of men and 70% of women were overweight [2]. A study conducted in 2005 revealed that 56.9% of women and 42.9% of men in Iran were overweight [3]. According to the WHO definition, adults with BMI between 25 kg/m2 and 30 kg/m2 are overweight, and those with BMI more than 30 kg/m2 are obese [1]. This condition is associated with increased mortality and morbidity, including an increased risk of heart disease, type 2 diabetes, and several types of cancer including thyroid, breast, colon, endometrial, prostate, liver, kidney, and gall bladder cancer. Besides, obesity can lead to adverse effects on metabolism and blood

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pressure, cholesterol, and triglycerides [4].

Various methods are available for the treatment of obesity, including diet therapy, exercise, behavior therapy, pharmacotherapy, and surgical methods. The current surgical options require hospital admission and are associated with potential postoperative complications, including numbness, pain, swelling, and long-term recovery as well as the inherent risks of surgery. Therefore, nutritionists are constantly looking for safe alternative methods to eliminate excess body fat without surgery. Recently, some noninvasive devices for body contouring have been invented based on radiofrequency (RF) and ultrasound (US) cavitation technologies. RF technology, which is the standard therapy in various fields of medicine including the elimination of acne scars and patches of raised skin, tightening the skin, reducing wrinkles and improving skin laxity, has been recommended as a novel application for reducing body fat [5]. The technique can be used in monopolar, bipolar, tripolar, or multipolar modalities [6]. It increases collagen production by stimulating dermal fibroblasts which, in turn, can improve the quality of fibrous septa of the adipose tissue—and also increases blood flow of the tissues and eventually stimulates lipolysis [7].

Another technology for body contouring is UC, which is based on the application of high-frequency ultrasound waves. Ultrasound waves can easily pass through the fat tissue and destroy fat cells by inducing negative pressure and micro air bubbles around these cells. Besides, owing to their thermal effect, they increase tissue metabolism, blood flow, and lymphatic drainage, as well as stimulating the fibroblasts to produce new collagen. This process leads to the strengthening of the fibrous septa of the adipose tissue and aids cellulite treatment [8].

Given the high prevalence of overweight and obesity and their related complications, as well as the failure of other body-contouring methods in obese individuals, application of RF and US cavitation technologies are proposed as two novel methods. However, the molecular mechanism underlying the effect of RF and US cavitation technologies on other aspects of the human body, including inflammatory response, still remains to be elucidated. Heat-shock proteins (HSPs) protect cells under stress conditions. Several studies have reported the association of HSPs with the pathophysiology of cardiovascular disease (CVD). HSP27, a member of the small HSPs family, is constitutively expressed in many cell types, including cardiomyocytes and endothelial cells, and possesses cardioprotective properties. Previous data showed that circulating levels of HSP27 and antibody titers against this protein (anti-HSP27) are higher in patients with established coronary artery disease and acute coronary syndrome [7]. However, further studies are warranted to elucidate the effect of noninvasive body-contouring methods on hematological markers. Since these devices enhance fat mobilization through the lymphatic system and liver by, we aimed to explore the effects of RF and UC methods on serum levels of the inflammatory marker anti-HSP27 antibody, blood parameters, and markers of liver function in overweight women.

Subjects and methods

Population

This was a randomized clinical trial conducted at Ghaem Hospital of Mashhad, Iran, from January 2014 to June 2014. According to the criteria, 50 women were enrolled in the current randomized clinical study (Figure 2). The inclusion criteria were being between 18 and 65 years old and having a BMI between 25 and 29.9 kg/m2. The exclusion criteria were having a history of cardiovascular diseases, diabetes, or other chronic diseases; taking medications including topical steroids, vitamin A, or isotretinoin; being pregnant; breastfeeding; having a skin infection, photosensitivity, anemia, or malignancy; or having a pacemaker. We used a computerized randomization list for randomizing the subjects into the intervention and control groups. All the participants gave written informed consent.

Intervention

A nutritionist evaluated the dietary intake of the participants and prescribed a diet providing a caloric deficit of 500 kcal/d for five weeks. The participants were visited in the first, third, and fifth weeks for the evaluation of diet adherence.

The intervention group received body-contouring treatment in addition to the diet. Each participant underwent two 40-min treatment sessions a week, on two different days, by the same specialist. Each therapy modality was applied once a week for each participant. Ultrasound cavitation was applied with the Megason cavitation device (EunSung Global Co. Ltd., Seoul, Korea). The vibration frequency was 32-36 kHz and the penetration was 6-8 cm. The RF therapy was applied using a Magicpot bipolar RF device (EunSung Global Co Ltd., Seoul, Korea). The frequency used for all the participants was 0.8 MHz. The abdominal area was the target zone in all subjects. Any adverse effects induced by the treatments, such as erythema, pain, or blistering, were recorded.

The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences and registered with the Iranian Registry of Clinical Trials (IRCT 2014042817475N1).

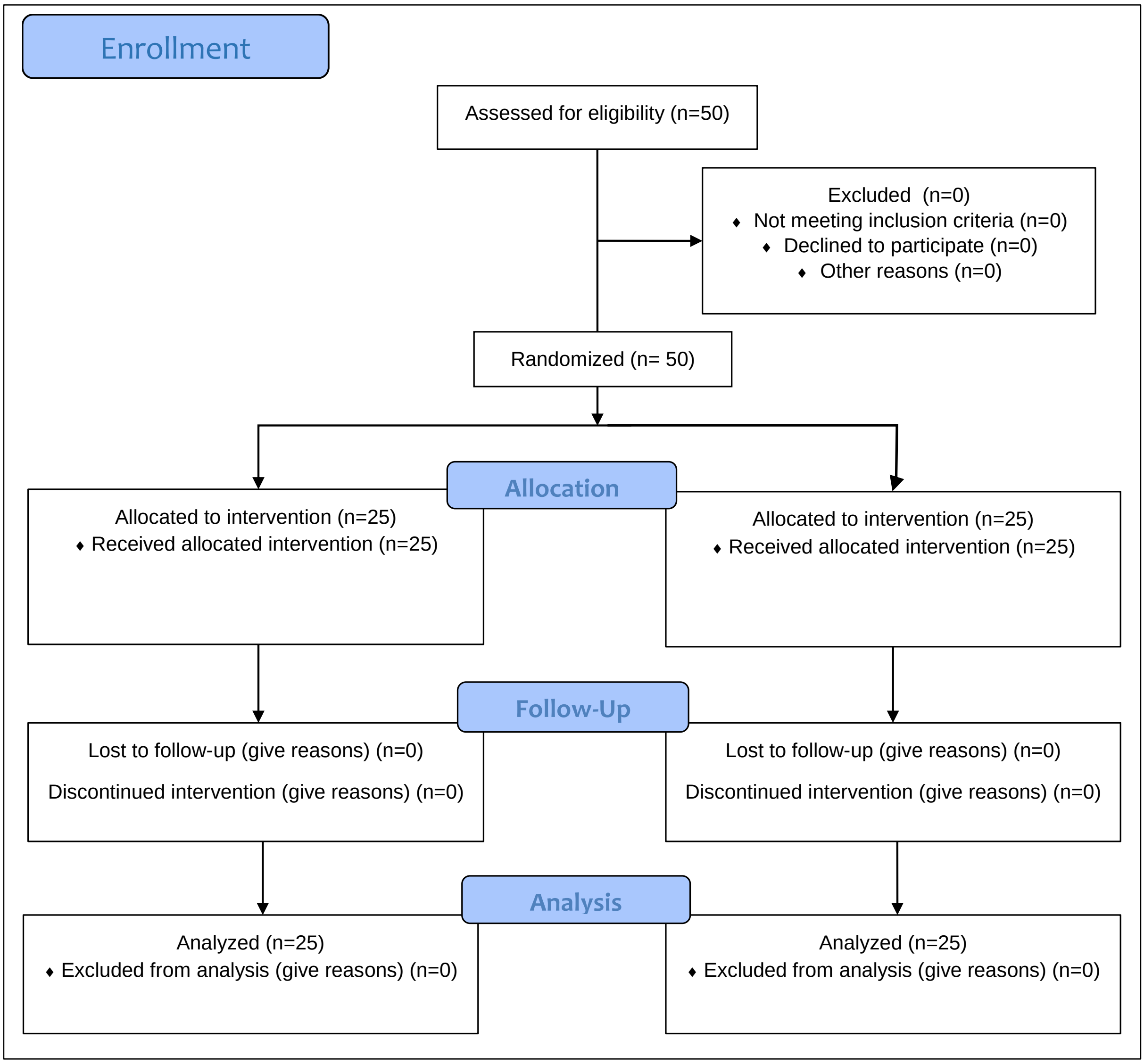

Figure 2. Flow diagram of the study

Anthropometric parameters

Anthropometric parameters including body weight and fat mass were measured using a Tanita BC-418 body composition analyzer (Tanita, Tokyo, Japan) [8]. Body mass index (BMI) was calculated as body weight (kg) divided by height in meters squared (m2). Systolic and diastolic blood pressures and abdomen and waist circumferences were also determined [8].

Hematological and biochemical parameters

Fasting blood samples were obtained before and after the intervention. Hematological markers (WBC, white blood count; RBC, red blood count; HCT, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; RDW, red blood cell distribution width) were determined using a Sysmex KX-21 hematology analyzer.

Biochemical biomarkers including total serum cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), serum C-reactive protein (CRP), fasting blood glucose (FBG), indirect and total bilirubin concentrations, and liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were measured by a BT300 auto analyzer device using Pars Azmoon kits [6, 9, 10]

Measurement of anti-HSP27

Serum anti-HSP27 is measurable by an in-house ELISA assay. In this measurement, we used microtitre plates (Nunc MaxiSorp, Nottingham, UK). The procedure was as follows: (1) Coating each well with 50 µl of carbonate buffer plus 100 ng of recombinant human HSP27; (2) Blocking with 250 µl of 2% goat serum in PBS; (3) Adding 100 µl of serum sample diluted 1:100 with 2% goat serum; (4) Adding the secondary antibody: 100 µl of 1:500 diluted peroxidase-conjugated anti-human IgG (Sigma-Aldrich, Poole, UK); (5) Adding 100 µl of TMB substrate to each well; (6) Stopping TMB with 50 µl of 2M HCl; (7) Reading the absorbance at 450 nm using an ELISA reader (iEMS Reader MF; Labsystems, Finland). We used the absorbance related to specific binding. This absorbance is equal to total binding (absorbance of antigen-coated wells) minus nonspecific binding (absorbance of uncoated wells). Finally, the results were expressed in absorbance unit (AU) [11].

Statistical analysis

Data were analyzed using SPSS 20 software (SPSS Inc., IL, US). The normality of data distribution was determined using the Kolmogorov-Smirnov test. Descriptive statistics, including mean ± standard deviation (SD), were used for normally distributed variables, while variables without normal distribution were expressed as median ± interquartile range. The t test was used for normally distributed data, while the Wilcoxon or Mann-Whitney U test was used for not-normally-distributed variables. The statistical significance level was set at 0.05.

Results

Anthropometric measurements

There was no baseline difference in age or BMI between the groups ($p > 0.05$) (Table 1). The mean reduction in abdominal circumference in the intervention group was 9.51 ± 2.66 cm versus 3.12 ± 1.88 cm in the control group ($p = 0.001$). Also, the mean reductions for waist circumference were 3.76 ± 1.69 cm and 2.40 ± 1.04 cm in the intervention and control group, respectively ($p = 0.006$). Differences in the mean reductions in BMI and body fat mass were not statistically significant between the groups ($p > 0.05$). The mean reduction for body fat mass was 2.31 ± 1.27 cm in the intervention group vs 1.99 ± 1.24 cm in the control group ($p = 0.34$).

Hematological parameters

Hematological parameters were assessed before and after the therapy in both groups (Table 2). HCT significantly decreased in the treatment group after five weeks. We found a significant increase in HCT in the control group ($p = 0.04$). Hemoglobin concentration decreased from 13.3 ± 0.6 g/dl at baseline to 12.6 ± 0.8 g/dl after the intervention ($p = 0.001$) in the intervention group, but there was no significant change in Hb concentration in controls. MCV decreased 3 units in the treatment group, which was significant ($p = 0.001$), while we did not find any changes in this parameter in controls. There was a significant decrease of 1.4 pg in MCH ($p = 0.001$) in the intervention group, but there was no change in controls. Platelets remained unchanged from before to after the intervention in the intervention and control groups ($p = 0.9$, $p = 0.1$ respectively). We found that RDW increased by 0.33% in the intervention group after the intervention, which was significant ($p = 0.004$), while it did not change in controls. Also, we found that WBC significantly decreased in cases after the intervention ($p = 0.003$). The differences in WBC ($p = 0.003$), RBC ($p = 0.015$), HCT ($p = 0.001$), MCV ($p = 0.008$), and MCHC ($p = 0.002$) between the intervention and control groups were significant.

Level of bilirubin and liver function markers

Total bilirubin significantly increased ($p = 0.005$) in the intervention group, but not in the control group. However, the level of indirect bilirubin did not change in either group (Table 3). After the 5-week therapy, liver enzymes AST and ALT demonstrated a significant difference between the intervention and control groups. The reduction in AST in the intervention group ($p = 0.03$) was significant after the study (Figure 1). Of note, all the changes were in the normal range.

Level of anti-HSP27 after therapy

Levels of anti-HSP27 were also measured to investigate its change in overweight subjects under therapy (Table 4). Our data showed that body-contouring treatment with US and RF had no significant effect on the levels of anti-HSP27.

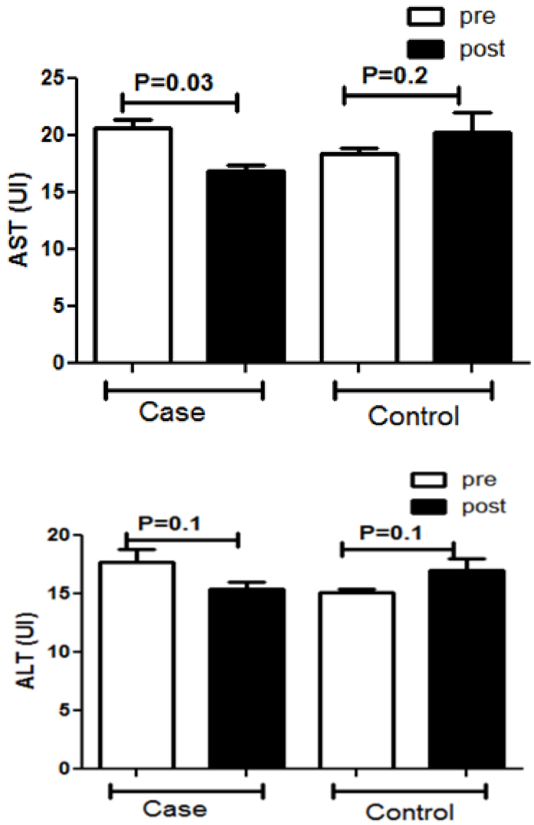

Figure 1. Liver function tests in the intervention and control groups.
Data are expressed as mean ± standard deviation

Table 1. Baseline characteristics of the participants

Variables	Intervention	Control	P value
Age, mean ± SD, y	36.5 ± 8.5	35.3 ± 8.6	0.62
BMI, mean ± SD, kg/m2	27.6 ± 1.4	27.07 ± 1.5	0.30

Table 2. Comparison of hematological parameters between the groups before and after the intervention

Parameter	Intervention			Control			P value*
	Before	After	P value	Before	After	P value	
WBC (×103/μL)	6.7 ± 1.5	5.5 ± 1.2	0.003	5.6 ± 1.4	5.8 ± 1.1	0.50	0.003
RBC (×106/μL)	4.5 ± 0.3	4.4 ± 0.4	0.055	4.4 ± 0.5	4.6 ± 0.4	0.06	0.015
HCT (%)	43.0 ± 1.6	41.0 ± 1.9	0.001	39.4 ± 3.5	41 ± 2.7	0.04	0.001
Hb (g/dL)	13.3 ± 0.6	12.5 ± 0.8	0.001	12.2 ± 1.2	11.6 ± 1.3	0.07	0.200
MCV (fL)	95.1 ± 3.9	92.4 ± 3.7	0.001	89.6 ± 7.1	89.2 ± 7.4	0.20	0.008
MCH (pg)	29.6 ± 1.6	28.2 ± 1.5	0.001	28.1 ± 3.4	25.3 ± 3.1	0.90	0.087
MCHC (gr/dL)	31.07 ± 0.95	30.50 ± 0.97	0.010	31.1 ± 1.6	28.2 ± 1.9	0.001	0.002
PLT (×103/μL)	235.8 ± 51.2	235.9 ± 54.2	0.900	231.6 ± 71.6	248.8 ± 61.8	0.10	0.200
RDW (%)	12.1 ± 0.6	12.4 ± 0.6	0.004	12.7 ± 1.2	13.3 ± 1.2	0.07	0.380
Lymphocyte (×103/μL)	2.01 ± 0.57	1.9 ± 0.4	0.300	2.1 ± 0.79	2.1 ± 0.4	0.43	0.200
Neutrophil (×103/μL)	3.5 ± 1.2	2.8 ± 1.2	0.004	2.83 ± 0.78	2.8 ± 0.8	0.80	0.018

WBC = white blood count, RBC = red blood count, HCT = hematocrit,
Hb = hemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, RDW = red blood cell distribution width.
Data are mean ± SD.
* Level of significance for between-group comparisons.

Table 3. Total and indirect bilirubin before and after the intervention

Variable	Intervention			Control			P value*
	Before	After	P value	Before	After	P value	
TBIL (mg/dl)	1.13 ± 0.05	1.29 ± 0.07	0.005	1.13 ± 0.10	1.23 ± 0.12	0.14	0.68
INBIL (mg/dl)	0.81 ± 0.32	0.85 ± 0.47	0.450	0.83 ± 0.46	0.91 ± 0.59	0.28	0.20

TBIL, total bilirubin; INBIL, indirect bilirubin.
* Level of significance for between-group comparisons.

Table 4. Serum levels of anti-HSP27 before and after the intervention in the two groups

	Median (Q1-Q3)	
	Intervention	Control
Week 0	0.24 (0.13-0.52)	0.19 (0.10-0.30)
Week 5	0.17 (0.08-0.48)	0.18 (0.09-0.28)
P value	0.29	0.977

Discussion

This is the first study to evaluate the influence of radiofrequency and ultrasound cavitation methods in addition to diet therapy on serum levels of anti-HSP27, hematological markers, and liver function markers in overweight Iranian women. Our data revealed that the combination of RF and focused US cavitation did not change the level of anti-HSP27. Also, we found that the levels of hematological markers and liver function markers might be influenced under this therapy.

There is a growing body of evidence showing that noninvasive fat removal devices based on RF and US waves are novel methods for managing overweight and obesity. These techniques are applied in many centers all over the world as well as in Iran. Some adverse effects were reported with these devices, including redness and edema for RF and tenderness, bruising, and edema for US, which are all localized side effects [6, 10]. However, the systemic side effects are still unclear. Therefore, in the present study, we assessed the effects of RF and US on serum levels of anti-HSP27. Moreover, we evaluated hematological parameters and liver function markers in overweight subjects before and after the five-week therapy. Hemoglobin (Hb) is the main protein component of red blood cells, responsible for carrying oxygen to other cells through the bloodstream. A Hb concentration of less than 12 mg/dl in females is considered anemia [1], which may cause fatigue, pale skin, weakness, shortness of breath, frequent infections, headache, dizziness, or even chest pain and affect physical performance in adults [12]. In this study, we found that using RF and US for body contouring significantly decreased Hb concentration, HCT, MCV, and MCH. In vivo evaluation of possible hematopoietic system disorders after RF radiation exposure in rats did not indicate any significant changes in the proliferation rate of bone marrow cells and erythrocyte maturation rate [9].

Because of the prevalent use of high-intensity focused ultrasounds (HIFU) in body contouring, Jewell et al [13] analyzed blood samples before and after the intervention and found no effects on lipid profile, inflammatory markers, coagulation, and renal function. However, they did not evaluate the effects of HIFU on hematologic parameters [13]. Takegami et al [14] evaluated the effect of HCT on HIFU efficacy. They indicated that RBC counts were important factors in heating and target coagulation. Moreover, we observed that RF and US therapy can alter the levels of hemoglobin and RDW, which is in line with the previous observation [14]. These findings show that RF and US body contouring could cause red blood cell destruction. It seems that given the significant changes in hematologic markers associated with these two methods, Hb concentration and the related parameters should be considered before the treatment. This is a cautionary suggestion to prevent some health conditions after treatment with these methods.

Furthermore, our data indicated changes in the level of liver function markers after the therapy, although these changes were in the normal range, which is in line with previous studies [13]. In particular, Jewell et al, evaluated the long-term safety of a high-intensity focused ultrasonography device on lipid profile, inflammatory markers, renal and hepatic function, and total bilirubin. They showed no significant changes from baseline in the laboratory values [13]. Another study reported that the combination treatment with focused ultrasound and RF for one session caused no significant change in liver function markers [15].

During this combined treatment, the US therapy causes disruption in adipocytes and RF subsequently increases the circulation and metabolism [15]. At the same time, using a low-calorie diet limits fat intake, thereby contributing to lower levels of liver enzymes compared with the controls. It seems that adding a low-calorie diet to this treatment procedure is a distinction of the present results compared with previous studies.

Tavallayi et al investigated the correlation of overweight and obesity with anti-HSP27 titer [16]. The subjects included 50 individuals with normal weight, 100 with overweight, and 100 with obesity. The results indicated that the antibody levels were higher in the obese compared with the overweight subjects, who in turn had higher levels than the normal group. Given the fact that anti-HSP27 is an immunologic and inflammatory marker, this finding implies an immunologic response during overweight and obesity [16]. In a study by Abdi et al [17] to investigate the effects acupuncture on obesity and a number of inflammatory markers, including anti-HSP27, 196 obese subjects were divided into two groups to receive a low-calorie diet combined with either authentic or sham acupuncture. Both groups experienced significant weight loss, but serum levels of antibodies against HSP27, HSP60, HSP65, and HSP70 were lower in the intervention group only, although the difference was not significant. Based on this evidence, there is no apparent correlation between weight loss due to a low-energy diet and immune responses. In the present study, the effects of weight loss by body-contouring on anti-HSP27 were comparable to that of weight loss by a low-energy diet. We observed no significant difference in anti-HSP27 from baseline to post intervention in either group. There is evidence that anti-HSPs, especially in higher titers, contribute to atherosclerosis through their immunologic roles [18]. It is also evidenced that anti-HSP27 has a positive relation with coronary heart disease and its severity, nominating it as a cardiovascular marker [19]. We found that applying this approach does not increase the level of anti-HSP27 and, probably, the risk of related diseases. This could be an encouraging inference about the safety of these noninvasive body-contouring methods.

The main limitation of the current study was its low sample size, stressing the need for exploring these findings in a larger population. Moreover, although all participants were assessed for daily caloric requirements and put on the same caloric deficiency, we cannot exclude the possible effect of a small variation in the diet on biochemical and hematological parameters.

Conclusion

Our findings demonstrated that the application of the RF-US combination does not have adverse effects on hematological parameters, liver function, and anti-HSP27 level in overweight women. Further studies in a larger multicenter setting are warranted to explore the value of these methods.

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* Equally contributed to the study

Informed consent: was collected from all participants using protocols approved by the Ethics Committee of Mashhad University of Medical Sciences.

Conflict of interest

None of authors have conflict of interests.

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