

Influence of Combined Low Frequency Ultrasound and Lymphatic Drainage Techniques on Body Fat Mass and Triglycerides in Atherosclerotic Patients

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Abstract: The aim of this study is to find out the effect of low frequency ultrasound and lymphatic drainage on body fat mass composition and blood triglycerides and testing the strength of the relationship, between the response of fat mass and the changes in the Total Body Triglycerides, **Methods:** Forty women patients with age ranged from 40 to 50 years were selected from out-patient clinic of cardiovascular diseases in Palestine hospital as they were diagnosed by chronic atherosclerosis. They were assigned into 2 groups according to their body mass index, based on the classification of the world health organization, (Group A (34.2 – 39.3 kg/m²) and Group B (42.9 - 49.11 kg/m²)). Both groups were evaluated before and after 24 sessions for body fat mass and blood serum triglycerides. Then they were enrolled in a combined treatment program of low frequency ultrasound and lymphatic drainage application of 20 minutes each for 24 sessions. **Results:** There was a statistical significant reduction of blood serum triglycerides and for body fat mass with a percentage of improvement (Gr. A: 8.5%, Gr. B: 10.6%) & (Gr. A: 12.4%, Gr. B: 10.2%) respectively after the end of the program moreover; there was a strong positive correlation between the reduction of total body fat mass and serum blood triglycerides. **Conclusion:** The combined treatments of low frequency ultrasound technique plus lymphatic drainage technique improve fat mass composition as well as blood serum triglycerides of chronic coronary atherosclerosis patients.

Keywords: Low frequency ultrasound/ Lymphatic Drainage/ Triglycerides/ Fat mass/ Coronary Atherosclerosis.

Introduction:

Triglyceride (TG, triacylglycerol, TAG or triacylglyceride) is an ester derived from glycerol and three fatty acids. Triglycerides are a blood lipid that helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides depending on the oil source. Some of these are highly unsaturated in the human body; high levels of triglycerides in the bloodstream have been linked to atherosclerosis and by extension, the risk of heart disease and stroke¹.

Hypertriglyceridemia is a prevalent risk factor for cardiovascular disease (CVD) and increasingly important in the setting of current obesity and insulin resistance epidemics. High triglyceride (TG) levels are markers for several types of atherogenic lipoproteins. Patients who have hypertriglyceridemia may be at significant risk for CVD even if low-density lipoprotein cholesterol levels are at goal, and therefore warrant treatment that optimizes diet, reduces overweight, and promotes regular exercise².

Men and women who have high triglyceride levels >150 mg/dl and a low level of HDL cholesterol <40 mg/dL are characterized by a significantly increased cardiovascular risk. The high triglyceride/low HDL cholesterol phenotype is a hallmark of the metabolic syndrome. The metabolic syndrome is closely associated with insulin resistance and is highly associated with the risk of CHD. It has a greater impact on the incidence of CHD in women than in men³.

Body fat composition which make up adipose tissue are specialized cells which contain and can synthesize globules of fat. This fat either comes from the dietary fat we eat or is made by the body from surplus carbohydrate or protein in diet. Adipose tissue is mainly located just under the skin, although adipose deposits are also found between the muscles, in the abdomen, and around the heart and other organs. The location of fat deposits is largely determined by genetic inheritance. Thus it is not possible to affect where the fat is stored . Nor is it possible to influence from which area the body burns fat for energy purposes⁴.

Respective of the location from which they are obtained, the fat cells in humans are composed almost entirely of pure triglycerides with an average density of about 0.9 kilograms per liter. Most modern body composition laboratories use the value of 1.1 kilograms per liter for the density of the "fat free mass", a theoretical tissue composed of 72% water (density = 0.993), 21% protein (density = 1.340) and 7% mineral (density = 3.000) by weight⁵.

Fat cells are not only energy depots, but are busy endocrine organs. They secrete cytokines, which regulate responses to infection, immune reactions, inflammation and trauma. In regards to inflammation regulation, fat cells secrete pro-inflammatory (TNF, IL-6, and C-reacting protein "C-RP") and anti-inflammatory (adiponectin) cytokines. Unfortunately, with visceral fat obesity accumulation, adiponectin levels are reduced, thus leading to a higher cardio metabolic disorders (e.g., heart disease and diabetes³).

High fat masses especially abdominal obesity is associated with an accelerated atherosclerosis, atherogenic blood triglycerides and other compartments of lipid profile, elevated platelet counts in females with chronic inflammation and increased rates of cardiovascular death^{6,7}.

Therapeutic ultrasound may have two types of benefit: Thermal effects and non thermal effects. Thermal effects are due to the absorption of the sound waves. Non thermal effects are from cavitations, micro streaming and acoustic streaming. Cavitation effects result from the vibration of the tissue causing microscopic air bubbles to form, which transmit the vibrations in a way that directly stimulates cell membranes. This physical stimulation appears to enhance the cell-repair effects of the inflammatory response⁸.

Lymph carries away large particles bacteria, Cell debris which can then be filtered out and destroyed by the lymph node . Lymph capillaries in the interstitial spaces have same structure as blood capillaries but their walls are more permeable to interstitial fluid constituents⁹.

As the lymphatic system is the platform for the immune system and lymph nodes are the sites of adaptive immune responses modulated by the surrounding adipose tissue, increased knowledge of how the lymphatic system contributes to triglycerides transport, distribution and metabolism and to the pathogenesis of chronic inflammatory conditions may provide the basis for the development of new therapeutic strategies and increased quality of life¹⁰.

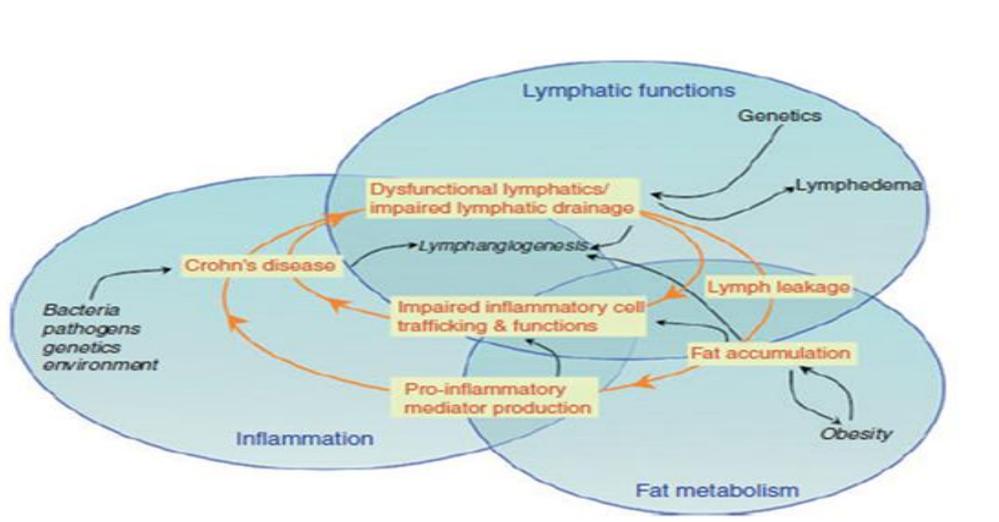


Fig. (1): Proposed working model for the interactions between lymphatic, fat and inflammation⁽¹¹⁾.

Methods and Procedures

Participants

The study was carried on 40 female patients with age ranges from 40 to 50 years were selected from outpatient clinic for cardiovascular diseases , Palestine hospital. They were chronic coronary atherosclerotic patients with moderate risk level of triglycerides (200-499mg\dl) and were assigned into 2 groups according to their **Body Mass Index** based on the classification of the world health organization **Group (A)**: 20 patients with BMI ranged between (35 – 39.9) kg/m²**Group (B)** : 20 patients with BMI ranged between (42.9 - 49.11) kg/m². All patients were assessed for weight and height to calculate their BMI, then for blood serum triglycerides by UDICHEM-300 Chemistry Analyzer and the Body Composition Analyzer (BCA) to assess the body fat mass before and after 24 sessions of combined treatment program of Low intensity low frequency(LILFU) and lymphatic drainage 3 times per week. Ethical consideration for all patients enrolled in the study ; they were informed about the nature and benefits of the treatment program; then signed a consent sheet prior to participation with concern that confidentiality was assured.

Procedures of treatment program :

A-Low Frequency Ultrasound Application: including abdominal area, upper arms, thigh and hips from supine position for 20 minutes , Scapular area , posterior aspect of the hips from prone position . (*The pulse duration : 200 msec. Frequency : 28-31 KHz . Stimulation mode (20sec. stimulation, 20sec pause)*) ; the duration of application of ultrasound was 40 minutes.

B-Vacuum Pressure Machine for lymphatic drainage Application: Starvac vacuum massage machine 866-312-7540 low power consumption 200W) cyclical vacuum starting from 80 mill bars ,(roller methods) was applied on the same previous areas for another 20 minutes.

Treatment duration for each session was 60 min including application of combined methods was applied 3 times per week for 24 sessions.

Results

1. Demographic Data:-

Group (A): include twenty female patients with age ranges from 40 to 50 years old with the mean value of (46 ± 3.77) yrs., the weight ranged from 80 to 100 Kg with the mean value of (92.41 ± 6.67) kg, the height ranged from 150 to 160 cm with the mean value of (158 ± 5.06) cm. The BMI ranged from 35 to 39.9 kg/m² with the mean value of (37.43 ± 1.98) kg/m² as shown in Table (1) and Figures (2,3,4 &5).

Group (B): include twenty female patients with age ranges from 40 to 50 years old with the mean value of (45 ± 3.01) yrs., the weight ranged from 110 to 120 Kg with the mean value of (115.5 ± 5.55) kg, the height ranged from 155 to 165 cm with the mean value of (155 ± 4.44) cm. The BMI ranged from > 40 kg/m² with the mean value of (44.5 ± 3.22) kg/m² as shown in Table (1) and Figures (2,3,4 &5).

Table (1): Demographic Data for both groups :-

Variable	Groups	Mean ± SD	median	Max - Min	t-value	p-value (2-tailed)
Age (years)	Group (A)	46 ± 3.77	46	43 – 50	1.379	0.176 NS
	Group (B)	45 ± 3.01	45.5	42 - 48		
Weight (Kg)	Group (A)	92.41 ± 6.67	90	84 - 98	-18.626	0.000 S
	Group (B)	115.5 ± 5.55	114	110 - 120		
Height (cm)	Group (A)	157 ± 5.06	155	153 – 163	-1.593	0.120 NS
	Group (B)	159 ± 4.44	160	155 – 163		
BMI (kg/m ²)	Group (A)	37.43 ± 1.98	37	34.2 – 39.3	-17.617	0.000 S
	Group (B)	45.5 ± 3.22	47	42.9 - 49.11		

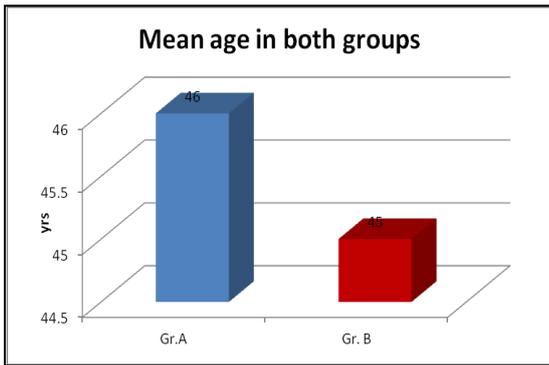


Fig. (2): Mean age in both groups

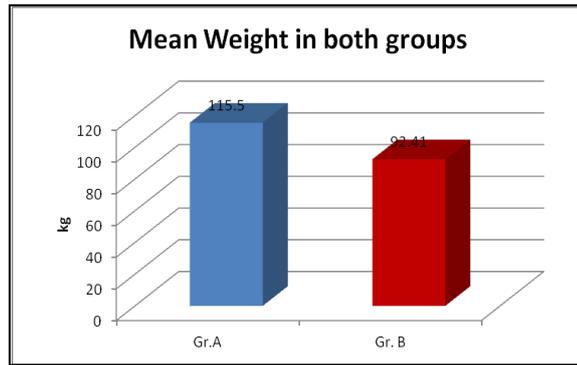


Fig. (3): Mean weight in both groups

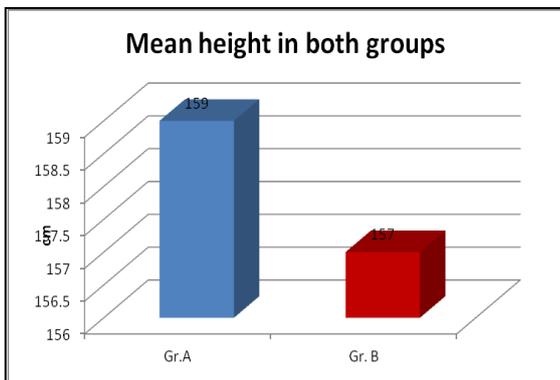


Fig. (4): Mean height in both groups

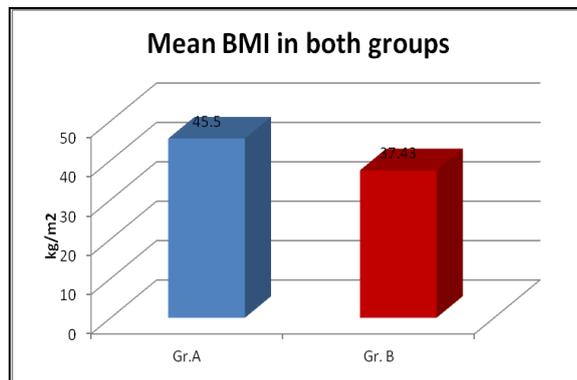


Fig. (5): Mean BMI in both groups

2. Fat Mass

Group A: The results revealed that, pre treatment mean value was 40.6 ± 7.23 , while that of post treatment was 34.76 ± 8.9 . According to Wilcoxon test $z = -3.912$; the significant improvement was revealed ($p > 0.001$) when comparing between pre and post treatment mean values (Median Difference= 5) as shown in Table (2) and Figure (6) and percentage of improvement (Reduction) = 12.4%.

Group B: The results revealed that, pre treatment mean value was 58.12 ± 12.9 , while that of post treatment was 51.87 ± 10.07 . According to Wilcoxon test $z = -3.912$; the significant improvement was revealed ($p > 0.001$) when comparing between pre and post treatment mean values (Median Difference= 5.6) as shown in Table (2) and Figure (6) and % of improvement (Reduction) = 10.02%.

Table (2): Comparing the median values of Fat Mass among the two groups.

Fat Mass	Group A Group		Group B Group	
	Pre treatment	Post treatment	Pre Treatment	Post treatment
Mean \pm SD	40.6 ± 7.23	34.76 ± 8.9	58.12 ± 12.9	51.87 ± 10.07
Median	39.5	34.6	55.85	50.25
Minimum –Maximum	26.4 – 51.8	16.8 – 48.9	41.8 – 96.3	37.5 – 80.2
Negative and positive rank	0 – 20		0 – 20	
% of improvement	12.4		10.2	
z-value	-3.921		-3.921	
p-value	0.001		0.001	
Level of Significant	S		S	

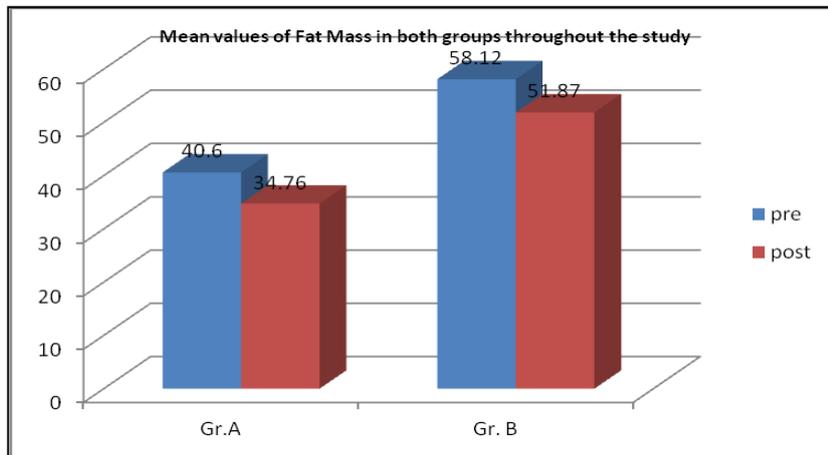


Fig 6: Pre and post treatment mean values of fat mass between the two groups.

3. Total Body Triglycerides

To determine the differences in the mean values of total body triglycerides among the groups, repeated measure analysis of variance (ANOVA F-Test) was performed as shown in Table(3)and figure(7) . The results of the Group A and Group B groups revealed that, there is a significant improvement (reduction) in the Total Body Triglycerides for the subjects after the treatment procedures

Table(3): Comparing the mean values of total body triglycerides among the two groups.

	Total Body Triglycerides	Mean ± SD	ANOVA Table					
				Sum of Squares	DF	Mean Square	F	Sig
Group A	Pre treatment	267.78 ± 34.3	Between Groups	8767.492	2	4383.746	3.973	.024
	Post treatment	245.01 ± 32.7	Within Groups	62900.358	57	1103.515		
				Sum of Squares	DF	Mean Square	F	Sig
Group B	Pre treatment	300.29 ± 66.1	Between Groups	15220.133	2	7610.0665	3.443	.038
	Post treatment	268.39 ± 57.2	Within Groups	125999.91	57	2210.5248		

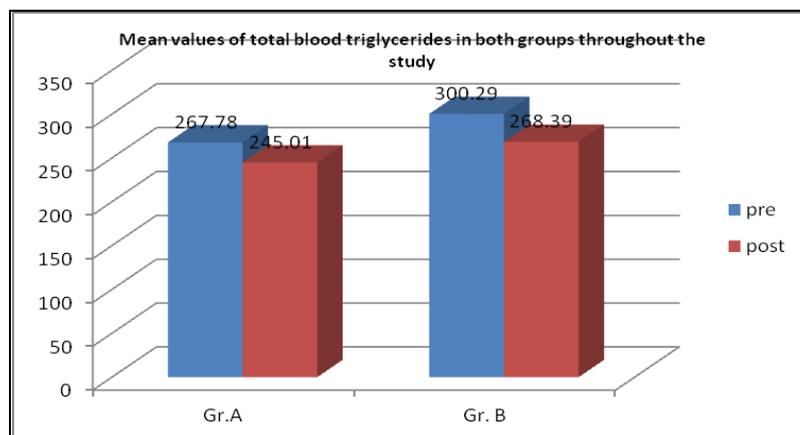


Fig. 7: Comparing the mean values of total body triglycerides between the two groups.

4. Correlation Analysis

This section is showing the inferential statistical analysis done on the sample, in order to test the significance and the strength of the relationship between the reduction in Fat Mass and the reduction in the Total Body Triglycerides using the Pearson bivariate correlation test to examine the one-to-one relationships of independent and dependent variables prior to running a linear regression

As shown in table (4) and figures (8) -which illustrates the bivariate correlations results for the whole sample -shows highly significant relationships for all the variables in the two groups at level of significant at the 0.001. In Group A, There is a positive significant correlation between the fat mass post-treatment and the total body triglycerides Post treatment with correlation coefficient $r = 0.702$ and significance ($p < 0.05$). In Group B, There is a positive significant correlation between the fat mass post-treatment and the total body triglycerides Post treatment with correlation coefficient $r = 0.929$ and significance ($p < 0.05$).

Table 4: Testing the correlation between the fat mass and the total body triglycerides

	Variables		r value	p value	Significance
Group A	Fat Mass Post-treatment	Total Body Triglycerides post Treatment	0.702	0.001	Significant Correlation
Group B	Fat Mass Post-treatment	Total Body Triglycerides Post Treatment	0.929	0.000	Significant Correlation

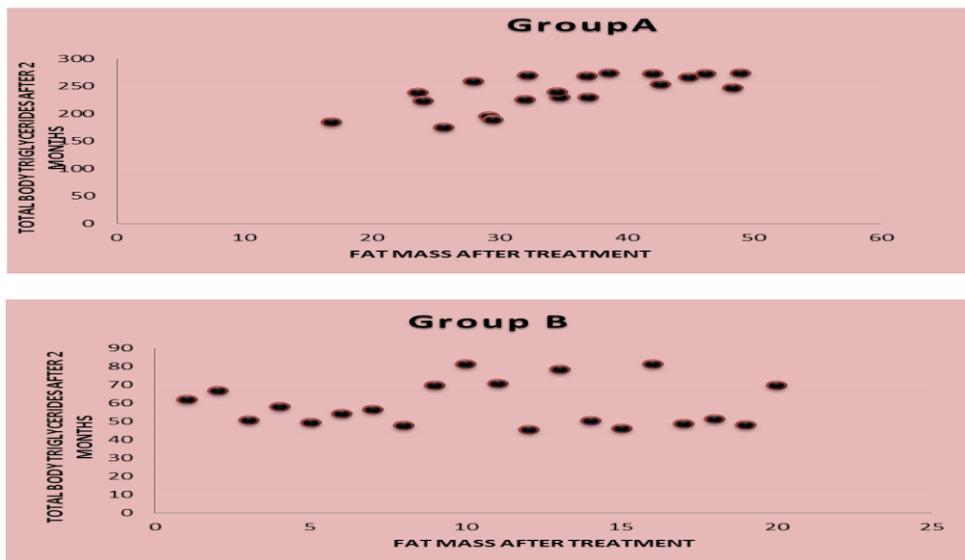


Fig. 8: Testing the correlation between the fat mass and the total body triglycerides

Discussion

This study was conducted to assess the responses of serum Triglycerides and fat mass to a combined treatment program by of low frequency ultrasound and lymphatic drainage in chronic atherosclerotic patients, The results showed that there were significant difference in Serum Triglycerides that is strongly correlated with the reduction of the body fat mass .

These results agreed with¹² which considered as an important **histomorphometrical** observations-, in both the ex vivo and in vivo experiments, lipid discharge was not accompanied by any morphological signs of adipocyte death and disruption or interstitial inflammation. Moreover, the noted cavitation-induced effects seem to be restricted to adipocytes, while blood micro vascular cells-showed normal features. These findings suggest that ultrasound-induced cavitation, at appropriate settings and timing does not create local adverse conditions that may favor tissue injury and subsequent inflammatory/fibrotic reaction, on the other hand pro inflammatory

mediators released from adipocytes, conceivably by lymphatic drainage. Indicating that ultrasound-induced cavitation causes a statistically significant release of triglycerides from the adipose tissue into the interstitial fluid, as previously reported. That gives an important clinical evidence base of the effect of low frequency ultrasound on fat mass and consequences enhancements of blood triglycerides and correlate this to the lymphatic system role on all of this findings of current study are coincided with that of ¹³ who studied the body composition by the bioelectrical impedance method in which waist hip ratio was correlated with serum triglyceride ($P < .0001$), that study concluded that abdominal adiposity is associated with metabolic disorders, level secondary to insulin resistance. Abnormality in triglycerides as previously demonstrated, whereas lymphatic dysfunction is mainly associated with ganoid adiposity. Besides microcirculatory disorders, changes in the secretory regulation of hormones.

That gives a clinical base evidence of the relationship among adiposities ,triglycerides and lymphatic system dysfunction which is the main core of our study.

These results came consistent with the results declared from a study by ¹² where their findings indicate that ultrasound-induced cavitation of human skin, achieved by Med2Contour ultrasound device, can yield a substantial reduction of subcutaneous fat and adipose cell size, confirming the previous clinical observations of a marked lipo reductive effect of this technique. This study also provides circumstantial evidence for the mechanism of action of ultrasounds on adipocytes. The histomorphometrical data indicate that exposure of full-thickness skin explants to 2 short ultrasound cycles (6 s each), with a similar energy output to that used for clinical purposes, resulted in a statistically significant shrinkage of subcutaneous adipocytes (-23% , $P < 0.001$).

In accordance with the results of our study ,came the results of ¹⁴ who evaluated the role of external ultrasonic plus liposuction as an adjunct to traditional liposuction on Ten patients underwent standard liposuction with the addition of 10 minutes of preoperative ultrasound therapy applied to one-half of their targeted treatment regions, the results showed that roughly half of the patients had an improvement results after postoperative course, with less swelling/edema, less bruising, and less postoperative pain/discomfort. The favorable results of this study warranted further studies about the effect of ultrasonic modalities in management of obesity without undergoing surgeries that is more preferable and safe to the patients.

On the other hand, our results was in opposite direction of the results that has been obtained from ¹⁵. They studied the effects of a new low frequency, high intensity ultrasound technology on human adipose tissue *ex vivo* were studied. In particular, they investigated the effects of both external and surgical ultrasound-irradiation, there was significant weight loss and fat release.), the effectiveness of ultrasound was much higher when the tissue samples were previously infiltrated with saline buffer, in accordance with the knowledge that ultrasonic waves in aqueous solution better propagate with a consequently more efficient cavitation process. the overall effects of ultrasound application did not appear immediately after treatment but persisted over time, a significant increase mainly of triglycerides and cholesterol has been noticed.

The data obtained in this study revealed statistical significance changes in fat mass and the significant improvement (reduction) in the Total blood serum triglycerides for the Group A and, Group B groups at the two stages of the measurements (Pre & Post), revealed that the combination of lymphatic drainage technique with the low frequency ultrasound reduce the Triglycerides rather than using the low frequency ultrasound only and revealed that there is a positive significant correlation between the reduction of Fat Mass post-treatment and the reduction of the Total Body Triglycerides which considered a main issue with atherosclerotic patients . Using of low frequency ultrasound alone may lead to increase of blood serum triglycerides as there will be no way out to get rid of adiposities waste as a consequence, applying lymphatic drainage modality is essential after using low frequency ultrasound equipment's.

With respect to ¹⁶ who examined the effects of the combination of ultrasound irradiation and exercise on the subcutaneous fat thickness of thighs inhuman. Adult men were assigned into three groups; [group A] took 10 min irradiation of 1MHz frequency and 10min walking, [group B] took 10 min irradiation of 500kHz frequency and 10 min walking, and [group C] also took 10 min. walking without ultrasound irradiation. The irradiation and walking were carried out once a day for 10 days. The site of ultrasound irradiation was the inner thigh of the right leg with the left side being used as control side. The results declared that the subcutaneous fat thickness of the irradiated (right) thighs significantly decreased compared with that of the non-irradiated (left) thighs after irradiation using 500kHz, but body weight between before and after exercise and ultrasound irradiation was not changed.

There was a statistical significant reduction of blood serum triglycerides with a percentage of improvement in Gr. A: 8.5% & Gr. B: 10.6% and for body fat mass with a percentage of improvement in Gr. A: 12.4% & Gr. B: 10.2% after the end of the program moreover ; there was a strong positive correlation between the reduction of total body Fat mass and serum blood triglycerides these results agreed with a pro-complementary study done by ⁽¹⁷⁾ that was done on obese patients with high body fat mass composition by using the combination Technique of low frequency ultrasound and lymphatic drainage modalities in order to improve body compositions including fat free mass, fat mass & body water composition that implicate a safe and well-tolerated method with no life-threatening side effect in treating cardiac patients especially whom at risks of complications of diabetes and/ or hypertension, providing an opportunity to improve the quality of life of cardiac patient.

In the present study the usage of lymphatic drainage which based on the activity of low frequency ultrasound combined with vacuum that increases the effect of the ultrasound. Vacuum gently sucks the skin and, improves the lymphatic drainage, the elimination of the fat tissue; the fat is spilled out without any need of conventional interference.

Conclusion

Low frequency ultrasound plus lymphatic drainage technique can be considered a safe and well tolerated method with no life threatening side effects that improves the blood serum triglycerides and fat mass in atherosclerotic cardiac obese patients and thus provide an opportunity to improve the quality of obese cardiac patient as many obesity surgery have a lot of hazards and sides effect that may affect the patient especially those with cardiac conditions, and that result could be achieved with the usage of LFUS plus LD technique in order to reduce the blood serum triglycerides, fat cells size and even destruction of adiposities with acceptable penetration and get rid of that cells out of the body preventing hazards and complication as thrombosis and many cardiovascular complicated that can be a result of high blood serum triglycerides and high total body fat mass.

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