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Role of Cold Laser as an Alternative Tool to Bariatric Surgeries in Modifying Sirtuin1 Gene Expression in Obese Down Syndrome Patients

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Abstract : Purpose: the current study was conducted to investigate the effect of cold laser as alternative tools to bariatric surgery on modifying SIRT1 gene expression in obese Down syndrome patients. **Methods:** Forty obese Down syndrome patients were included in current study. Their IQ level was greater than 60. Their age were ranged from 14 to 18 years old, their body mass index (BMI) was ranged from 30kg/m2 to 39,9 kg/m^2, These patients were divided randomly and equally into two groups G1 composed of twenty obese patients with down syndrome. They were exposed to cold laser on abdominal adipose tissue two times/ week for 3 months plus indoor ergonomic exercise two times / week for 3 months plus balanced diet and G2 composed of twenty obese patients with Down syndrome. They were received indoor ergonomic exercise two times/ weeks for three months plus balanced diet, **Methods**: data were obtained for each patient from BMI, abdominal circumference, skinfold and SIRT1 level. Measurements were performed before study (pre-test) and after three months (post-test). **Results:** statistical analysis revealed that there was reduction of BMI, waist circumference and skin fold and fold change within both groups after treatment in compare to pretreatment ($p<0.05$). Also there was a significant reduction in skin fold in group I compared with group II) $P<0.05$). In spite of there was no significant difference in the BMI, waist circumference, and fold change between both groups) $P>0.05$) after treatment. As regards , in post treatment there was a clinical difference of BMI in favor to G2 (11.69%) compared to G1 (6.93) and waist circumference in G1 (18.29) compared with G2 (11%), As regards the fold change was favor in G2 (40%) while it was 27.27% in G1. **Conclusion:** Clod laser and exercises with balanced diet has a significant effect in reduction BMI and skin fold. As regards more favor effect of cold laser in waist circumference reduction, than exercises. Fold change as indicator for sirtuin1 gene expression was changed in G2 and G1 respectively (40%, 27.27%). These indicated that BMI reduced with exercises, cold laser respectively which may be an effective tool in modifying Gen expression in Down syndrome obese patients.

Key word : Obesity- Down syndrome- Bariatric surgery- Sirtain I- cold laser.

Introduction

Down syndrome (DS) is a genetic disorder caused by the presence of a part or a full extra copy of chromosome 21 resulting in 47 chromosomes, and the most common type is meiotic non-disjunction causing trisomy 21 (95%); other types are Robertsonian translocation and mosaic type. DS children have typical dysmorphic features and cognitive impairment. They are known to be shorter than their normal counterparts and may suffer a multitude of debilitating problems, including congenital heart disease, gastrointestinal anomalies, leukemia, alzheimer's disease, immune dysfunction, hypothyroidism, diabetes mellitus, and vision and hearing problems [1].

Furthermore, Down syndrome has a higher risk for developing obesity. Childhood obesity, which is associated with health problems throughout life, is a particular threat to children with Down syndrome and may slow or reverse other health gains. Obesity is a stigmatizing condition and can be another characteristic that identifies those children as different[2].

The primary care provider can assist the family in preventing or managing obesity by recognizing the physiological and behavioral factors that place children and adolescents with Down syndrome at increased risk to become obese, and establishing a screening and management plan early to prevent or treat excess weight gain [3].

SIRT1 stands for sirtuin (silent mating type information regulation 2 homolog)1, is also known as NAD+-dependent deacetylase. It is a protein that in humans encoded by the SIRT1 gene [4].

SIRT1 is an enzyme that deacetylates proteins that contribute to cellular regulation, it also affects the activity of both members of the PGC1-alpha/ERR-alpha complex, which are essential metabolic regulatory transcription factors [5].

Sirtuin1 (SIRT1) is activated during calorie restriction and appears to be related to energy balance through glucose or lipid metabolism and insulin signaling. These findings suggest that SIRT1 may play a significant role in the pathophysiology of visceral obesity. SIRT1 expression levels were negatively correlated with body mass index, waist circumference, and abdominal visceral fat area. The significant association between abdominal visceral fat accumulation and SIRT1 gene expression in PBMCs let suggest that SIRT1 may be a new therapeutic target for the prevention of disease related to obesity, especially visceral obesity [6].

On the other hand, Bariatric surgery, also known as weight-loss surgery, refers to surgical procedures usually performed on people who are morbidly obese for the purpose of losing weight and to treat, as well as prevent, obesity-related comorbidities. Bariatric surgery has evolved since its introduction in the 1950s, with some procedures that were popular initially (like jejunoileal bypass) having been abandoned because of unacceptable complication rates [7].

Bariatric surgeries are operations on the stomach and/or intestines that help patients with extreme obesity to lose weight. These surgeries are option for people who cannot lose weight by other means or who suffer from serious health problems related to obesity and has failed to lose weight by non-surgical treatment (exercises, diet and physical therapy devices) [8].

Essential factors to be consider before taking the decision for operation include the patient mental status , patient's BMI, eating habits, health conditions related to obesity, and previous stomach surgeries [9].

Moreover, Low-level laser therapy (LLLT) (635 - 680 nm) is commonly used in medical applications as a non-invasive reduction of weight and body contouring intervention modality through loss of fat on fat cells [10].

It is believed that, cold laser activates the lymphatic system to help remove the remaining fatty wastes and toxins from the tissues and muscles [11].

Therefore this current study is an attempt to investigate the effect of cold laser as an alternative tool to bariatric surgery on modifying SIRT1 gene expression in obese Down syndrome patients.

Subjects:-

Forty obese Down syndrome patients were included in current study. Their IQ level was greater than 60 .Their ages was ranged from 14 to 18years. Their body mass index was between30 Kg/m² And 39.9 Kg/m² (moderate obesity). They were free from any pathological disorders or pervious surgery which may affect the study and application of treatment modalities. The subjects were divided equally and randomly into two groups : Group (1):In this group, twenty obese patients with Down syndrome were exposed to cold laser on abdominal adipose tissue (each session was 30 minutes, two times per week for 3 months) plus a balanced diet and indoor ergonomic exercises therapy (each session was 30 minutes, 2 times per week for three months) Group (2): In this group, twenty obese patients with Down syndrome were received balanced diet and indoor ergonomic exercises therapy (each session was 30 minutes, two times per week for 3 months).

Measurement procedures:

Equipment:-

Measurement Equipment:

- Weight and height scale: to measure the weight and height for detection of BMI
- Plastic tape to measure abdominal / circumference.
- Electronic skin caliper to measure skin fold.
- Quantitative real-time polymerase chain reaction (qRT-PCR) was applied for detection the quantitative SIRT1 gene mRNA expression.
- Gene expression of SIRT1 gene

Therapeutic Equipment:-

- **Bicycle ergometer:**

Electronic bicycle ergometer (Universal, made in New York, USA) equipped with pedals, electronic break, and adjustable seat, and handle bar, display screen and foot straps also provided with programmable control unit.

- **Cold laser therapy device:**

The Lapex 2000 BCS (LipoLaser) is a 100% Non-invasive, laser-based, spot fat reduction and body contouring system. The Lapex 2000 BCS (LipoLaser) uses laser energy to safely (and painlessly) penetrate the skin and target specific adipose (or fat cells) [12].

- Technical specifications of the laser machine were:
- Model name : Lapex2000 BCS
- Serial number : 000065285
- Manufactured in: Korea
- Wave length : 632.8nm
- Time of application : 30min
  Energy density: 16j/cm2

Procedures of the study:

A) Measuring procedures:

- Measurement of Height and Weight:

Weight was determined, height was measured, then the body mass index was calculated where: Body mass index (BMI) = body weight (Kg)/ height (m^2) [13].

-**Plastic tape measurement** to measure abdominal circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch resistant [14].

-Abdomen skin folds skinfold were measured in cm with the patient in supine position by skin fold caliper.

The most prominent point in the abdomen was marked, skin and subcutaneous tissues at that point was pinched between the thumb and the forefinger and gently pulled away from the underlying muscles. Then, while the grasp was maintained, the calliper will be placed over the skin fold and left in its place for 3 seconds, and then the value was recorded [15].

- Gene expression of SIRT1 gene: 5ml blood was collected from each participant after an overnight fast in two separate tubes containing EDTA. 20 participant from group1 and 20 participants from group2 before the study, as pre-test and after 3 months as posttest.

Gene expression of SIRT1gene in obese children of the two groups was studied to investigate the effect of cold laser therapy in Sirtuin 1 gene expression in obese Down syndrome children. Five ml anti-coagulated Blood samples was taken from each participants RNA was extracted according to standard procedures from PBMCs using ready-made kits, followed by RNA reverse transcription for cDNA synthesis. quantitative real-time polymerase chain reaction (qRT-PCR) was applied for quantitative SIRT1 gene mRNA expression [16].

- B) Therapeutic procedures:

- Low level laser therapy:

Procedures of Lapex 2000BCS laser application:

Each patient was placed in a comfortable supine lying position. The therapist was position stride standing beside patient for application of laser probes and observation any patient's is discomfort. Both the patient and the therapist wore protective eye glasses. Two smaller probes lasers were placed over the appropriate lymphatic glands and hold in place for 10 minutes. The Lapex BCS lipolaser probs were re-located and was turned on for other 15 minutes; above umbilicus then below umbilicus this procedure was performed to ensure that all the spot fatty areas were treated, in 30 minutes. All session was performed in 40 minutes. Patient was instructed to stay 15 minutes before leave. The treatment was applied two day per week for three months**.**

Program of aerobic training:

Each patient was sit on stationary bicycle ergometer with her back in relaxed position, before exercise the limit of subject tolerance would be assessed by exercise test which was comprised a 3 minute control period of unloaded pedaling, followed by an incremental ramp on a cycle ergometer at a rate of 10 W per minute to the limit of subject tolerance. Then the subject cycle was sit at 30 W for warming up, and then the intensity was increased every 60 seconds by 15 W until exhaustion, then the subject cycle was 30 W for cooling down. Duration was for month, 3 times /week.

Exercises were performed on the electronic bicycle ergometer as the following stages:

-First stage (warming up): Consisted of 5 minutes warming up in the form of pedaling at speed of 60 revolutions per min without load.

-Second stage (active stage): Consisted of: Duration: 30 minutes. Mode: pedaling at speed of 60 revolution per min with; Load: adjusted load to achieve 60% of the predictive age maximal heart rate which was calculated by the following equation: Maximal heart rate = 220-age in yearsModerate work load = 60% of maximal heart rate.The heart rate was measured through pulsometer attached to the patient's ear.

-Third stage (cooling down): Consisted of 5 minutes cooling down in the form of pedaling at speed of 60 revolutions per min without load Frequency: 3 sessions per week for one month.

Low caloric diet protocol:

Patients were beginning adding thicker liquids that are high in protein and low in fat and sugar. They were used high –protein, low –calorie liquid supplement drinks their requirements during this period. Daily caloric intakes were not exceed 1500 calories. They were drinking 1 to 1.5 liters of water or other non-caloric liquids per day.

Recommended thicker liquids:

- Nonfat or 1% milk, it participant could.
- Lactose-free or soy based low calorie drinks.
- Sugar free pudding
- Sugar free nonfat yogurt
- Low fat cottage cheese
- Blended broth based soup or other low fat soups.

Statistical procedures

Data collection: Data were collect two times as follow: before starting (pre test) & after three months post – test including body weight BMI, abdominal circumference, skinfold and sirtun I .

Data analysis: Statistical analysis was conducted using SPSS for windows, version 20 (SPSS, Inc., Chicago, IL). The current test involved two independent variables. The first independent variable was the (interventions); between subjects factor which had two levels (group I receiving Laser therapy and exercises group II receiving exercises). The second independent variable was the (measuring periods); within subject factor which had two levels (pre, post). In addition, this test involved three tested dependent variables (body weight, waist circumference, and skin fold). Accordingly, 2×2 mixed design MANOVA was used to compare the tested variables of interest at different tested groups and measuring periods. With the initial alpha level set at 0.05.

Results:

1-General characteristics (age, weight and height) between two groups of the study.(Table 1)

Items	Group I	Group II	Comparison		Level of significant
	Mean ± SD	Mean ± SD	t-value	P-value	
Age (yrs)	16.11±1.36	15±1.95	-2.76	0.05	NS
Height (cm)	147.11±7.70	140.25±13.89	-1.33	0.199	NS

***SD: standard deviation, P: probability, S: significant. NS: non-significant**

2- Body mass index:

Table (2) revealed the results for the Body mass index pre and post treatment between two groups of the study. There was no significant difference in pre treatment values. But there was a significant difference in the posttreatment values ($P<0.05$) .

Table (2): Mean ±SD and p values of body mass index at both groups.

Body mass index (Kg)	Pre treatment	Post treatment	MD	% of change	p- value
	Mean± SD	Mean± SD			
Group I	34.18±3.31	30.29±3.89	2.371	6.93	0.0001*
Group II	33.23±8	30.86±7.89	3.887	11.69	0.0001*
MD	0.94	-0.57			
p- value	0.744	0.844			

***Significant level is set at alpha level <0.05 SD: standard deviation MD: Mean difference p-value: probability value**

3-Waist circumference:

Table (3) revealed the results for the Waist circumference pre and post treatment between two groups of the study. There was no significant difference in pre treatment values. But there was a significant difference in

the posttreatment values (P<0.05) .

Table (3): Mean ±SD and p values of Waist circumference at both groups.

Waist circumference (Cm)	Pre treatment	Post treatment	MD	% of change	p- value
	Mean± SD	Mean± SD			
Group I	**107.44±9.22**	**87.77±10.29**	**19.66**	**18.29**	**0.0001***
Group II	**101.08±19.24**	**89.95±17.26**	**11.12**	**11**	**0.0001***
MD	**6.361**	**-2.181**			
p- value	**0.373**	**0.741**			

***Significant level is set at alpha level <0.05 SD: standard deviation MD: Mean difference p-value: probability value**

4-Skin fold thickness:

Table (4) revealed the results for the Skin fold thickness pre and post treatment between two groups of the study. There was no significant difference in pre treatment values. But there was a significant difference in the posttreatment values (P<0.05) .

Table (4): Mean ±SD and p values of Skin fold thickness at both groups.

Skin fold (Cm)	Pre treatment	Post treatment	MD	% of change	p- value
	Mean± SD	Mean± SD			
Group I	4.77±0.44	1.11±0.33	3.66	76.72	0.0001*
Group II	4.91±0.28	2.25±1.13	2.66	54.17	0.0001*
MD	-0.139	-1.139			
p- value	0.393	0.009*			

***Significant level is set at alpha level <0.05 SD: standard deviation MD: Mean difference p-value: probability value**

5- Sirtuin 1 Gen Expression:

Table (5) show median score, U, Z and P-values of the fold change (A fold change is basically a ratio (post treatment and pre treatment). It indicates the number of times gene expression has changed in comparison to an original amount) at both groups. In group I, the median score of fold change for gene expression was 0.07205. While in group II, the median score of fold change for gene expression was 0.26375. "Mann-Whitney tests" revealed there was no significant difference between the both groups in fold change (U = 39, Z = -1.385, and P = 0.166).

Table (5): Median, U, Z, and p values of fold change at both groups.

Fold change	Group I	Group II	U-value	Z-value	p- value
Median	0.07205	0.26375	**39**	**-1.385**	**0.166**

*Significant level is set at alpha level <0.05 p-value probability value

6-Correlation among fold change and post treatment of BMI, waist circumference, and skin fold at group I:

As illustrated at figure (1 to 3) the correlations among fold change and post treatment of BMI, waist circumference, and skin fold at group I were studied through the Pearson product moment correlation coefficient. It revealed that there was no significant correlation between fold change and BMI (r= 0.261, p= 0.466). While, there was positive strong significant correlation between fold change and waist circumference

(r= 0.69, p= 0.019*). While, there were no significant correlation between fold change and skin fold (r=0.347, p= 0.295).

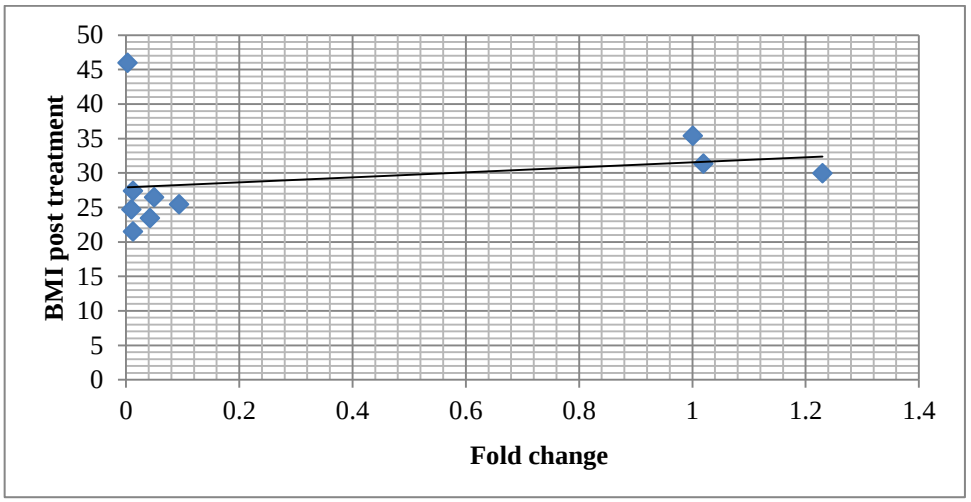

Figure (1). Scatter plot for the bivariate correlation between fold change and BMI post treatment at group I.

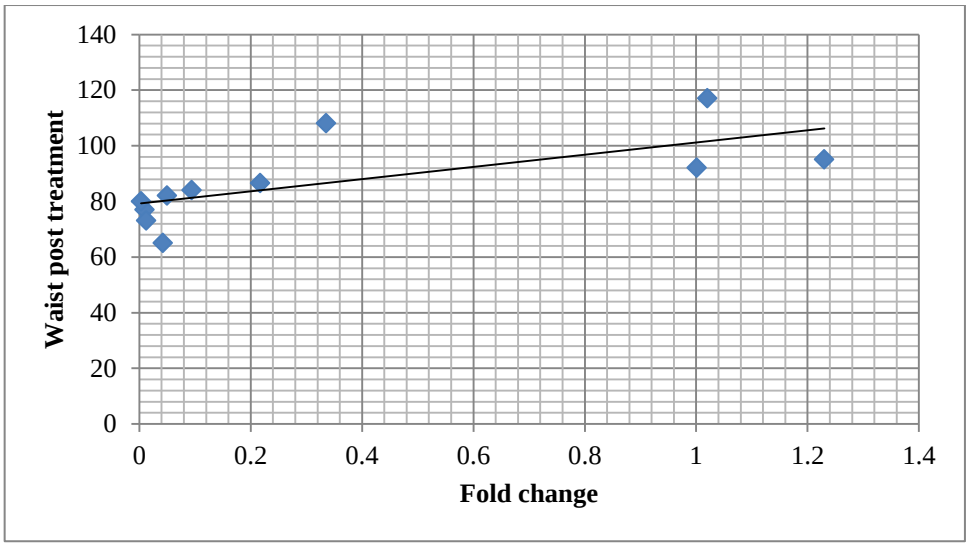

Figure (2). Scatter plot for the bivariate correlation between fold change and waist post treatment at group I.

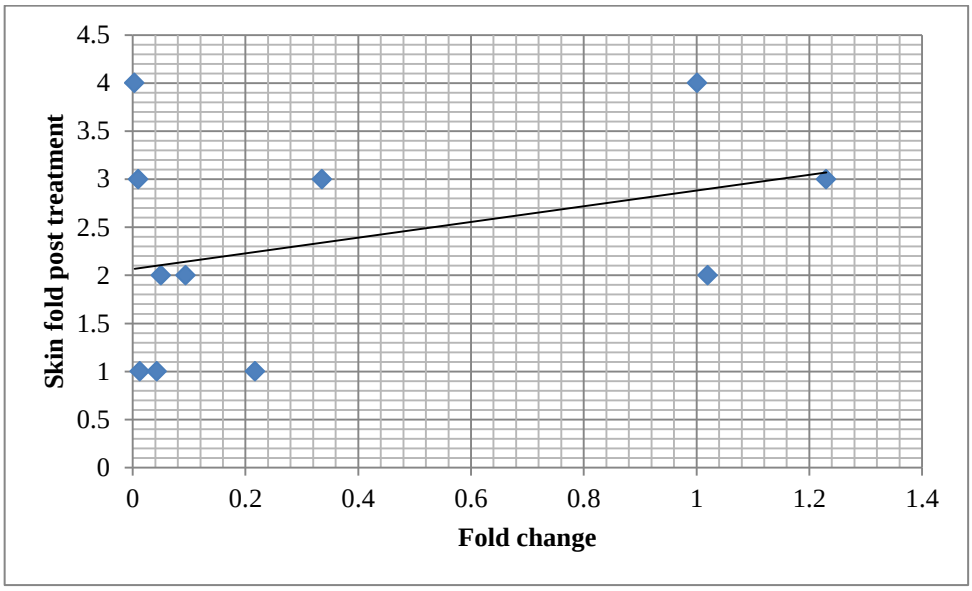

Figure (3). Scatter plot for the bivariate correlation between fold change and skin fold post treatment at group I.

7-Correlation among fold change and post treatment of BMI, waist circumference, and skin fold at group II:

As at figure (4 to 6) the correlations among fold change and post treatment of BMI, waist circumference, and skin fold at group II were studied through the Pearson product moment correlation coefficient. It revealed that there was no significant correlation between fold change and BMI (r= -0.099, p= 0.801). As well as, there was no significant correlation between fold change and waist circumference (r= 0.001, p= 0.998). Additionally, there were no significant correlation between fold change and skin fold (r=0.042, p= 0.915).

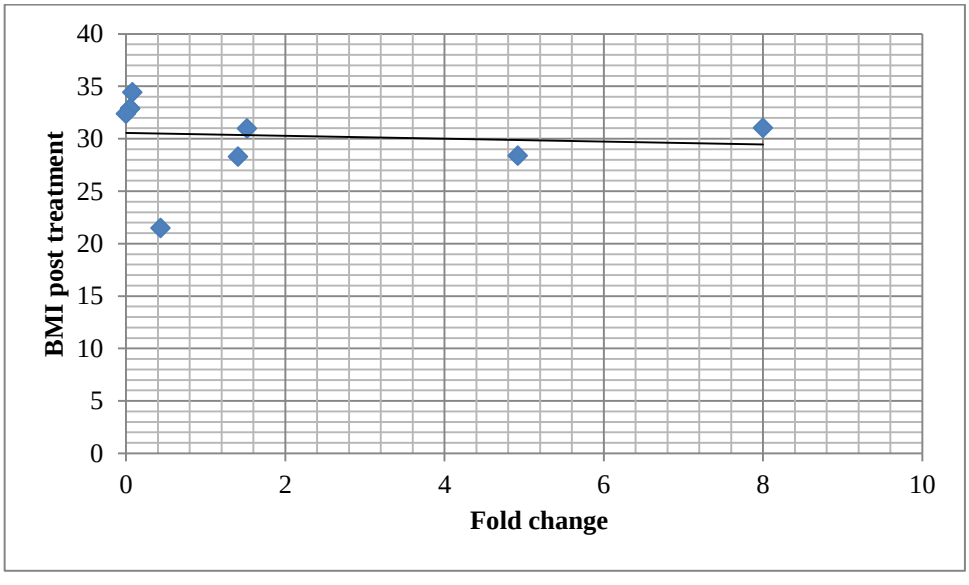

Figure (4). Scatter plot for the bivariate correlation between fold change and weight post treatment at group II.

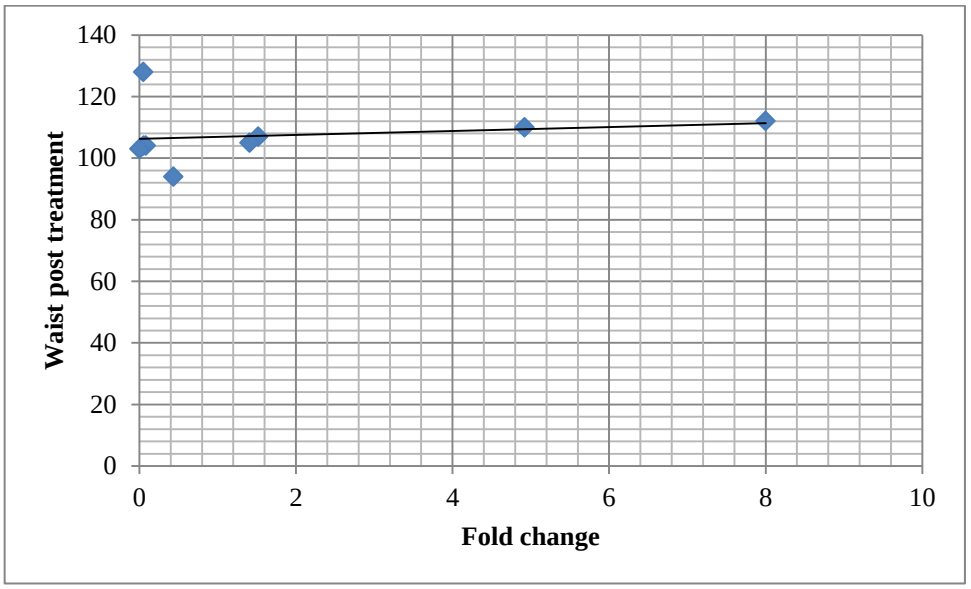

Figure (5). Scatter plot for the bivariate correlation between fold change and waist post treatment at group II.

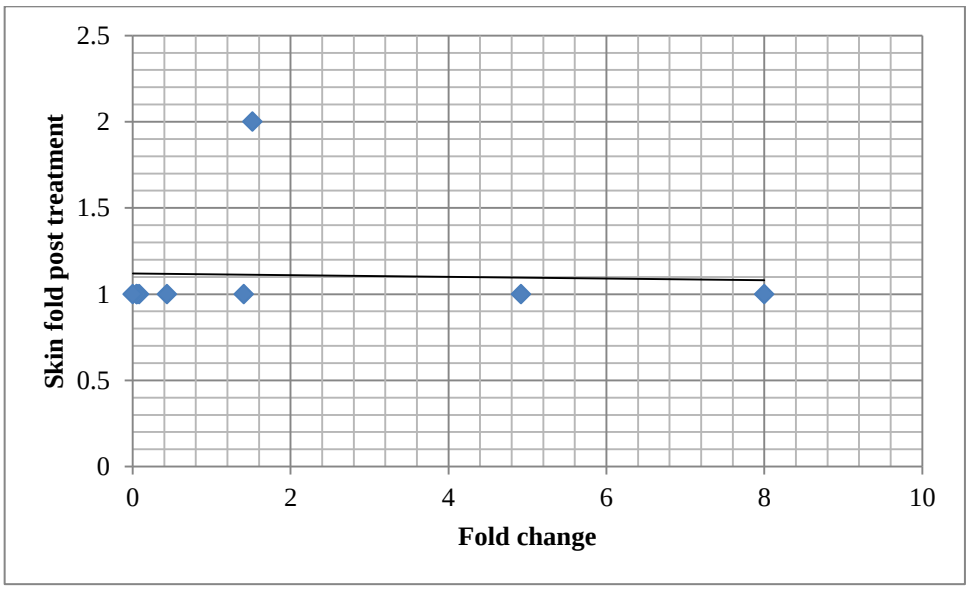

Figure (6). Scatter plot for the bivariate correlation between fold change and skin fold post treatment at group II.

Discussion:

The results were explained as following:

Role of LASER in controlling of obesity:

Studies on low level laser therapy (LLLT) indicate "Liquefaction" or release of stored fat in adiposities by opening of the cell membrane after a short treatment. Fat cells that were not exposed to the laser treatment looked like round grapes. Eighty percent of the fat was released from the fat cells after 4 min of laser light exposure and 99 % was released after 6 min of exposure. After exposure to the laser light, pores in fat cells were visible by scanning electron microscope. It was presumed, but not demonstrated, that the fat was released from these pores, taken up in the lymphatic and reesterified in other tissues or metabolized for energy [17].

Decrease in body mass index and abdominal fat by training on a cycle ergometer for 3 days/week for 6 weeks because there was decrease in total amount of stored calories. This decrease in energy stores is obviously

the results of a negative energy balance so that exercise produces decrease in energy intake leading to a reduction in weight [18].

The biochemical mechanism of action of LLLT appears to increase adenosine monophosphate (cAMP) production via cytochrome C oxidase activation, which causes the breakdown of cell lipids in adipocytes and formation of transitory pores in their cell membrane with subsequent cell collapse. Therefore, LLLT appears to provide a safe and effective alternative for the reduction of subcutaneous tissue volume [19].

LAPEX 2000 Lipolaser gives a significant waist girth loss that is sustained over repeated treatments and is cumulative over 4 weeks of eight treatments. This waist girth loss was almost 1 inch (2.54 cm) in magnitude. Therefore, the LAPEX 2000 Lipolaser gave a clinically meaningful, a cosmetically datable, and a statistically significant improvement in appearance. The fat loss was probably a consequence of laser creating temporary pores in fat cells through which triglycerideLAPEX 2000 Lipolaseres were leaked, a process that requires serum, but is not complement-mediated [20].

Role of exercises therapy in controlling of obesity:

According to [21] Increased lipoprotein lipase activities lower LDL and chylomicron triglyceride levels and enhance clearance of cholesterol-rich LDL and chylomicron remnant. LDL, and triglycerides are exchanged for cholesterol ester in LDL, and HDL, a process mediated by cholestryl ester transfer protein, and the triglycerides in HDL, and LDL is then hydrolyzed by lipases, causing a decrease in the size of the particles. Exercise and weight loss also reduce the level of cholestryl ester transfer protein perhaps because a fraction of this protein is made in adipose tissue **.**

On the other hand, [22] showed a decrease in body mass index and abdominal fat by training on a cycle ergometer for 3 days/week for 6 weeks because there was decrease in total amount of stored calories. This decrease in energy stores is obviously the results of a negative energy balance so that exercise produces decrease in energy intake leading to a reduction in weight.

Studies have demonstrated that cycling can be an effective form of exercise for weight loss and weight control. Also, improvements were seen in cardiovascular function with a reduction in total body weight and percentage of body fat [23].

Moreover, [24] found that exercise on obese women causes a long term weight loss and decrease body mass index. They revealed that this loss, due to regular physical activity that enhance fat oxidation and partially prevent the age related increase in central body fatness.

Modifying Sirtun 1 Gene expression :

According to [25] Mechanisms that underlie the control of gene expression are becoming increasingly well understood. Every conceivable step in the process is subject to dynamic regulation in the cell. This includes structural changes in the chromatin to make a particular gene accessible for transcription, transcription of DNA into RNA, splicing of RNA into mRNA, editing and other covalent modifications of the mRNA, translation of mRNA into protein, and, finally, post-translational modifiion of the protein into its mature, functional form.

On the other hand it was reported that Mutations in the obese (ob) gene lead to obesity. This gene has been recently cloned, but the factors regulating its expression have not been elucidated. To address the regulation of the ob gene with regard to body weight and nutritional factors, Northern blot analysis was used to assess ob mRNA in adipose tissue from mice [lean, obese due to diet, or genetically (yellow agouti) obese] under different nutritional conditions. ob mRNA was elevated in both forms of obesity, compared to lean controls, correlated with elevations in plasma insulin and body weight, but not plasma glucose [26]**.**

Farther more SIRT1 has been shown to repress PPARγ, by docking to the negative cofactors of the nuclear receptor, and thereby down regulating genes such as the mouse aP2 gene. Thus, when mice are starved, SIRT1 is induced to bind the aP2 promoter in WAT, repress gene expression, and promote mobilization of fat into the blood. Moreover, in differentiated adipose cells, upregulation of SIRT1 leads to decreased fat storage and increased lipolysis [27].

Consistent with these findings, treatment of mice on a high fat diet with resveratrol was shown to reduce weight gain. These findings demonstrate that SIRT1 acts in concert with lipid regulating transcription factors to adapt gene transcription to changes in nutrient levels [28,29].

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