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Role of obesity and pro-inflammatory leptin in autoimmune thyroiditis independent on hypothyroidism

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ABSTRACT

Relation between obesity and thyroid autoimmunity, with the adipocyte hormone leptin, appearing to be the key factor linking these two conditions. The present study aims to investigate the role of pro-inflammatory leptin on autoimmune disease independent on hypothyroidism. This study carried out on forty women (20 control and 20 obese) participated as volunteers with mean age 43 year. The obese women followed a well-balanced regimen and reassessed after 2 months of weight loss program. Clinical examination, blood pressure, anthropometric measurements and basal metabolic rate (BMR) were reported. Serum levels of glucose, insulin, total cholesterol, triglycerides, high density lipoprotein (HDL-C), thyroid hormones (TSH, Ft3, and Ft4), anti-thyroid peroxidase antibodies (Anti-TPO), leptin and hs-C reactive protein (hs-CRP) were measured. Low and very low density lipoprotein (LDL, VLDL), risk factor (total cholesterol/HDL-C) as well as homeostasis model assessment of insulin resistance (HOMA-IR) were calculated. Thyroid stimulating hormone (TSH), Anti-TPO, Leptin, and hs-CRP levels were significantly higher in obese than control women. TSH showed a significant positive correlation with Body Mass Index (BMI), leptin and Anti-TPO in obese group. Fasting blood sugar (FBS), lipid profile, TSH, Anti-TPO, leptin and hs-CRP were improved and showed significant reduction after weight loss. The data of this study revealed that obesity has a probable impact on autoimmune thyroiditis through the role of adipocyte pro-inflammatory leptin, indicating a high risk for obese persons to develop thyroid dysfunction. Healthy effect of weight reduction was confirmed.

Keywords: Obesity, Leptin, autoimmune thyroiditis**,** hypothyroidism

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INTRODUCTION

Autoimmune chronic thyroiditis (AT) is the most widespread thyroiditis form and it is amongst the most common thyroid diseases, it's affecting up to 2% of the population [1]. Its incidence is definitely more frequent in the elderly and in female gender [2] Up to 10-20 folds higher in women than in men [3]. It is characterized by a chronic lymphocytes infiltration of thyroid and presence of circulating auto antibodies as anti-peroxidase (AbTPO) and anti-thyroglobulin (AbTg). The inflammatory process leads to a follicular destruction [4].

Obesity has more than doubled worldwide since 1980. In 2014, more than 1.9 billion adults, 39% of adults aged 18 years and over were overweight and 13% were obese. At least 2.8 million people each year die as a result of being overweight or obese **[**5].

Leptin, a peptide hormone, has been shown as one of the most important hormones secreted by adipose tissue. Also, it plays a regulatory role in inflammation and immunity status, as can affect thymic homeostasis, and has an impotent role in the regulation of immune responses [6]. Even though, a regulatory effect of leptin on immune cells, its expression and release is reciprocally under the control of different inflammatory stimuli. Leptin affects the

neuroendocrine system at several levels, including the hypothalamic-pituitary-adrenal, thyroid, gonadal, and growth hormone axes [7, 8].

Individuals with high BMI and percentage total body fat have an elevation in leptin level [9, 10]. Several studies (*in vitro* and *in vivo*) documented that leptin has a vital role in the path physiology of autoimmune diseases, infections and endocrine/metabolic diseases [11-14].The incidence of autoimmune thyroid disease in obesity reported to be 12.4% in children and between 10 and 60% in adults [15,16]. Regarding the probable relationship between obesity, thyroid function and autoimmune thyroiditis, Rotondi *et al.,* [15] concluded that a higher rate of subclinical hypothyroidism was in patients with morbid obesity.

Although Knudsen *et al*., [17] showed an association between thyroid stimulating hormone (TSH) levels and body mass index (BMI) independent of hypothyroidism. Little is known about the impact of obesity on autoimmune thyroid disease risk, despite several evidences linking obesity to thyroid hormones

Our objective in this study was to spot light on the effect of obesity on autoimmune thyroiditis through the role of pro-inflammatory leptin.

MATERIALS AND METHODS

Patients
The study was conducted in the Department of nutrition and food science at National Research Center, Cairo, Egypt.

Ethical approval
The protocol of the study was approved by the National Research Center Ethics Committee (registration no.14-090). In addition, an informed consent was obtained from each volunteer. They were well informed in advance about the purpose of the study.

The present study was carried out on forty women (29-51 years), participated as volunteers.

Each woman was subjected to full history with particular emphasis on age, family history and any systemic diseases like diabetes, hypertension and dyslipidemia.

Included criteria
Obese women have BMI ≥30 kg/m^{2}and control healthy women have BMI < 25 kg/m^2 according to WHO criteria 2010

Excluded criteria:
Subjects were excluded for any of the following reasons:
1. History and /or thyroid diseases
2. History and / or being on medication that may affect metabolic profile and thyroid function
3. No women were prescribed hypo caloric diets or therapies for weight control for at least 3 months before examination.

Study Design:
Consisted of three groups; Group *I* (Control group) this group comprised 20 non obese apparently healthy women with BMI <25 kg/m^2; Group *II* (*Obese group*) comprised 20 obese women had a BMI ≥30 kg/m^2 and *Group III* (*After regimen group*) comprised of the 20 obese women reassessed after 2 months of weight loss program. They consumed a balanced (60% carbohydrate, 20% protein, 20% fat) hypo caloric regimen (900-1000 calorie/day); and also followed a program based on physical exercise.

Methods
1-Anthropometric and blood pressure measurements
Weight (kg), height (m) and mid waist circumference (MWC) were measured using standard methods [18]. Body mass index (BMI) and waist hip ratio (W/R) were calculated, as weight divided by squared height (kg/m^2) and mid waist circumference divided by hip circumference, respectively. Percent body fat (%BF) and basal metabolic rate were measured using *Geratherm Body Fitness (B-5010), Germany.*

Systolic and diastolic blood pressure (SBP and DBP) were measured three times by a sphygmomanometer (Mercurial pressure measuring device**) [19]** while the subjects were seated, and the two last measurements were averaged.

2-Blood sampling and Biochemical analysis

Overnight fasting blood samples were collected in plain tubes. Non hemolysed sera were prepared by centrifuging blood samples at 4000 rpm for 5 min. Serum samples were aliquoted and stored at -70^{o}c until analysis, except for glucose which was determined on the same day without delay.

Serum glucose was determined by colorimetric method according to Trinder et al (1969) [20]. Serum total cholesterol and triglycerides were determined by colorimetric methods of Allian *et al.* (1974) [21], and Wahlefeld, (1974*)* [22], respectively, while high density lipoprotein-cholesterol (HDL-C) was determined by enzymatic colorimetric assay method of Finley et al., (1978) [23]. Glucose and all lipid profiles were determined using a commercial assay kit (Stanbio, USA). The levels of serum LDL-C and VLDL-C were calculated according to Freidewald formula (1972) [24]

$$\frac{\text{Serum Triglycerides}}{5}$$

Thyroid hormones, antibody and inflammatory marker were measured by commercial ELISA kit. Thyroid hormones (TSH, Ft_3, Ft_4) and Anti-TPO were measured according to Hopton and Harrap (1986) [25] (MonobindInc.,USA), Wild (1994) [26] (MonobindInc., USA), Midgley (2001) [27] (MonobindInc.,USA) and Schatz and Lobig (1989) [28] (MonobindInc.,USA), respectively.

Serum insulin, leptin and High sensitive-C reactive protein levels were measured by commercial ELISA kits according toYallow and Bawman, (1983) [29] (BioSource, USA), Imagawa *et al.*, (2001) [30] (DIA source Immuno Assays, Belgium) and Kimberly et al (2003) [31] (MonobindInc., USA), respectively.

Homeostasis model assessment (HOMA) was calculated according to Matthews et al. (1985) [32].

HOMA -IR= (Fasting blood glucose (mmol/l) × fasting insulin (μIU/ml)/ 22.5).

Statistical analysis:

The collected data was statisically analyzed using SPSS, (version16.0 software of Microsoft windows). Results were expressed as Mean±SD. Comparison of different variables in various groups was done using one way analyses of variance (ANOVA) The difference among groups means were tested using the least significant differece (LSD) for parametric variables. Correlationbetween the different parameters was tested by Pearson correlation test. For all tests a probability (p) less than 0.05 was considered significant (Dawson and Trapp, 2001) [33].

RESULTS

Data in table (1) revealed significant elevation ($p<0.05$) in body weight, BMI, MWC, hip circumferences, WHR and % BF in obese women with percent differences reached to 51.73%, 48.53%, 34.96%, 24.74%, 8.35% and 76.03%, respectively, compared to control group. After two months on regimen, women exhibited a significant reduction in their weights, MWC and hip circumferences and WHR reached to 4.91%, 8.63%, 5.52% and 4.80%, respectively, compared to their previous baseline data. Moreover, they represented a significant improvement in some parameters which nearly approach the control values, in WHR and BMR, with percent differences 103.15% and 111.73%, respectively.

Furthermore, basal metabolic rate (BMR) clarified a significant elevation in obese women by 69.19% than control group. After regimen, non significant reduction (3.13%) was observed as compared to their baseline.

Systolic blood pressure was significantly increased ($p<0.05$) by 10.75 % in obese, while diastolic blood pressure was unchanged, compared to control women. After two months of well balanced regimen, women presented no significant improvement in systolic blood pressure as presented in table (1).

Table (1): Statistical significance of anthropometric parameters, body fat (%), basal metabolic rate (BMR) and blood pressure in obese women before and after dietary intervention, compared to control group

Parameters	Groups		
	Control	Obese	
		Baseline	After regimen
Age (years) Mean± SD Range	43.65±3.29 (30-51)	42.85±6.9 (29-51)	42.58±6.9 (29.51)
Weight (kg) Mean± SD Range	60.21±6.27 (43.8-70.90)	91.36±9.76[a] (65.70-124.00)	86.37±11.37[ab] (63.4-123.0)
Mid Waist (cm) Mean± SD Range	70.45±5.49 (60.0-82.0)	95.08±8.85[a] (79.0-115.0)	86.87±7.34[ab] (63.40-123.8)
Hip (cm) Mean± SD Range	96.45±5.49 (87.0-105.0)	120.32±9.81[a] (100.5-137.0)	113.67±10.11[ab] (93.0-132.0)
WHR(cm/cm) Mean± SD Range	0.730±0.037 (0.7-0.8)	0.791±0.040[a] (0.7-0.9)	0.753±0.045[b] (0.7-0.9)
BMI (kg/m^2) Mean± SD Range	24.86±2.30 (19.0-29.3)	36.91±4.39[a] (27.8-47.8)	35.76±4.21[a] (27.8-47.8)
Body Fat (%) Mean± SD Range	31.37±4.96 (21.0-41.0)	55.22±10.34[a] (35.0-60.0)	44.15±5.33[b] (26.0-50.0)
BMR (Kcal/day) Mean± SD Range	1856.56±501.20 (1268-2853)	2141.20±322.39[a] (1599-2623)	2074.40±307.56[b] (1238-2883)
Systolic BP(mm/Hg) Mean± SD Range	109.25±19.53 (90-160)	121.0±14.74[a] (90-150)	116.0±10.83 (90-130)
Diastolic BP(mm/Hg) Mean± SD Range	69.75±5.34 (60-90)	71.25±9.90 (50-100)	72.50±5.30 (60-90)

a:Significance vs. control group at (p< 0.05).
b: Significance vs. obese (baseline)group at (p< 0.05).

Table (2): Statistical significance of serum lipid profiles and risk factors in obese women before and after dietary intervention, compared to control group

Parameters	Groups		
	Control	Obese	
		Baseline	After regimen
Total Cholesterol (mg/dl) Mean± SD Range	136.32±15.31 (114-173.1)	175.23±28.08[a] (128.4-230.6)	167.80±24.14[a] (121.9-206.3)
Triglyceride (mg/dl) Mean± SD Range	100.53±13.41 (80.0-125.8)	146.54±25.84[a] (100.8-173.3)	125.37±10.92[ab] (114.2-144.0)
HDL-C (mg/dl) Mean± SD Range	84.57±10.04 (56.7-85.0)	68.84±14.80[a] (47.2-70.2)	68.38±7.20[a] (48.2-72.2)
LDL-C (mg/dl) Mean± SD Range	37.34±8.21 (22.6-60.1)	92.09±16.20[a] (61.8-129.1)	83.77±24.46[a] (26.7-107.9)
VLDL-C(mg/dl) Mean± SD Range	8.02±1.64 (4.53-12.02)	18.74±3.24[a] (12.37-25.83)	16.75±4.89[a] (5.36-21.58)
TC/HDL Mean± SD Range	1.71±0.25 (1.07-2.23)	2.88±0.37[a] (1.98-4.88)	2.50±0.43[ab] (1.65-3.60)

a: Significant difference vs. control group at (p<0.05).
b: Significant difference vs. obese (baseline) group at (p<0.05).

Table (2) illustrates that obesity has a deterioration effect on lipid profiles, when obese group compared to control group, serum levels of total cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-C), and very low density lipoprotein-cholesterol (VLDL-C) were significantly higher by 28.17%, 45.76%, 146.62% and 146.78%respectively. Also, significant low values of HDL-C, which reached 25.62%, were observed in obese as compared to control group. Lipid profile was improved in women followed the well balanced regimen as indicated

by the significant reductions in triglycerides and total cholesterol/HDL-C, which reached 14.44%, 13.19% respectively of obese group.

Glucose and insulin showed no significant differences between control and obese women before and after intervention. On the contrary, HOMA-IR showed significant reduction by 8.57% after obese women followed two months of weight loss program, compared to their base line values, as shown in table (3).

Table (3): Statistical significance of glucose, insulin and homeostasis model of insulin resistance (HOMA-IR) in obese women before and after dietary intervention, compared to control group

Parameters	Groups		
	Control	Obese	
		Baseline	After regimen
Glucose(mg/dl) Mean± SD Range	93.05±8.47 (77.7-107.7)	97.84±9.38 (84.0-107.1)	100.37±10.46 (86.0-107.4)
Insulin(μIU/ml) Mean± SD Range	1.23±0.352 (0.7-2.5)	1.49±0.63 (1.1-3.6)	1.42±0.69 (0.4-3.2)
HOMA-IR Mean± SD Range	0.31±0.02 (0.04-0.96)	0.38±0.022[a] (0.08-0.94)	0.35±0.03[ab] (0.05-0.83)

a: Significance vs. control group at (p<0.05).
b: significance vs. obese (baseline) group at (p<0.05).

Comparing to control group, obese women showed a significant change in the thyroid hormones (TSH, FT3) levels, where, TSH value was elevated by 179.85% while value of FT3 decreased by 16.09%. FT4 showed no significant difference between the two groups. After two months, TSH and FT4 levels showed a reduction by 35.05% and 15.09% respectively, while FT3 increased by 12.37 as compared to their base line values.

Anti-TPO and pro-inflammatory leptin values showed significant difference in obese women, presented as higher levels with percent difference reached to 12193.30%, 312.94%, respectively, compared to control women. The recommended regimen reduced these values significantly by1695.55% and 96.87% respectively as compared to control group.

In addition, the results illustrated that weight loss of obese women ameliorated the high significant level of hs-CRP which was higher by 654.45% compared to control women to 54.57% when compared to their previous values as clarified in table (4).

Table (4): Statistical significance of serum thyroid hormones, Anti-Thyroid peroxidase antibodies (Anti-TPO), pro-inflammatory leptin and high sensitive C-reactive protein (hg-CRP) in obese women before and after dietary intervention, compared to control group

Parameters	Groups		
	Control	Obese	
		Baseline	After 2 months
FT3 (ng/dl) Mean± SD Range	3.11±0.81 (1.5-3.5)	2.67±0.51[a] (1.5-4.0)	3.01±0.64 (1.5-4.0)
FT4 (pg/ml) Mean± SD Range	1.03±0.24 (0.4-1.4)	1.06±0.13 (0.8-1.4)	0.90±0.21[b] (0.5-1.2)
TSH (μIU/ml) Mean± SD Range	0.61±0.118 (0.4-1.8)	1.94±0.70[a] (0.7-7.1)	1.26±0.52[b] (0.3-3)
Anti-TPO (IU/ml) Mean± SD Range	0.225±0.02 (0.1-0.6)	27.66±9.95[a] (10.0-145.8)	4.04±1.0[b] (0.1-26.1)
Leptin (ng/ml) Mean± SD Range	2.24±0.42 (1.4-3.4)	9.25±2.43[a] (1.9-13.5)	4.41-0.83[ab] (2.1-10.4)
hs-CRP (μg/ml) Mean± SD Range	1.85±0.63 (0.4-4.1)	13.98±0.90[a] (3.4-29.1)	6.35±1.31[ab] (1.3-11.0)

a: Significance vs. control group at (p<0.05).
b: Significance vs. obese (baseline) group at (p<0.05).

Positive correlations were observed between serum TSH and BMI, leptin and Anti-TPO (r = 0.640, 0.421 and 0.568, respectively) as observed in figures (1-3). In addition, leptin was positively correlated with Anti-TPO (r =0.451) as illustrated in figure 4.

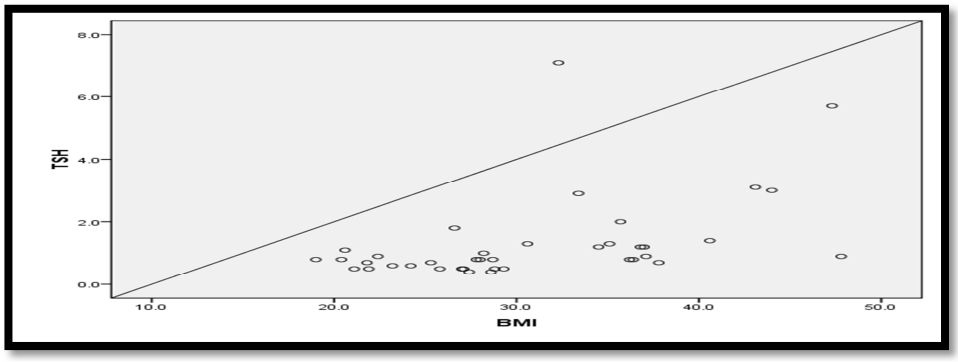

Fig. (1): Correlation between serum level of TSH (мIU/ml) and BMI (kg/m^2) in obese group

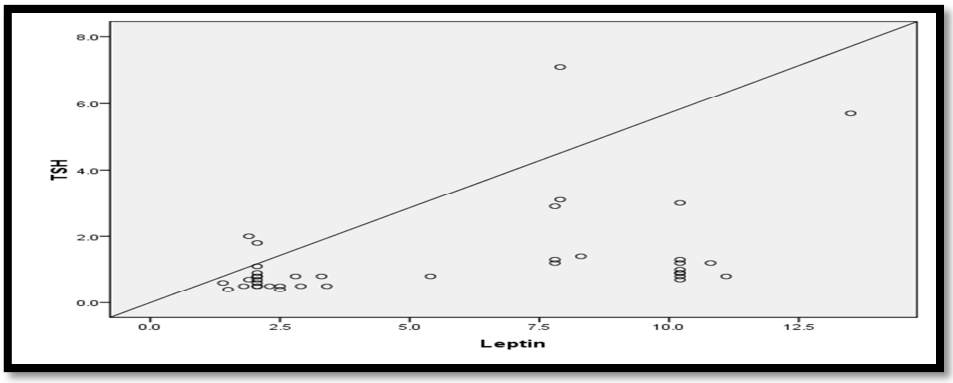

Fig. (2): Correlation between serum level of TSH (мIU/ml) and Leptin (ng/ml) in obese group

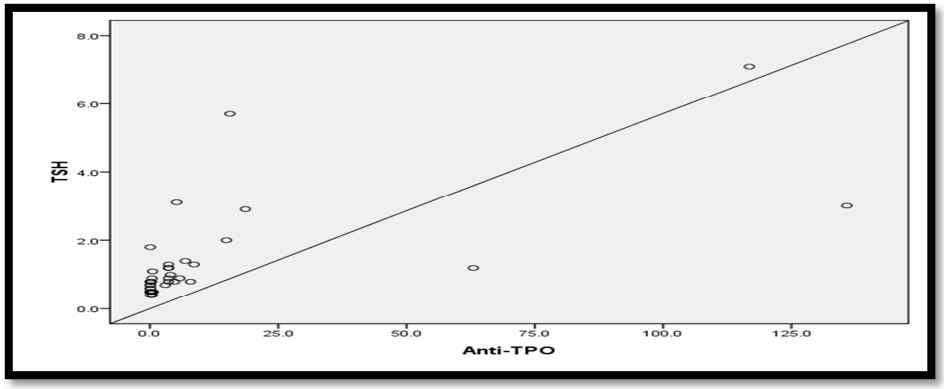

Fig. (3): Correlation between serum level of TSH (мIU/ml) and Anti-TPO(IU/ml) in obese group

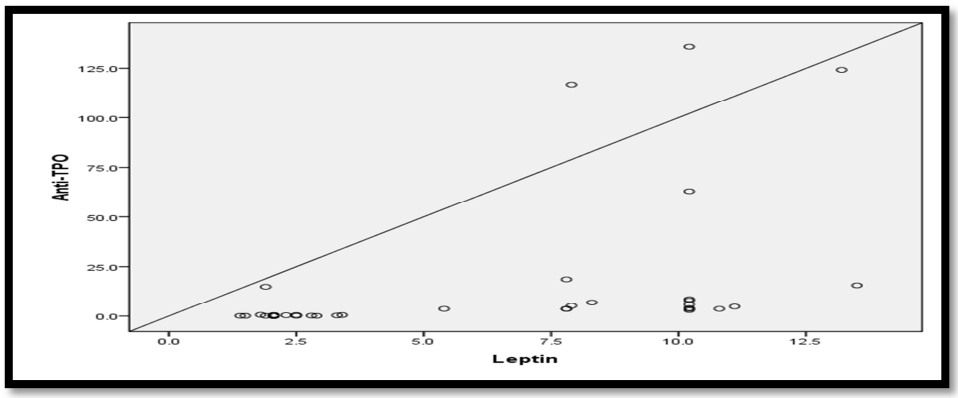

Fig. (4): Correlation between serum level of Anti-TPO (IU/ml) and Leptin (ng/ml) in obese group

DISCUSSION

Relation between obesity and autoimmune thyroiditis become more interesting topic recently. Where, the prevalence of obesity is in increasing worldwide. However, there are conflicting data in the literature regarding the relationship between obesity and thyroid hormones.

The present data revealed that obese women had significantly higher values of mid waist circumference (MWC), WHR, BMI and % of body fat, as well as metabolic syndrome as illustrated by the dyslipidemia, abdominal adiposity and insulin resistance. These results are in line with those of Mohamed *et al.*, [34] and Yoon *et al.*,[35] who stated that metabolic syndrome (MetS) was positively associated with body weight, waist circumference, blood pressure, blood glucose levels, TC, TG and negatively associated with the HDL-cholesterol level and its incidence is increased in obese subjects. Martins *et al.*, [36], Lichnovská *et al.*, [37] and Garg *et al.*, [38] reported that subjects with MetS had more insulin resistance (HOMA-IR) and less insulin secretion (HOMA-β) than healthy controls.

On the other hand, Karakurt *et al.*, [39] found that obesity was associated with high TSH, fasting insulin levels and HOMA-IR independent of serum T3 and T4 levels. Christ-Crain and colleagues [40] reported significant elevation in the levels of CRP in patients with subclinical hypothyroidism (SCH); however, several recent studies have reported that increased CRP levels were not associated with SCH. [41, 42]

The present study showed that obese women had a significant increase in TSH and decrease in FT_3 with no change in FT_4 comparing to control women. These results are in accordance with those reported by Farhangi *et al.***,** [43] and Bhowmick *et al.*, [44]. It seems that this elevation in serum TSH concentration in obesity is not associated with alterations in serum T4 concentration and, according to previous reports; serum T4 concentration is independent of the body weight status [45]. Nannipieri *et al*., [46] stated that, in spite of the higher plasma TSH levels, TSH receptors are less expressed on adipocytes of obese opposed to lean. This reduction may induce down-regulation of thyroid hormone receptors and thyroid hormone action, which further increasing plasma TSH and FT3 concentrations and constituting a condition of peripheral thyroid hormone resistance. Our results again confirm this concept that, obesity is a causal pathway between thyroid function and metabolic syndrome. In this line the present study revealed a significant positive correlation between TSH with BMI, this finding is in accordance with other studies Siemińska *et al.*, [47] and Nymes *et al.***,** [48].

Moreover, leptin, produced by adipocytes, regulates appetite and energy expenditure and influences thyrotropic axis and TSH secretion [49]. These effects of leptin reveal the endocrine role of adipose tissue in thyroid dysfunction. Consequently, the elevation of serum TSH level noted in obese women may be a result of fat accumulation.

Data of this study revealed that TSH levels in obese women hada significant elevation in the levels of CRP, pro-inflammatory leptin and Anti-TPO than control group.

Leptin stimulates proliferation of native T cells, and promotes T cells differentiation toward T-helper 1 (Th1), producing pro-inflammatory cytokines such as interferon gamma (IFNγ) and IL-2, and suppressing the production of the Th2 cytokines IL-4 and IL-10. Furthermore, it inhibits the proliferation of T-regulatory cells (Treg), known as critical mediators of immune tolerance [50]. Many studies have indicated that high leptin levels may be associated with autoimmunity [12, 14, 51] through its role in shifting the T helper balance toward a Th1 phenotype and suppress the function of T-regulatory (Treg) cells, which results in more TPO-Ab production [50, 52, 53].

In addition, high BMI increased the risk of TPO-Ab positivity but not Tg-Ab positivity this documented that the obese ones are more prone to become thyroid autoimmunity [16, 54, 55]. In this line, the present results clarified a significant positive correlation between TSH with leptin and leptin with Anti-TPO which make a probable association between obesity, leptin, autoimmunity and subclinical hypothyroidism. This observation is in harmony with Marzullo *et al*.**,**[16] who stated that the prevalence and characteristics of thyroid autoimmunity in obese men and premenopausal obese women showed that leptin increases susceptibility to autoimmune thyroid disease (AITD) by regulating immune processes, where, the prevalence of hypothyroidism was higher in obese patients than in a control group of age- and sex-matched subjects with normal BMI, as documented by lower FT3 and FT4 plasma levels and a deranged lipid profile.

The metabolic syndrome criteria observed in obese patients with elevation in TSH, leptin, Anti-TPO and hs-CRP were ameliorated and significantly reduced by weight loss. These events can be explained as a result of reduction in body weight which restores the size and function of mature adipocytes [46], this is in line with previous data which reported that abnormal thyroid function and TSH level are commonly normalized after weight loss whether consequent to diet or to bariatric surgery [56, 57]. The finding that TSH, Anti-TPO, hs-CRP and leptin levels are increased in obese subjects and weight loss lead to a significant reduction in serum levels supports the hypothesis that obesity has a probable impact on thyroid function where can be reversed by losing weight.

One of the limitations of the present study is the limited number of obese subjects. It is therefore crucial to substantiate these findings in larger obesity groups. The next one is the inclusion of only female rather than both sexes. However, from our point of view, what support this study and give it beneficial scientific value; it is the first study carried on obese euthyroid women and data obtained proof the association between obesity and autothyroiditis as well as the healthy effect of weight loss as confirmed by the reassessment of all the anthropometric parameter and biochemical indicators after weight reduction program.

CONCLUSION

This study revealed and documented that obesity and high level of the pro- inflammatory leptin were associated with elevated levels of TSH hormone and Anti-TPO. So obesity may contribute and predisposes to autoimmune thyroid disease. Weight reduction lead to improvement of most of these parameters and may ameliorate such a condition.

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