ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

OBSTATIN AND NUTRITION DISORDERS INEGYPTIAN OBESE WOMEN WITH AUTOIMMUNE THYROID DISEASES.

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Manuscript Info

Manuscript History:

Received: 19 January 2016
Final Accepted: 29 February 2016
Published Online: March 2016

Key words:
autoimmune thyroid disease, nutritional disorders, obstatin;DEXA**.**

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Abstract

Background:-Obesity is a major global health problem. Obesity and iron deficiency are two of the most common nutritional disorders worldwide.Iron deficienciescan be the cause of thyroid disorders. Obstatinis an important appetite and energyregulating peptide, secreted by the stomach. This gut peptide and thyroid hormones are involved in nutrition regulation. Few studies have been reported variations of gut hormones in thyroid dysfunction.We aimed to evaluate the possible relationships between serum obstatin, nutrition disorders, and thyroid dysfunction in thyroid peroxidase antibody positive obesewomen.

Methods:- case–control study included 150Egyptian patients with autoimmune thyroid diseasesand 50women as controls .Complete blood count, iron, total iron binding capacity, ferritin, thyroid stimulating hormone (TSH), free thyroid hormones (FT4 and FT3), anti-thyroglobulin (anti-TG) ,anti-thyroid peroxidase (anti-TPO) andobstatinwere measured .Fat mass (FM) and fat free mass (FFM) were evaluated by DEXA.

Results:-Serum obstatinwere statistically higher in hyperthyroidism patients than in euthyroid cases. However, the serum obstatinlevels were statistically lower in hypothyroidism patients than in those euthyroid. In autoimmune thyroiditis patients, serum obstatin level was positively correlated with hemoglobin, hematocrit, ferritin,serum iron, transferrin saturationratio (TSF), anti TPO, anti TG, TSH, FT3, and FT4. On the contrary, there were significant negative correlations between serum obstatin level and indices of obesity; mid arm circumferences (MAC).body mass index (BMI), FM, FFM and FFM.In autoimmune thyroiditis patients, linear regression analysis showed thatserumobstatin levels were independently correlated with hemoglobin,hematocrit and serum iron levels,

Conclusion:- In this study,serum obstatinwere higher in hyperthyroidism patients and lower in hypothyroidism patients than in euthyroid.Moreover obestatn, iron deficiency parameters (hemoglobin, hematocrit serum iron, ferritin, TFS and TIBC) and body composition parameters(FM, FMI, FFM and FFMI)associated with different types of thyroid dysfunction.Our results suggested thatthe gut peptide, obstatin can be a valuable diagnostic marker of nutrition disorders especiallyin obeseautoimmune thyroiditis patients.

Introduction:-

Hashimoto's thyroiditis is the most common cause of hypothyroidism in iodine-sufficient areas of the world. Hypothyroidismcan affect up to 10 percent of worldwide the population and its prevalence increases with age [1]. It is characterized clinically by gradual thyroid failure, with or without goiter formation, due to autoimmune-mediated destruction of the thyroid gland involving apoptosis of thyroid epithelial cells. Nearly all patients have high serum concentrations of antibodies against one or more thyroid antigens; diffuse lymphocytic infiltration of the thyroid, which includes predominantly thyroid-specific B and T cells; and follicular destruction, which is the characteristic hallmark of thyroiditis[2]

The cause of Hashimoto's thyroiditis is thought to be a combination of genetic susceptibility and environmental factors. The familial association with Graves' disease and the fact that Graves' disease may sometimes evolve into Hashimoto's thyroiditis indicate that the two disorders are closely related pathophysiologically, albeit not functionally [3].

Normal thyroid status is dependent on the presence of many trace elements e.g., iron, iodine, selenium, and zinc for both the synthesis and metabolism of thyroid hormones. Deficiencies of these elements can impair thyroid functions.Other nutrient deficiencies usually observed in patients suffering from ATD are: protein deficiencies, vitamin deficiencies (A, C, B6, B5, and B1) and mineral deficiencies (phosphorus, magnesium, potassium, sodium, chromium). [4]

Studies reported that obesity was associated with low concentrations of serum iron [5, 6, 7]. A report ofNational health and nutrition examination survey(NHANES) population revealed that the risk for iron deficiency, defined as low transferrin saturation and low serum ferritin, was increased to be twice as high in overweight adolescents compared to normal weight adolescents [8].The association between obesity and iron deficiency could be explained bydifferent factors such as genetic factors, physical inactivity leading to insufficient breakdown of myoglobin, reduction in iron levels which released into the blood stream, impaired intestinal iron absorption, inadequate dietary iron intake, and increased iron requirements [9,10]. Thus, low iron status in overweight individuals may be due to combination of nutritional and functional factors [11].

Thyroid hormones are involved in the regulation of body metabolism. Their effects include the stimulation of resting metabolic rate, increase in energy expenditure, modulation of responsiveness to catecholamines, and thermogenesis in adipose tissue [12]. Disturbances in thyroid function lead to changes in body weight, muscle mass and fat tissue. Thyroid-stimulating hormone (TSH) receptors have been found in the adipose tissues, indicating that they play a role in the regulation of the adipocytokines which are involved in the regulation of energy balance [13].

Ghrelin and obstatin are important appetiteand energyregulating peptides, secreted by the stomach. These gut peptides and thyroid hormones are involved in metabolism regulation. It is suggested thatobstatin does not have any activity by itself [14]. In our Egyptian population,nutritional disorder especially; iron deficiency, obesity, as well as thyroid disordersare very commonand they have not been fullyinvestigated up till now.To date, very few studies have been reported about gut hormones in thyroid dysfunction with controversialresults. Therefore, the purpose of current novel study is to investigate obstatin in patients with autoimmune thyroiditis. Moreover, we aimed to clarify the possible relationships of serum obstatin, body composition parameters; [fat mass (FM) and fat free mass (FFM),fat massindex (FMI), and fat free mass index (FFMI), iron deficiency parameters (hemoglobin, hematocrit serum iron, ferritin, transferrin saturation ratio (TFS) and total iron binding capacity(TIBC)], as well as thyroid antibodies; anti TPO and anti TG and thyroid function tests in Egyptian obese patients with different types of thyroid dysfunction .

Subjects and methods:-

Subjects:-

This study included 200 unrelated women. One hundred fifty women withautoimmune thyroiditisrecruited from diabetes and endocrinology outpatient clinic of Internal Medicine Department of Zagazig University Hospitals and 50 healthycontrols, were matched to cases by age, gender, and ethnic origin. Women were stratified into one of the following five groups based on the guidelines for the use of thyroid function tests. [15]. Euthyroid was defined as a normal thyroid function test [16]. Hyperthyroidism was defined as a TSH concentration < 0.40 μIU/ml with an elevated free thyroxine(FT4) and free triidothyronine(FT3) levels [17]. Subclinical hyperthyroidism(SCH) was defined as a TSH concentration < 0.40 μIU/ml with a normal FT4 and FT3 concentration. [18].on the other hand,hypothyroidism was defined as a thyroid stimulating hormone(TSH) concentration > 4.20 μIU/ml with an FT4 and FT3 concentration level below normal. [19]. SCH was defined as a TSH concentration of > 4.20 μIU/ml with a normal FT4 and FT3 concentration [20].Notably, the American thyroidassociation(ATA) recommends the combined use of TSH and FT4 as the most efficient combination of blood tests for diagnosis and follow-up of both ambulatory and hospitalized patients [21].

All patients were subjected to thorough history taking and full clinical assessment including blood pressure. Height, waist circumference (WC) and hip circumference (HC) were measured to calculate obesity indices. Anthropometric variables including Body mass index (BMI) was calculated as weight in kg/ height in (meters) ^{2},waist-to-hip ratio (WHR), waist circumference (cm)/hip circumference (cm), mid upper arm circumference (MAC) taken midway between the olecranon process of the ulna and the acromion process of the scapula in cm [22].

Womenwith history of stroke, respiratory disease, heart failure, cancer, severe hepatic, renal diseases, Iron therapy, acute illness, hormonal therapy, any active inflammatory diseases and abdominal surgery that could have an impact on abdominal fat distribution, as well as receiving medications that affect glucose metabolism or for weight reduction or participating in a dietary or exercise programs were excluded from the study. The ethical committee of Faculty of Medicine, Zagazig University approved our study protocol, and all participants assigned written informed consent.

Blood sampling:-

Blood samples were drawn from all subjects after an overnight fast and divided into 3 portions: 1 ml of whole blood was collected into evacuated tubes containing EDTA, forhemoglobin, hematocrit,HbA1c; 1 ml of whole blood was collected into evacuated tubes containing potassium oxalate and sodium fluoride (2:1) for fasting blood glucose. Sera were separated immediately from remaining part of the sample and stored at −20 °C until analysis.

Biochemical measurements:-

We determined fasting blood glucose using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol,HDL cholesterol, and triglycerides were measured by routine enzymatic methods (Spinreact, Girona, Spain). LDL cholesterol was calculated using the Friedewald formula [23] Fasting serum insulin concentrations was measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) The homeostasis model assessment of IR (HOMA-IR) index was calculated as follows: HOMA-IR = fasting insulin (mIU/l) × glucose (mg/dl)/405.Serum ironlevels were estimated by commercially available kits (Spinreact, Girona, Spain).Serum ferritin concentrations were measured using sandwich linked immunosorbent assay (ELISA) kit provided by (Biosource Europe S.A., Nivelles, Belgium).Serum TIBC by calorimetrically method using the commercially available kit (Biolabo,Maizy, France). Transferrin saturation percent (TFS %) was determined by dividing the serum iron concentration by the TIBC and multiplying by 100 [4].

Immunochemical measurements:-

The thyroid function tests includingFT3, FT4 , anti-thyroglobulin antibodies (anti-TG) ,anti-thyroid peroxidase antibodies (anti-TPO) , and TSH were measured using chemiluminescence immunoassay (CLIA) assay kit provided by (Immunospec Corporation, Canoga Park, CA, USA). The normal reference range for FT3 is 1.8–4.6 pg/ml, for FT4 is 1.0–1.8 ng/dl and for TSH is 0.3–4.2 lIU/ml according to Bemben et al. [24]and Bell et al. [25]. Cases with elevated TSH and low thyroid hormones levels were categorized as clinical hypothyroid (CHT) patients. While,those with high TSH and normal thyroid hormones concentrations were considered as subclinical hypothyroid (SCHT) patients. Similarly, low TSH with raised thyroid hormones and normal thyroid hormones levels was called clinical hyperthyroid and subclinical hyperthyroid, respectively [26].Additionally, the levels of antibodies were considered positive if they were >1 IU/ml for anti-TG and >50 IU/ml for anti TPO.

We estimated fasting serum insulin levels using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit provided by (DRG International, IRC, USA). The insulin resistance (IR) was calculated using the homeostatic model assessment-IR (HOMA-IR) index, which is defined as fasting plasma insulin value (μU/ml) × fasting plasma glucose value (mg/dl) / 405.

Finally, the serum obstatin level was measured using a quantitative sandwich ELISA method according to manufacturer's instructions (RayBio Human procalcitonin ELISA kit, Norcross, GA, USA). All standards, diluted samples and quality controls were incubated for 2 h at room temperature in microtitration wells pre-coated with monoclonal anti-human obstatinantibody. Biotin-labeled second monoclonal antihuman obstatin antibody was added and incubated with captured obstatin for 60 min. After washing twice with wash buffer; streptavidin–horseradish peroxidase (HRP) conjugate was addedincubated for 1 h at room temperature. After second washing step for five times, the remaining conjugate was allowed to read with substrate solution hydrogen peroxide and tetra-methylbenzidine (TMB). After 30 min incubation at room temperature in the dark, the reaction was stopped by the addition of acidic solution (0.2 M H2SO4), and absorbance of the resulting yellow product was measured spectrophotometrically at 450 nm. The absorbance was proportional to the concentration of obstatin. A standard curve was constructed by plotting absorbance value versus obstatin concentration of standards, and concentrations of unknown samples are determined using this standard curve.

Dual-energy X-ray absorptiometry (DEXA):-
The accurate and precise values of the body composition parameters were estimated from the DXA scan of the total body, which included; fatmass (FM), fat-free mass (FFM),additionally, the FM index (FMI; FM/height^2), FFM index (FFMI; [BMC+LM]/height^2), were calculated.

Statistical analysis:-
Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). Data were expressed using descriptive statistic (mean ± standard deviation) and were analyzed. One-way analysis of variance (ANOVA) test was done to compare different parameters between more than two groups. Pearson correlation coefficient was used to assess the association between obstatin, obesity indices,hematological, thyroid function tests,and other studied metabolic parameters in women with autoimmune thyroiditis. A linear regression analysis was performed to detect the main predictors of obstatin levels in autoimmune thyroiditis.P-values were considered significant if <0.05.

Results:-

Clinical, Anthropometric and biochemical characteristics of the studied groups are summarized in Table 1:-
Patient with anti TPO positive had significantly higher values of systolic and diastolic blood pressure, fasting blood glucose, HbA1c values, fasting serum insulin, HOMA-IR, triglycerides, total cholesterol, and LDLcholesterol as compared to controls. Moreover,hemoglobin, hematocrit, ferritin, serum iron, TSF and anthropometric indices (BMI, FM, FMI, FFMI, waist/hip ratio, MAC), were significantly higher in anti TPO positive patients than in healthy subjects. On the contrary, anti TPO positive patients had significantly lower levels of FFM, HDLcholesterol and TIBC compared with controls. Regarding thyroid function tests;FT3, FT4, and were significantly higher in anti TPO positivecases as compared to controls (P< 0.05). **(Fig. 1**respectively**)**

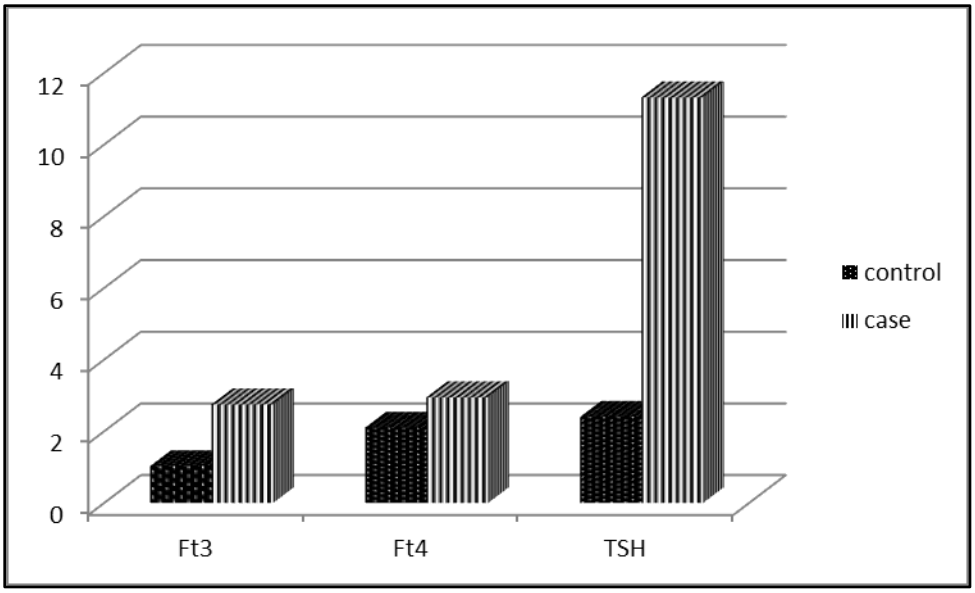

Fig1.serum FT3,FT4 and TSH levels in the studied groups.

Table 1:-Clinical ,Anthropometric and biochemical characteristics of the studied groups.

Parameter	Healthy controlsgroup (*n*=50)	Patient with anti TPO positive group (*n*=150)	*P* value
Age (years)	43.94±4.51	44.29±2.36	<0.001*
Systolic blood pressure (mmHg)	125.34±7.25	137.52±7.07	<0.001*
Diastolic blood pressure (mmHg)	85.66±4.5.	85.66±4.52	<0.001*
Waist/hip ratio	.983±0.089	1.14±0.126	<0.001*
Body mass index (kg/m^2)	21.90±1.98.	37.70±4.46	<0.001*
MAC(cm)	27.74±1.89.	2.384±0.275	<0.001*
FM(kg)	14.9±1.35.	41.02±4.85	<0.001*
FMI(Kg/m2)	5.40±0.42	61.54±7.28	<0.001*
FFM(kg)	44.69±4.05	13.53±1.266	<0.001*
FFMI(Kg/m2)	16.21±1.27	20.30±1.899	<0.001*
Total cholesterol (mg/dl)	168.6±19.6	202.16±13.49	<0.001*
HDL cholesterol (mg/dl)	51.56±6.8	36.77±6.06	<0.001*
LDL cholesterol (mg/dl)	66.3±27.0	110.5±42.4	<0.001*
Triglycerides (mg/dl)	201.4±5.8	259.64±34.52	<0.001*
Fasting blood glucose (mg/dl)	83.9±8.3	101.96±13.16	<0.001*
Fasting serum insulin (µU/ml)	13.0±3.30	48.61±23.7	<0.001*
HOMA-IR	2.69±0.68	12.85±7.66	<0.001*
HbA1C (%)	5.764±0.16	6.00±0.17	<0.001*
Hemoglobin (g/L)	12.5±1.004	7.613±1.45	<0.001*
Hematocrit (%)	42.08±3.31	28.45±3.85	<0.001*
Serum Iron level (µg/dl)	58.92±4.6	26.74±4.94	<0.001*
TIBC(µg/dl)	322.3±18.5	476.97±44.1	<0.001*
Serum ferritin (ng/ml)	18.02±3.06	10.79±1.81	<0.001*
TSF (%)	18.10±3.06	5.68±0.74	<0.001*

anti-thyroid peroxidase antibodies: anti-TPO; HOMA-IR: homeostasis model assessments of Insulin resistance; BMI, body mass index; MAC, mid arm circumferences; FM, fat mass; FMI, fat mass index; FFM, fat free mass; FFMI, fat free mass index; TIBC: total iron binding capacity; TFS :transferrin saturation.

* $P< 0.05$ when compared with control group.

Association of thyroid disorders and biochemical characteristics:-

Anti TPO positivepatientswere classified according to the thyroid state, There were statistically significant increases of systolic and diastolic blood pressure, fasting blood glucose, HbA1c values, fasting serum insulin, HOMA-IR, triglycerides, total cholesterol,LDLcholesterol, anti TPO, anti TG, TSH and in subclinical hypothyroidism (SCHT; n=60) and clinical hypothyroidism (CHT; n=28) as compared to euthyroidcases (n = 40)(P< 0.05).On the other hand, there were significantly lower values of HDLcholesterol, FT3 and FT4 (P< 0.05). Our results demonstrated statistically significant higher values of anti TPO, anti TG,total cholesterol and lower values of FT3 and FT4 in patients with subclinical hyperthyroidism (n=10)than in euthyroid patients. Alsoamonghyperthyroidism patients (n=12), there were statistically significant higher levels of anti TPO and anti TG, and significant lower levels of triglycerides,HDLcholesterol, fasting serum insulin, and HOMA-IR than ineuthyroidcases (P< 0.05) **(Table 2).**

Table2**:-** Anthropometric and biochemical characteristics in autoimmune thyroiditis patients according to their thyroid state.

Parameters	SCHT (*n*=60)	CHT (*n*=28)	Subclinical hyperthyroidism (*n*=10)	hyperthyroidism (*n*=12)	Euthyroid (n=40)
Systolic blood pressure (mmHg)	146.2±5.7*	136.8±6.1*	138.1±2.1	133.8±5.3	133.3±5.3
Diastolic blood pressure (mmHg)	88.1±4.7*	92.1±5.6*	86.8±4.3	85.8±2.9	88.2±3.3
Total cholesterol (mg/dl)	199.9±11.7*	208.4±11.8*	211±18.01*	193.5±25.2	201.4±9.3
Triglycerides (mg/dl)	266.9±24.4*	301.5±33.56*	232.6±8.8	209.6±9.9*	241.2±14.0
LDL cholesterol (mg/dl)	128.9±36.8*	148.5±4.9*	85.9±30.84	63.7±10.60	76.5±31.8
HDL cholesterol (mg/dl)	33.7±4.935*	33.78±2.15*	43±2.549	44.8±3.97*	39.5±6.12
Fasting blood glucose (mg/dl)	102.4±9.6*	121.1±9.0*	93.4±6.80	89±7.21	94±6.86
Fasting serum insulin (µU/ml)	13.5±54.2*	5.34 ±0.85*	23.39±3.39	18.67±3.93*	29.7 ±7.2
HOMA-IR	13.87±4.28*	25.42±2.55*	5.80±1.135	4.14±1.07*	6.91±1.85
HbA1C	6.03±0. 99*	6.18±0.122*	5.85±0.88	5.95±0.21	5.89±0.12
FT3(pg/ml)	2.81±0.52*	2.12±0.35*	2.44±0.33*	3.01±0.416	3.11±0.34
FT4(ng/dl)	3.01±0.52*	2.32±0.811**	2.64±0.733*	3.22±0.45	3.32±0.34
TSH (µIU/ml)(	8.62±1.56*	38.48±10.82	0.113±0.01	0.107±.01	2.5±0.97
Anti TPO(IU/ml)	77.32±3.58*	85.2±2.62*	53.1±2.522*	50.4±3.03*	58. ±3.9
AntiTG(IU/ml)	2.49 ±0.12*	2.75±0.46*	1.72±0.651*	1.63±0.58*	1.87±0.62
Obstatin(pg/ml)	98.2 ± 8.65	79.1±12.6*	98.7 ± 6.7	114.9 ± 13.9*	103.2 ±9.6

HOMA-IR: homeostasis model assessments of Insulin resistance ,BMI, body mass index; SCHT, subclinical hypothyroidism; CHT, clinical hypothyroidism; TSH ,thyroid stimulating hormone; FT3 free triidothyronine ,FT4;free thyroxine , Anti TG ;anti thyroglobulin antibodies, anti-TPO; anti-thyroid peroxidase antibodies

* *P*< 0.05 when compared with euthyroid group.

Obesity indicesin autoimmune thyroiditis patients according to the thyroid state:-

Hyperthyroid patients had significant lower values of WHR compared to euthyroidwomen. On the contrary, there was non-significant difference as regard WHR among other patients with thyroid disorder. Cases with clinical hypothyroidism had higher values of MACas compared to euthyroidsubjects (P< 0.05).Moreover, there were significantly differences as regard obesity indices; BMI, FM, FMI, FFM, and FFMI among women with thyroid disorders (P< 0.05)compared to euthyroidwomen. Higher values were observed in subclinical hypothyroidism and hypothyroidism. In contrast, subclinical hyperthyroidism and hyperthyroidism patients had significant lower values than euthyroidpatients (P< 0.05) **(Table 3).**

Table 3: Obesity indices inautoimmune thyroiditis patients according to the thyroid state

Parameters	SCHT (*n*=60)	hypothyroidism (*n*=28)	Subclinical hyperthyroidism (*n*=12)	hyperthyroidism (*n*=10)	Euthyroid (n=40)
Waist/hip ratio	1.146±0.11	1.16±0.180.	1.21±0.11	1.21±0.11*	1.10±0.108
BMI(Kg/m^2)	39.82±2.6*	42.57±1.72*	30.9±1.7*	32.0±1.58*	34 ±2.47
MAC(cm)	31.16±1.3	33.71±1.54*	32.6±3.44	31.6±2.70	31.01±0.67
FM(kg)	43.32±2.8*	46.32±1.87*	33.62±1.85*	34.82±1.72*	37.6±2.6
FMI(Kg/m2)	14.2±0.71*	14.15±0.71*	11.56±0.53*	11.9±0.47*	12.6±0.72
FFM(kg)	64.9±4.2*	69.48±2.81*	50.4±2.78*	52.2±2.58*	56.4±4.03
FFMI(Kg/m2)	21.2±1.07*	22.34±0.68*	17.4±0.79*	17.85±0.71*	19 ±1.08

BMI, body mass index, MAC, mid arm circumferences, FM, fat mass, FMI, fat mass index; FFM, fat free mass, FFMI, fat free mass index; SCHT, subclinical hypothyroid; HT, .hypothyroidism.
* *P*< 0.05 when compared with euthyroid group.

The comparison of iron and hematological profiles in autoimmune thyroiditis patients according to the thyroid state:-

Patients with subclinical hypothyroidism and hypothyroidism had significant higher level of TIBC than euthyroidpatients. On the contrary, they had significant lower values of hemoglobin, hematocrit, ferritin and serum iron as compared to euthyroidwomen (P< 0.05).Moreover, there were significantly lower levels of hemoglobin and hematocrit in women with subclinical hyperthyroidism (P< 0.05) compared to euthyroid women. While, There were no statistically significant different between hyperthyroid and euthyroid patients as regard hemoglobin, hematocrit, TIBC, ferritin and serum iron. Also, There were no statistically significant different between patient with autoimmune thyroiditis as respect to TFS (P> 0.05) **(Table 4).**

Table 4**:-**The comparison of iron and hematological profiles in autoimmune thyroiditis patients according to the thyroid state

Parameters	SCHT (*n*=60)	hypothyroidism (*n*=28)	Subclinical hyperthyroidism (*n*=12)	hyperthyroidism (*n*=10)	Euthyroid (n=40)
Hemoglobin (g/L)	7.73±1.33*	5.95±0.9*	8.63±1.58*	6.78±0.76	8.50±.88
Hematocrit(%)	27.22±3*	25.9 ± 0.96*	30.21±5.55*	29.7± 2.69	29.75±3.08
Serum Iron(µg/dl)	26.70±3.6*	19.78± 3.16 *	32.83±3.97	28.20± 1.92	29.50±2.76
TIBC(µg/dl)	507.1±39.6*	484.8± 18.2 *	438.3±26.3	461.5± 21.9	441.6±36.45
Serum ferritin (ng/ml)	10.68±1.44*	8.42±0.99*	13.13±1.58	11.28± 0.76	11.80±1.10
TSF (%)	5.84±.60	5.18±0.783	5.91±.89	4.75±.0.53	5.95±0.61

TIBC: total iron binding capacity; TFS: transferrin saturation.
* *P*< 0.05 when compared with euthyroid group.

Serum obstatin levels in studied patients:-

Our results revealed statistically significant lower serum obstatinlevels in autoimmune thyroiditis patients (97.83±13.95 pg/ml) compared to controls (115.11±6.61pg/ml) (P< 0.001)**(Figure 4)**. Moreover, the levels of serum obstatinwere statistically higher in hyperthyroidism patients than in those euthyroidsubjects (114.9±13.89pg/ml vs. 103.2±9.6, P< 0.001)**(Table 2)**.However, levels of obstatin were statistically lower in hypothyroidism patients than in those euthyroid (79.1±12.6 vs. 103.2±9.6pg/ml, P< 0.001)**(Table 2).**

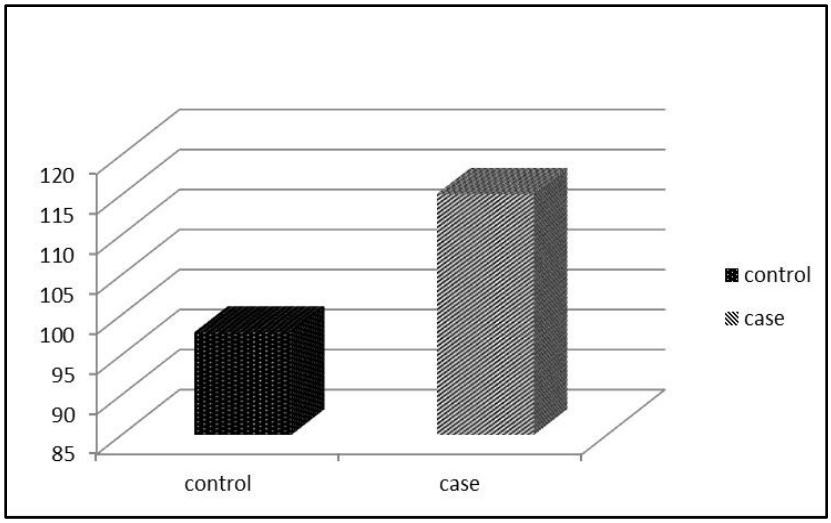

Fig 4. serum obstatin levels in studied groups.

Correlations between serum obstatin levels with various clinical, anthropometric, and biochemical parameters in autoimmune thyroiditis patients:-

In euthyroidpatients, serum obstatin level was positively correlated with hemoglobin, hematocrit, ferritin, serum iron, TSF, anti TPO, anti TG, TSH, FT3 and FT4. In total hypothyroid patient (n=44), serum obstatin level was positively correlated with hemoglobin, hematocrit, ferritin, serum iron, TSF, anti TPO, anti TG, TSH,FT3 and FT4 . On the contrary, there were significant negative correlations between serum obstatin levels and obsityindices; MAC BMI, FM, FMI, FFM and FFMI. In total hyperthyroidism (n=11), there were positive correlations between serum obstatin level andhemoglobin, hematocrit, ferritin, serum iron, TSF, anti TPO, anti TG, TSH, FT3 and FT4 **(Table 5).**

Table5.Pearson Correlations of serum obstatin levels (pg/ml) with anthropometric and biochemical characteristics inautoimmune thyroiditis patients

Characteristics	Euthyroidism (n=40)		Total Hypothyroidism (n=88)		Total hyperthyroidism (n=22)	
	r	*P*	r	*P*	r	P
Waist/hip ratio	0.168	0.480	0.045	0.771	0.124	0.717
BMI(Kg/m^2)	0.334	0.150	-0.698	<0.001	0.485	0.130
MAC(cm)	0.117	0.623	-0.623	<0.001	0.089	0.794
FMI(Kg/m2)	0.334	0.150	-0.698	<0.001	0.485	0.130
FFMI(Kg/m2)	0.334	0.150	-0.698	<0.001	0.485	0.130
Hemoglobin (g/L)	0.831	<0.001	0.609	<0.001	1.000	<0.001
Hematocrit(%)	0.831	<0.001	0.966	<0.001	1.000	<0.001
Serum Iron(µg/dl)	1.000	<0.001	1.000	<0.001	1.000	<0.001
TIBC(µg/dl)	0.129	0.587	0.359	0.017	0.638	0.035
Serum ferritin (ng/ml)	1.000	<0.001	0.835	<0.001	1.000	<0.001
TSF (%)	0.831	<0.001	0.609	<0.001	0.985	<0.001
FT3(pg/ml)	0.831	<0.001	0.746	<0.001	0.911	<0.001
FT4(ng/dl)	0.831	<0.001	0.746	<0.001	0.911	<0.001
TSH (µIU/ml)(	0.623	0.003	0.736	<0.001	0.638	0.035
Anti –TPO(IU/ml)	0.623	0.003	0.774	<0.001	0.638	0.035
Anti-TG(IU/ml)	0.623	0.003	0.774	<0.001	0.638	0.035

Linear regression analyses in autoimmune thyroiditis patients to test the influence of the main independent variables against obstatin levels (dependent variable):-

In autoimmune thyroiditis patients (n=150), stepwise linear regression analysis showed that, serum obstatin levels were independently correlated with hemoglobin,hematocrit and serum Iron levels, (P< 0.001; (**Table 6**).

Table 6**:-**Linear regression analyses in autoimmune thyroiditis patients to test the influences of the main independent variables against obstatin levels (dependent variable).

Model	Unstandardized Coefficients		Standardized Coefficients	t	P	95% C.I	
	B	S E	Beta			Lower Bound	Upper Bound
Constant	-6.708-	34.237		-.196-	.845	75.475-	62.06
Systolic blood pressure (mmHg)	0.014	0.038	.007	.366	.716	-.063-	0.091
Diastolic blood pressure (mmHg)	0.001	0.002	.003	.204	.839	-.004-	0.005
Hip circumference (cm)	-.079-	0.200	-.085-	-.397-	.693	-.481-	0.322
Waist circumference (cm)	0.131	0.189	.119	.692	.492	-.248-	0.509
Waist/hip ratio	11.479-	18.824	-.104-	-.610-	.545	49.289-	26.33
MAC	0.108	0.090	.018	1.195	.238	-.073-	0.28
Total cholesterol (mg/dl)	-0.021-	0.017	-.020-	1.234-	.223	-.056-	.013
Triglycerides (mg/dl)	0.014	0.010	.034	1.362	.179	-.007-	0.034
LDL cholesterol (mg/dl)	0.011	0.012	.033	.914	.365	-.013-	0.035
HDL cholesterol (mg/dl)	0.090	0.074	.039	1.219	.229	-.058-	0.239
Fasting blood glucose (mg/dl)	0.036	0.062	.034	.580	.564	-.089-	0.161
Fasting serum insulin (µU/ml)	0.039	0.134	.066	.288	.774	-.231-	0.309
HOMA-IR	0.061	0.491	.033	.124	.902	-.926-	1.048
HbA1c (%)	1.109	1.816	.014	.611	.544	-2.538-	4.757
FM(kg)	1.684	2.536	.586	.664	.510	-3.409-	6.778
FFMI(kg/m)	-3.813-	6.444	-.519-	-.592-	.557	16.755-	9.130
FT4(ng/dl)	-1.903-	2.444	-.075-	-.779-	.440	-6.812-	3.006
TSH (µIU/ml)	-0.029-	.042	-.030-	-.697-	.489	-.112-	0.054
Anti-TG(IU/ml)	-1.649-	2.190	-.049-	-.753-	.455	-6.047-	2.750
Hemoglobin (g/L)	-3.210-	1.127	-.334-	2.849-	.006	-5.473-	-.947-
Hematocrit(%)	0.878	0.199	.243	4.407	<0.001	.478	1.278
Serum Iron(µg/dl)	4.123	0.205	1.461	20.140	<0.001	3.712	4.535
Serum ferritin (ng/ml)	-0.769-	0.887	-.100-	-.867-	.390	-2.551-	1.012
TIBC(µg/dl)	0.007	0.007	.022	.966	.339	-.008-	0.022

Thyroid autoantibodies levels in the studied groups:-

Our results revealed statistically significant higher Serum Anti TPO levels in autoimmune thyroiditis patients (69.87±12.88IU/ml) compared to controls (38.9±3.06IU/ml) (P< 0.001)**(Figure 2)**. Moreover, levels of Serum Anti TPOwere statistically lower in subclinical hyperthyroidism and hyperthyroidism patients (53.07±2.522; 50.41±3.026IU/ml, respectively) than in those euthyroid (58. 0±3.90, P< 0.001) (**Table 2)**.However, levels of Anti TPO were statistically higher in subclinical hypothyroidism and hypothyroidism patients (77.32±3.58; 85.21±2.618IU/ml, respectively) than in those euthyroid (58.0 ±3.9028IU/ml, P< 0.001)**(Table 2).**

In autoimmune thyroiditis patients, there were statistically significant higher Anti-TG levels (2.25±0.416) compared to controls (0.699±0.081IU/ml,P< 0.001)**(Figure 3)**. Moreover, levels of Anti-TG were statistically higher in subclinical hypothyroidism and hypothyroidism (2.499±0.115; 2.75±0.464IU/ml,respectively) than in those euthyroid (1.87±0.616IU/ml, P< 0.001). On the other hand, levels of Anti-TG were statistically lower in subclinical hyperthyroidism and hypothyroidism (1.629±0.587; 1.715±0.651IU/ml, respectively) than in those euthyroid(1.87±0.616IU/ml, P< 0.001)**(Table 2)**.

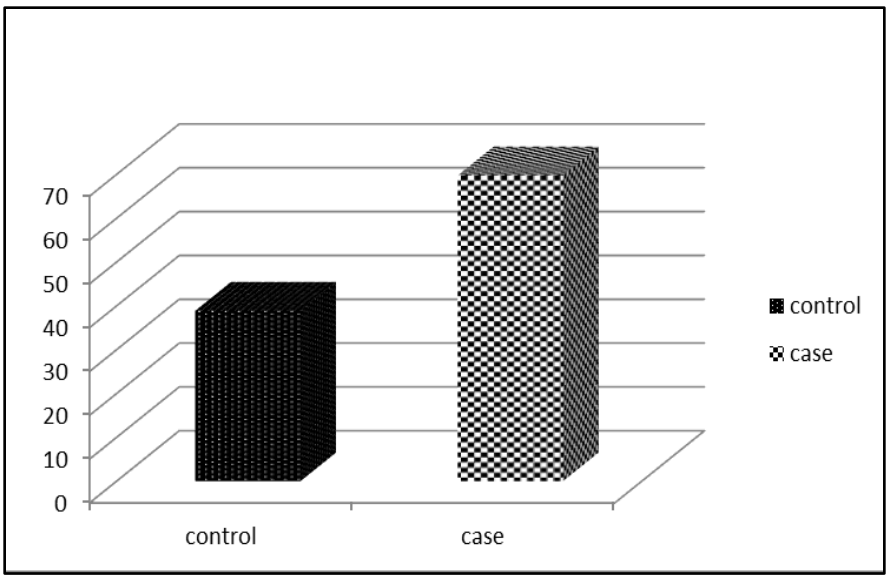

Fig2.serum Anti TPO levels in the studied groups.

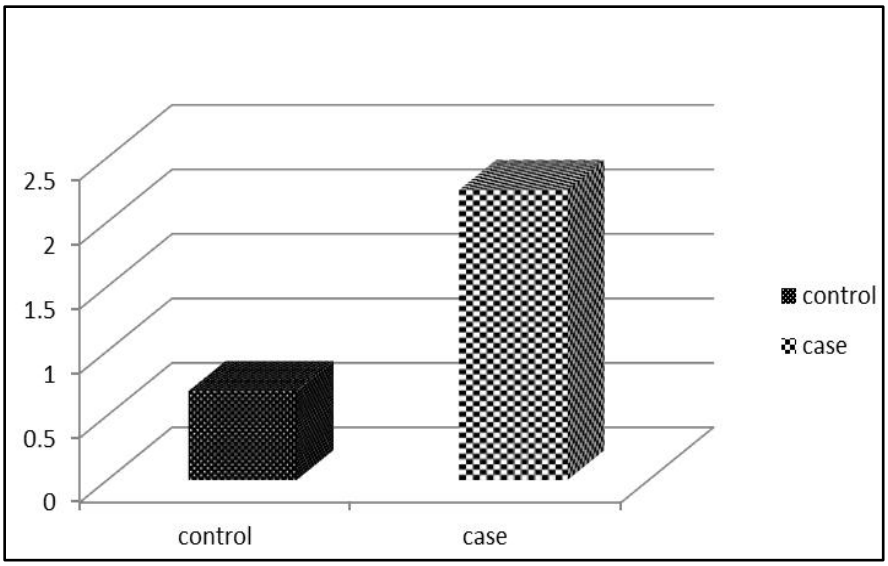

Fig3.serum anti TG levels in studied groups.

Pearson correlations between iron deficiency parameter,, anthropometric, and obesity indices intotal hypothyroidism patients:-

In patients with total hypothyroidism (n=88) obesity indices; FM,FMI,MAC and BMI were significant negative correlated with hemoglobin, hematocrit, ferritin, serum iron, andTSF.In contrast, iron deficiency parameters were positively correlated with FFM and FFMI. On the contrary, there were no significant correlations between waist hip ratio; as well as TIBC (P>0.05(**Table 7**).

Table7. Pearson correlation coefficient between anthropometric indices and parameters of iron deficiency among autoimmune thyroiditis.

		hemoglobin	hematocrit	Serumiron	TFS	ferritin	TIBC
Waist /hip ratio	r	0.199	0.257	0.054	0.257	0.195	0.247
	p	0.196	0.092	0.727	0.092	0.205	0.107
Body mass index (kg/m^2)	r	-0.714	-0.469	-0.798	-0.469	-0.737	0.258
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.091
Mid arm circumference	r	-0.598	-0.495	-0.657	-0.495	-0.630	0.190
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.216
Fat mass index	r	-0.714	-0.469	-0.798	-.469	-0.737	0.258
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.091
Fat free mass index	r	0.714	0.469	0.798	0.469	0.737	0.258
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.091
Fat free mass	r	0.718	0.473	0.798	0.473	0.739	0.259
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.089
Fat mass	r	-0.718	-0.473	-0.798	-0.473	-0.739	0.259
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.089

Discussion:-

The most common dysfunctions of the thyroid gland are hypothyroidism, Graves-Basedow disease and Hashimoto's disease. Hashimoto's thyroiditis can be the main cause of primary hypothyroidism of the thyroid gland. Auto immune thyroiditis is the most common cause of hypothyroidism in the United States after age 6 years, affected about 2 to 4 % of women and up to 1% of men.The pathophysiology of AITDs is genetic and environmental factors, as well as hormonal influences [27]

Obesity and its associated health comorbidities (e.g., insulin resistance and type 2 diabetes mellitus are currently at unprecedented rates of prevalence worldwide [28]more than 60% of all adults are classified as overweight or obese in most westernized societies, and as the prevalence of obesity increases it is responsible for an ever larger proportion of the overall burden of disease [28].

Obesity increases the inflammation-related hematological indices [29]. As obesity is accompanied by a state of low grade and chronic inflammation [30]. It seems that serum ferritin levels are naturally higher in obese people due to inflammatory state caused by obesity. Iron deficiency anemia is one of the world's most widespread health problems especially among children. In Egypt 27% of Egyptian children have iron-deficiency anemia. [2] Since iron is essential for all cells, many systems are affected in iron deficiency in addition to anemia. Normal thyroid status is dependent on the presence of many trace elements e.g., iron, iodine, selenium, and zinc for both the synthesis and metabolism of thyroid hormones. Deficiencies of these elements can impair thyroid functions. [4]

Ghrelin and obstatin are important appetite and energy-regulating peptides, secreted by the stomach. These gut peptides and thyroid hormones are involved in metabolism regulation. Although subclinical thyroidism is common, to date, very few studies have been reported about gut hormones in thyroid dysfunction, and their results are controversial.We investigated in the present study, for the first time, the possible association of gut peptides; obstatin, and thyroid hormones to suggest new regulatory relation between thyroid and gut. Moreover, we investigated relationships of iron deficiency parameters (hemoglobin, hematocrit serum iron, ferritin, TFS and TIBC) and body composition parameters(FM, FMI, FFM and FFMI)withthyroid function testsas well as thyroid antibodies; anti TPO and anti TG as markers of autoimmune thyroiditis in Egyptian obese women with different types of thyroid dysfunction.

Overt thyroid disease is associated with marked changes in energy expenditure and body weight [31], and typically weight loss in hyperthyroidism, and the overweight inhypothyroidism . Moreover, small changes in T4 dose in patients in long-term T4 treatment have been shown to modify resting energy expenditure significantly [32].

The main finding of the present study is that, Serum concentrations ofobstatinwere statistically higher in hyperthyroidism patients than in thoseeuthyroid.however, levels of obstatin were statistically lower in hypothyroidism patients than in those euthyroid.

In agreement with our results,**Emami et al.,**found that, obstatindecreased in subclinical hypothyroid subjects compared to the control group,however, obstatin increased in subclinical hyperthyroid subjects compared to the control group. Also,obstatin showed strong correlations with TSH, FT3 and FT4 [33].
Against to our results,Kosowicz and collegues demonstrated that hypothyroidism is associated with high and hyperthyroidism is associated with low levels of obstatin [34]. Gurgul et al. found a positive association between TSH levels, ghrelin, and obstatin in hypothyroidism [35].

In order to evaluate the associations between obstatin ,iron deficiency ,obesity indices and thyroid function parameters, we found that , patients with euthyroid,total hyperthyroidism and total hypothyroid serum obstatin level was positively correlated withiron deficiency and thyroid function parameters . On the contrary, there were significant negative correlations between serum obstatin level indices of obesity; for further analysis,,in autoimmune thyroiditis patient, stepwise linear regression analysis showed that, serum obstatin levels were independently correlated with Hemoglobin, Hematocrit and Serum Iron levels,

Results of **Sawicka** et al., observed that, there were positive correlation between obstatin level and HOMA-IR index in children with subclinical hypothyroidism in the course of Hashimoto's thyroiditis on the contrary, no significant correlations between obstatin and antithyroid antibodies as well as lipids in patients with untreated hyperthyroidism and subclinical hypothyroidism. [36]

In the present study we observed that, hyperthyroid patients had significant lower values of Waist/hip ratio compared to euthyroid women .,moreover, women with clinical hypothyroidism had higher values ofMACas compared to euthyroid , .also BMI,FM,FMI,FFM and FFMIwere higher in subclinical hyporthyroidism and hypothyroidism, on the other hand , subclinical hyperthyroidism and hyperthyroidism had significant lower values than euthyroid patients .

This was expected as there is fat tissue could affect thyroid hormones in different ways.first, leptin, an adipocyte-derived hormone, could affect thyroid hormone status through the stimulation of hypothalamic thyrotropin-releasing hormone (TRH) gene expression [37] and influence thermogenesis [38]. However, as described in a recent review, the role of leptin seems to be restricted to energy-deficient states such as acute fasting and exercise, and evidence in humans is still scarce [39]. Second, the effect may be due to a change in deiodinase activity in central and/or peripheral tissue [40,41]. Third, hormone resistance in obese subjects as a result of a T3 receptor decrease has been suggested, Fourth, a partially bio inactive TSH protein in obese subjects has been speculated [42].

Ourfindings are in concordance with **Kitahara et al.,**who found that measures of overall and central adiposity were associated with higher circulating levels of TSH and fT3 in euthyroid adults. Also,they observed no association with fT4 levels. Although weight loss and weight gain are well-known consequences of overt thyroid dysfunction, our results suggest that, within the euthyroid range, excess body weight may induce changes in thyroid hormone levels [31]

Mamtani et al., demonstrated that WC, as a measure of central obesity, is a significant and independent indicator of thyroid dysfunction in Mexican Americans. Also, their results indicated that central obesity may be not only an important player in the multifactorial web of thyroid dysfunction but may also contribute to the development of future clinical hypothyroidism by predicting the subjects who are currently at a high risk of subclinical hypothyroidism [33]

Similar results confirmed by **Bjergved et al.,**they observed significant, positive association between change in serum TSH and change in body weight [34]

Manji et al could not confirm the association between body weight and thyroid dysfunction. Possible explanations for the lack of association, could be the small sample size (N = 400), the different clinical spectrum of the studied population (euthyroid hospital-referred patients with thyroid nodules or goiter), and the use of correlations without adjustment for confounders [32].

Our study demonstrated that, Inautoimmune thyroiditis patients, there were statistically significant higher Anti-TG level compared to controls. Moreover, levels of Anti-TG were statistically higher in subclinical hypothyroidism and hypothyroidismthan in those euthyroid. On the other hand, levels of Anti-TG were statistically lower in subclinical hyperthyroidism and hypothyroidism than in those euthyroid

El-Hadidi et al., found that patients with anti-TPO and anti-TG antibodies had statistically elevated TSH levelscompared to those without antibodies, but, FT3 and FT4 levels showed no significant differences among patients with and without either of antibodies [44]

in order to investigate the association between iron deficiency and thyroid disorder ,we found that, women with subclinical hypothyroidism and hypothyroidism had significant higher level of TIBC than euthyroid patients ,on the other hand, they had significant lower values of hemoglobin, hematocrit, ferritin and serum iron as compared to euthyroid , .moreover , there were significantly lower levels of hemoglobin and hematocrit in women with subclinical hyperthyroidism compared to euthyroid women , There were no statistically significant different between hyperthyroid and euthyroid patients as regard hemoglobin, hematocrit, TIBC, ferritin and Serum iron. Also, There were no statistically significant different between patient with autoimmune thyroiditis as respect to TFS.

Metwalley et al., found that, Egyptian primary school children with iron deficiency anemia especially severe type are liable to develop subclinical hypothyroidism and intellectual dysfunction [45]

Celani et al., results indicate that tissue hypoxia due toanaemia is probably not a significant cause of thyroiddysfunction as has been previously postulated [46] .Correction of anaemia after iron treatment partially normalizedthyroid abnormalities and restored normal thermoregulation.Although these findings were interpreted tosuggest that disordered thermoregulation was mediated byabnormal thyroid function, it is possible that thyroid hormoneabnormalities in cold stress experiments are related to theeffects of stress on catecholamine excretion by cold exposurerather than inherent thyroid dysfunction due to anaemia.[47]

Ferritin is an acute phase protein that may be increased during inflammationHence, unlike the usual non-inflammatory state where ferritin levels below 15 is considered as iron deficiency, in the case of inflammation and infection, values less than 30 will be interpreted as iron deficiency since in these cases (i.e. inflammation and infection) ferritin levels will be elevated [48]

However, according to our results, ferritin level was not elevated in obesity or thyroid dysfunctions these finding may due to, we excluded any cases with infection from the study, so our patient had low ferritin levels due to irondeficiencies.

Similar to our results,studies from an Israeli [49]. , Iranian and Chinese population [50]found that, low serum iron and ferritin in obese subjects. Also, US study found a strong link between iron deficiency and BMI across all races, ages and amounts of dietary intake [51].. These studies unequivocally demonstrated lower serum iron availability with increasing adipose tissue mass in adolescents. Furthermore, [52].

On the contrary, study by Sanad et al. in Egypt found that, serum hepcidin was significantly lower in non-obese children with iron deficiency anemia and significantly higher in obese children with iron deficiency anemia (53), also,a study in mostly lean or only mildly overweight adolescents without severely obese subjects concluded that higher serum ferritin concentrations and transferrin saturation correlated with increasing BMI in adult populations [54].

Our results found that, patients with total hypothyroidism ; subclinical and overt hypothyroidism,obesity indices; FM,FMI,MAC and BMI were significant negative correlated with hemoglobin, hematocrit, ferritin, Serum iron, and TSF, on the other hand, iron deficiency parameters were positively correlated with FFM and FFMI

HoweverGhadiri et al. study in Iranian Population showed no difference in serum iron, TIBC, transferrin saturation index, and ferritin among normal weight, overweight, and obese persons. Excluding diabetic patients and maybe the well nutritional status of obese people, for example, intake of high iron foods can be proposed to explain their results [55]

Conclusion:-

The prevalence of nutrition disorders in patients with autoimmune thyroiditis was high, distinctively in SCH and hypothyroidism. Obesity related parameters and indicesare variables in thyroid dysfunction according to pattern of dysfunction. We demonstrate that serum obstatin associated with iron deficiency parameters and obesity indices so, obstatin found to be good diagnostic marker of nutrition disorders especially in obesity with thyroid dysfunction. Indeed, further in-depth studies are needed to identify other nutrition disorder in Egypt and its association with thyroid dysfunctions and obesity.

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