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Estimation of pro and anti-inflammatory cytokines related with obese individuals in Babylon/Iraq

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Abstract : The current study was designed to assess pro and anti-inflammatory cytokines (IL-6 and TGB-β1)that may be related to obese adults. The study included 52people(13 males and 39 females) suffering from obesity (excluded patients undergoing from chronic diseases) and 37 healthy individuals (19 males and 18females) as a control group with age ranging between 19-40 years. The obese groups were divided into three classes I , II and III depending on body mass index(BMI) .The results showed that the majority suffering from obesity were females , and there werea significant difference($P \leq 0.05$) between obese and control groups regarding the age, sex, and waist circumference (WC) . The major distribution of the obese groups according to their classes was located in class 1, whereas the class III showing the lowest percentages among other classes. The obese group revealed that a significant increase ($P \leq 0.05$) in the level of TGB-β1 compared with control group, but there was no significant difference in the concentration of IL-6 between obese and healthy groups ,also there were no significant difference in the level of both cytokines between sex of both groups and among the three classes of obesity. The data concluded that the serum level of TGB- β1may be one the main signals for increasing the appetite and development of obesity.
Keywords : Obesity; Cytokines.

Introduction

Obesity is a chronic disease affecting over billion adults all over the world and it is predicted that its prevalence will have doubled by the year 2030 and that the obesity epidemic is going to become the biggest health problem of the century, where annually obesity contributes to an estimated 112,000 preventable death [1-3]. In addition, the WHO[1], estimated that there are more than 600 million obese adults out of total 1.9 billion adults (more than 18 years of age) who are overweight with most of these people in the developed world. The prevalence of overweight and obese people is increasing worldwide at an alarming rate in both developing and developed countries of the region between 1980 and 2014[3]. Furthermore, 52% of the European adult's population is considered overweight or obese[4,5], while in middle east countries is no exception with its overall obesity prevalence among adults reaching alarming levels 24.5%[6]. Certain researchers[7] revealed that increased production of transforming growth factor-β (TGF-β) corresponding to increased fat mass , as the essential role maintenance of immune homeostasis without promoting their subsequent differentiation[8] .Furthermore, in obese animals or humans , adipose tissue is characterized by increased local and systemic production of pro-inflammatory cytokines such as IL-6 , which induce the production of reactive oxygen species (ROS)[9] , and these intracellular level act in many signaling pathways in different peripheral organs , but also in hypothalamus where they control food intake and metabolism by acting on different types of neurons[10]. The aim of this study

was to find a relationship between cytokines (IL-6, TGF-β) and energy balance, appetite control as well as obesity in the young adults of both sexes who did undergo from chronic diseases.

Materials and Methods

1. Patients and control:

This work was performed on89 individuals , patients suffer from the obesity 52 (13 males and 39 females) were selected from the nutrition clinic at the teaching hospital as obesity group with age ranged between 19-40 years, while the control group included 37apparently healthy people (19 males and 18 females) .

2. **Blood Samples:**

The blood samples were drawn from each obese patients and controls (5 ml) by vein puncture using disposable syringes and placed in disposable tube, kept to clot at room temperature, and then centrifuged at 3000 rpm[11] for 10 minutes, after that sera samples were carefully transferred to Eppendorf tubes and stored in aliquotes at deep freezing until used .

3. **Immunological Assays**:

Human IL-6 and TGF- β1 were estimated by ELISA according to the manual procedure of ELA science - China Company.

5. **Statistical Analysis:**

The results were analyzed by using statistical system spss version -15. The data were expressed by means , standard error (± SE), one sample T-test , one-way ANOVA and chi-square test fisher exact test and Odds ratio to identify the risk factors of obesity with their 95% confidence interval , were used to find out the association between the categorical variables .

Results :

The data illustrated in table (1) showed that the majority of obese groups were females (75%) , and all of them have abnormal waist circumference (WC) (≥88cm) , in addition there was a significant correlation (p≤0.05)between obese groups in comparison with the control group regarding the age (OR 7.46 , CI {2.51-22.18}) , sex (OR 3.16 , CI {1.28-7.78}) and WC (Fisher < 0.001) .

Table(1) : The relationship between variables of study groups

Variables	Control groups	Obese groups	χ2	Sig.	Odds ratio	95% CI
Age						
(19-29) year	32(86.49%)	24 (46.15%)	**14.90**	**<0.001***	**7.46**	**2.51-22.18**
(30-40) year	5 (13.51%)	28 (53.85%)				
Sex						
Male	19 (51.35%)	13 (25.0%)	**6.44**	**0.01***	**3.16**	**1.28-7.78**
Female	18 (48.65%)	39 (75.0%)				
WC						
High (≥102cm) Male	0(0%)	8(61.54%)		**<0.001*[a]**		
High ≥88cm Female	0(0%)	39(100%)				
Normal (≥102cm) Male	19(100%)	5(38.46%)				
Normal ≥88cm Female	18(100%)	0(0%)				

*P ≤ 0 .05.

[a]: Fisher test.

OR=(95%CI)

The distribution of the obese groups according to their classes was shown in figure (1) , in which the major distribution of the groups located in the class 1 , in addition the BMI ranged from 30 to 34.9 kg/m²which including 46.15(34.61% for females and 11.54% for males), while the obesity in class II, their BMI were ranged between 35 to 39.9 kg/m² , representing 32.7%(21.15% for females and 11.54% for males) , whereas the class III showing a lowest percentages among other classes , in which BMI was ≥40 kg/m² that included 21.15%(15.38% for females and 3.77% for males) .

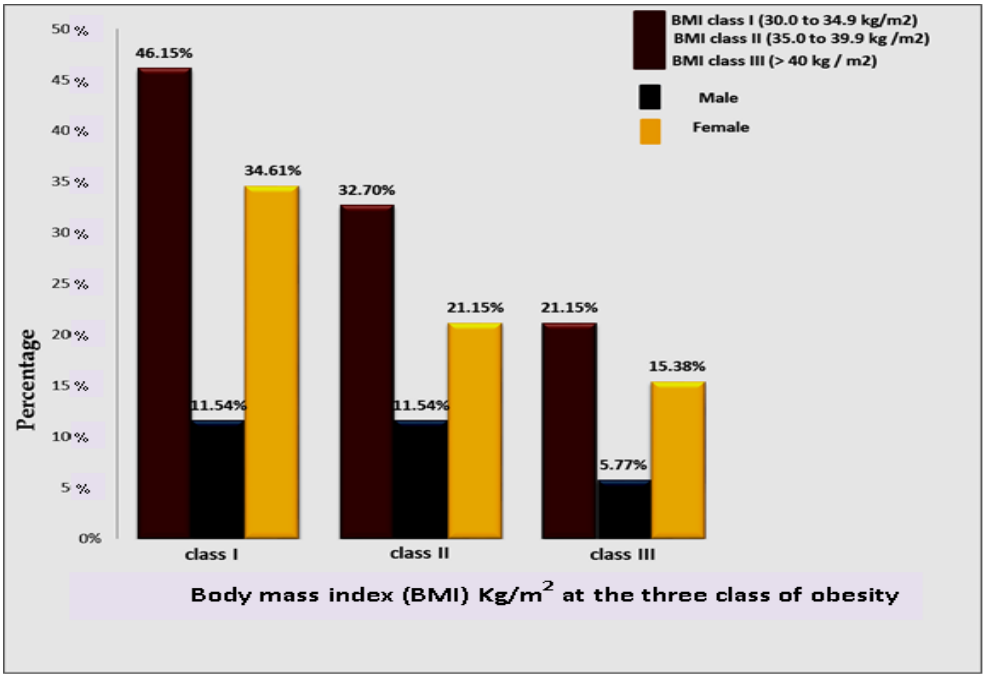

Figure (1): The distribution of obese groups into three classes.

Table (2) illustrated that the levels IL-6 and TGF-β1 cytokines among obese and control groups. It was clear that TGF-β1 level has been elevated significantly ($p \leq 0.05$) in the sera of obese groups (602.23 ±26.401 pg/ml) compared with healthy subject group (502.50±22.974 pg/ml), whereas the concentration of IL-6 revealed no significant differences among obese groups (1.053±25.36 pg/ml) compared with control groups (0.82±23.92 pg/ml) .

Table (2): The concentrations of IL-6 and TGF-β1cytokines (pg/ml)of thestudy groups.

Group / **Parameters**	**Control (Mean ± S.E) n=37**	**Obese (Mean ± S.E) n= 52**	**P-value of Group**
Interleukin-6(pg/ ml)	0.82±23.92	1.053±25.36	0.27
Transforming growth factor- β1(pg/ml)	22.974±502.50	26.401±602.23	***0.03**

*P ≤ 0 .05.
S.E: Standard error.

The results showed that there were no significant differences in the levels of these cytokines in among sex in both groups as shown in thetable (3).Meantime, the concentrations of IL-6 and TGF- β1 revealed that

there were no significant differences in all classes of the groups as illustrated in table (4), also the correlation analysis pointed out that no significant correlation between BMI and the levels of cytokines (table 5) .

Table (3): The concentrations of IL-6 and TGF-β1(pg/ ml) among sex of study groups

Group / Parameters	Mean ± S.E				P-value of Groups
	Control	Obese	Control	Obese	
	Male n=19	Male n=13	Female n=18	Female n=39	
Interleukin-6 (pg/ ml)	24.70 ± 1.22	24.49 ± 1.27	23.10 ± 1.10	25.65 ± 1.34	0.21
Transforming growth factor - β1(pg/ml)	465.43 ± 37.76	628.44 ± 64.84	541.63 ± 22.97	593.49 ± 28.22	0.10

t-test.
*P ≤ 0 .05.
S.E: Standard error.

Table (4): The concentrations of cytokines IL-6 and TGF-β1at the three classes of obese groups

Group / Parameters	Obese group (Mean ± S.E)			P-value of groups
	Class I n= 24	Class II n= 17	Class III n= 11	
Interleukin-6(pg/ ml)	26.57 ± 1.51	24.86 ± 2.16	23.49 ± 1.70	0.52
Transforming growth factor- β1(pg/ml)	585.24 ± 40.91	606.10 ± 47.94	633.31 ± 50.58	0.79

F-test
*P ≤ 0 .05.
S.E: Standard error.

Table (5):Thecorrelation coefficient between BMI and the concentrationsimmunologicalparameters inobese groups.of

Groups / Parameters	BMI (Kg/M^2)	
	R	P-value
Interleukin-6(pg/ ml)	-0.13	0.34
Transforming growth factor- β1 (pg/ml)	0.14	o.29

The correlation coefficient (r).
* Correlation is significant ≤ 0.05 level .

Discussion:

The current study showed that no significant differences in the level of IL-6 between obese and control groups and between both sexes of both groups. The present study disagreed with results of[12,13] whom observed that elevated levels of IL-6 in obese groups, but data regarding the relevance of this cytokines was debated could be partially attributable to the complex etiology of obesity, consisting of the interaction of genetics, diet, and physical activity level, additionally influenced by environmental, socioeconomic, and behavioral factors .In addition[14] observed that the circulating levels of IL-6 increased with the enlargement of fat mass , and in morbid obesity both arterial and subcutaneous venous IL-6 levels have been found increased as compared with none-obese subjects, meantime elevated plasma level of it was associated with decreased muscular mass or decreased muscle activity, but [15]pointed out that no significant differences in fasting levels of IL-6 between obese and normal weight adults men; this finding relatively suggests that obesity does not affect the level of IL-6 .

The level of IL-6 in the class I was more than other classes of obese groups, this may be due to the differences in distribution of percentages of obesity classes, where class III was the least percentages with relatively small size of the group, or due to IL-6 secretion associated with obesity as a regulatory mechanism attempting to correct excess body weight and achieve negative energy balance, as hypothesized for obesity-related increases in leptin hormone[16].Also, may be physical activity daily in the obese groups was not different with control groups, where high physical activity levels result in low basal IL-6 levels, in contrast, high basal of this cytokine accompanied low levels of physical activity[17].In addition , exercise was directlyaffectedby the immune system and a reduction in visceral fat , including the diminished release of pro-inflammatory cytokines and chemokines from adipocytes[18].

The concentration of TGF-β1was significantly increases in obese groups compared with control group.[19]found that serum TGF-β1levels increased with increasing BMI in both sexes, possibly reflecting physiological elevated its production corresponding to increasing fat mass, where the high levels of this cytokine in obese animal models could also reflect an important early role in supporting the undifferentiated population in a calorie-rich environment[20]. Other studies [21]indicated that circulating TGF-β1 levels are decreased in obese women, while other researchers have showed that obesity in human results in increased expression of this cytokine in adipose tissues[22], and its concentration has also positive correlation with obesity, BMI, and leptin levels in hypertensive patients[23], furthermore, the TGF-β1 and BMI are closely associated in human adipose tissue during morbid obesity[24].

The current study pointed out that the level of TGF-β1 was higher in the class III of obese group in comparison with other classes , this agrees with the results of [25,26] where concluded that this cytokine level in adipose tissue is strongly associated with class III of obesity, while the correlations showed that no significant correlation between BMI and TGF- β1 in both sexes of both groups; this agree with[27], who observed apositive association between BMI and serum TGF- β1 level in patients with essential hypertension obesity , in addition[2] reported that the concentration of this cytokine was significantly elevated in hypertensive obese patients compared with hypertensive patients with normal BMI .

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