Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 1, 2023, pp. 483-496..

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/
e-mail: bullpharm@aun.edu.eg

OBESITY IS ASSOCIATED WITH AUTOPHAGY DYSREGULATION IN EGYPTIAN WOMEN

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Background: *Obesity causes a reduced life quality and economic burden to Egyptians. Therefore, it is of utmost importance to investigate its risk factors to establish potential preventive strategies for it and its future complications. Obesity and its associated stress insults can often interfere with the autophagic process through various mechanisms, which can result in further aggravation of obesity-related metabolic pathologies. This case control study aimed at investigating autophagy dysregulation in Egyptian obese women.* ***Methods:*** *This study comprised 85 women that were classified into 5 groups (17 persons each):1- healthy, 2-overweight, 3-moderate obese, 4-severe obese and 5-morbid obese group. The expression of autophagy related gene (ATG5) was estimated by qRT-PCR, whereas, the serum activity of P70S6 kinase was assayed by ELISA.* ***Results:*** *The data revealed elevated mRNA expression levels of ATG5 with a significantly high serum P70s 6 kinase-1 in a stepwise fashion in obese subgroups.* ***Conclusion:*** *The present study demonstrated increased levels of both autophagy parameters in obese women that mean autophagy dysregulation. ROC curve analysis showed that p70S6 kinase-1 is a useful biomarker for both diagnosis and prognosis of obese subgroups. On the other hand, ATG5 was able to discriminate between the severe and moderate obese women. These data may represent a possible protective value for obese women, in addition to establishing potential strategies for autophagy inhibition in obesity and in turn, alleviation of its future complications.*

Keywords: *Obese, ATG5, P70S6 kinase-1*

INTRODUCTION

Obesity is a complex and multifactorial disease affecting over one third of the world's population today[1-4]. In Egypt, the prevalence of obesity has increased in adults to reach about 40% of its population[5].

Obesity is attributed as a risk factor for several diseases such as type 2 diabetes, hypertension, cardiovascular diseases, hepatic diseases, and cancer[5]. Consequently, obesity causes a huge clinical and economic burden to Egyptians as individuals and as a society.

Autophagy is one of the major degradative mechanisms that can eliminate excessive nutrients, toxic protein aggregates, damaged organelles, and invading microorganisms. In response to obesity and obesity-associated lipotoxic, proteotoxic and oxidative stresses, autophagy plays an essential role in maintaining physiological homeostasis. However, obesity and its associated stress insults can often interfere with the autophagic process through various mechanisms, which can result in aggravation of obesity-related metabolic pathologies. Paradoxically, dysregulation of autophagy and transition from an adaptive phase to a maladaptive phase has been linked to many diseases, especially obesity[6]. Inhibition of autophagy, within

Received in 18/11/2022 & Accepted in 13/12/2022

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specific contexts, indirectly produces beneficial effects that can alleviate several detrimental consequences of obesity[6].

In the present study, we aim to examine the status of autophagy dysregulation associated with obesity to establish potential preventive strategies for obesity and future complications.

MATERIALS AND METHODS

Patients

The present study is a case control study that was conducted in both the Biochemistry Department, Faculty of Medicine, Assiut university & Internal Medicine Department, Assiut University Hospital, Assiut, during the period from August 2021 to August 2022.

The study comprised a total of 85 Egyptian women that were family unrelated and selected randomly from the attendants of the Out-patients Clinic of Internal Medicine Department. The study included 17 normal, age matched, completely healthy control women, with no apparent evidence of any medical disorders. The ages of participants ranged between 18-53 years. Their heights ranged between 130-185 cm and their weights ranged between 51-135 kg.

The sample size was calculated using G*power software 3.1.3, the estimated minimum required sample size was calculated to be 80 subjects (16 in each group).

The study participants were subdivided into 5 groups according to BMI as follows[7].

- **Group (1):** included 17 healthy controls (normal), with a BMI of 18.5-24.9 kg/m^2.
- **Group (2):** included 17 overweight women, with a BMI of 25-29.9 kg/m^2.
- **Group (3):** included 17 moderate obese women (class I), with a BMI of 30-34.9 kg/m^2.
- **Group (4):** included 17 severe obese women (class II), with a BMI of 35-39.9 kg/m^2.
- **Group (5):** included 17 morbid obese women (class III), with a BMI of ≥ 40 kg/m^2.

An informed consent was obtained from each patient and control, and all study procedures were approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (IRB no: 17200758). All participants were subjected to full history taking; in addition, detailed clinical and menstrual history was obtained at the time of consent. Personal and relevant data were collected by a questionnaire designed to ask about risk factors and co-morbidities of obesity.

All participants were subjected to a thorough clinical examination in the form of complete physical examinations of the chest, heart and abdomen, measurement of blood pressure, body weight, height, waist circumference and hip circumference. Routine laboratory investigations were performed as blood picture, liver functions and kidney functions.

Exclusion criteria included patients with co-morbidities (diabetes, hypertension or insulin resistance (IR), any chronic illness (liver, renal, heart, gastrointestinal, endocrine disorders or thyroid disease), coronary artery diseases, cerebral vascular accidents, active smoking, patients who received any previous treatment for about 6 months later, patients with malignancies and hematologic disorders in addition to lactating and pregnant women.

Obesity indices calculations

1. Body mass index (BMI): was calculated by dividing the weight in kg by square the height in m^2.
2. Body fat percentage (BFP) was calculated as follows [8-9]:
3. BFP = (1.2 × BMI) + (0.23 × age in years) – (10.8 × sex) – 5.4, sex is set for zero in women.
4. Fat mass index (FMI): was calculated by dividing the fat mass (FM) in kg by square the height in m^2 where FM = BFP x body weight (Kg) / 100[10-11]
5. Waist circumference/hip circumference ratio (WHR): was calculated by dividing waist circumference by hip circumference.

Sample collection and handling

Four milliliters of antecubital venous fasting blood sample were withdrawn from each patient and control and divided into 2 tubes. Two milliliters were collected in a tube containing EDTA and preserved at -70 ºC for RNA extraction. Half milliliter was taken on

fluoride for estimation of blood glucose. One and a half milliliters of blood were put in a Wassermann test tube, left to clot at room temperature for 10-20 min, then were centrifuged at 3000 rpm for 20 min. Sera were separated and divided into aliquots. A part was used fresh for serum routine laboratory tests and lipid profile. The remaining sera were stored at -80 °C for ELISA.

Lipid profile

Total blood cholesterol, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C) and triglycerides were assayed using colorimetric kits, supplied by spectrum diagnostics, Egypt.

Estimation of Human Phospho-P70S6 kinase-1 (P70S6 kinase-1)

P70S6 kinase-1 concentrations were determined using a commercial ELISA Kit (Catalog No.: SG-15355, SinoGeneclon Comp., LTd, Hangzhou, China) according to the manufacturer's specifications.

RNA extraction and real-time PCR

Total RNA was extracted from whole blood using a Gene JET RNA Purification Kit (catalog no #K0731 Thermo Scientific Inc, USA) according to the manufacturer's instructions. After quantitation using Nanodrop spectrophotometer, RNA (500 ng) was used for reverse transcription to complementary DNA (cDNA) with the High-Capacity cDNA Reverse Transcription kit (catalog no, #K1622, Thermo Scientific, USA). cDNA was then amplified with Maxima SYBR Green qPCR Master Mix kit, (Catalog no. #K0251, Thermo Scientific, USA and used as a template for ATG5. The amplification was performed using the primer sets described in **Table 1.** A two-step reaction protocol was performed: an initial denaturation cycle of 95 °C for 1 min, followed by 40 amplification cycles of 95 °C for 15 sec and 60 °C for 1 min using the Applied Biosystems 7500 Fast Real-time PCR machine (Applied Biosystems, Germany). Gene expression values were expressed as fold-change versus normal samples, by using the $2^{-\Delta\Delta CT}$method with GAPDH as reference gene.

Statistical analysis

The entry and data analysis were performed using IBM-SPSS version 26 (Statistical Package for Social Science) for windows software. Continuous data were expressed as number, mean ± standard deviation (Mean ± SD). The distribution of data was determined using the Shapiro-Wilk test. Statistical comparison of differences between test groups was evaluated by Kruskal Wallis test for non-parametric data. The correlation coefficients analysis was performed using Pearson's coefficients. Medcalc was used to calculate sensitivity, specificity, (positive and negative predictive values) (PPV and NPV) and Receiver operating characteristic (ROC) curves used to assess the best cut-off value. A two-tailed P-value was considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Results

Anthropometric data of subjects

The anthropometric data of the four subgroups of patients (overweight and obese classes I, II & III) are shown in **Table 2** compared to the controls (group 1). There was no statistical significance regarding age and height between the four groups. There was an increase in weight, waist and hip circumferences of obese women that was significant regarding both waist and hip circumference when compared to the controls ($p< 0.001$ for each).

Table (1): The sequences of the PCR primers.

Gene	5′-3′ primer sequence
ATG 5	**Forward:** TTGAATATGAAGGCACACCACTGAA **Reverse:** GCATCCTTAGATGGACAGTGCAGA
GAPDH	**Forward:** GCACCGTCAAGGCTGAGAAC **Reverse:** TGGTGAAGACGCCAGTGGA

Table (2): Anthropometric data of different study groups.

	Controls (n=17)	Overweight (n=17)	Moderate Obese (n=17)	Severe Obese (n=17)	Morbid Obese (n=17)
Age (Year)					
Range	21 - 45	25 - 50	18 - 45	20 - 50	27 - 53
Mean±SD	31.41±8.11	34.71±6.30	31.41±7.92	33±9.63	37.76±8.07
P1		NS	NS	NS	NS
P2			NS	NS	NS
P3				NS	NS
P4					NS
Weight (Kg)					
Range	51 - 82	65 - 100	59 - 125	70 - 106	70 - 135
Mean±SD	69.24 ± 12.79	79.06±8.71	81.94±14.45	92.35±10.39	105.71±17.35
P1		NS	NS	<0.001	<0.001
P2			NS	<0.001	<0.001
P3				<0.01	<0.001
P4					<0.05
Height (Cm)					
Range	155 - 185	150 - 185	135 - 172	135 - 165	130 - 170
Mean±SD	170.41±13.09	165.82±9.17	156.59±8.85	156.35±8.43	151.06±10.19
P1		NS	<0.01	<0.01	<0.001
P2			<0.01	<0.01	<0.001
P3				NS	NS
P4					NS
Waist Circum.(Cm)					
Range	77 - 95	88 - 130	90 - 137	85 - 150	111 - 160
Mean±SD	84.82±5.58	101.82±11.48	101.76±11.12	112.76±16.10	130.41±13.99
P1		<0.001	<0.001	<0.001	<0.001
P2			NS	<0.05	<0.001
P3				<0.05	<0.001
P4					<0.01
Hip Circum.(Cm)					
Range	99 - 120	108 - 160	106 - 149	100 - 170	118 - 174
Mean±SD	109.88±8.26	123.65±14.14	121.88±10.40	129.59±16.92	143.82±14.31
P1		<0.01	<0.01	<0.001	<0.001
P2			NS	NS	<0.001
P3				NS	<0.001
P4					<0.05

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups. **P3**: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Obesity indices

BMI, waist/hip ratio (WHR), fat mass index (FMI) and body fat percent (BFP) were compared among obese subgroups and controls. Highly significant differences were found between controls and other obese subgroups regarding all parameters ($p< 0.01$) and the morbid obese group exhibited the highest values. **Table 3**

Biochemical investigations

Table 4 shows the biochemical data of different studied groups. Kidney function was normal in all groups. Regarding liver function tests, despite their normal reference range, however, significant differences were observed between controls and obese subgroups ($p< 0.001$ for each). Also, mean values of serum total cholesterol, LDL-C, HDL-C and triglycerides levels were remarkably significantly higher in obese women than controls ($p< 0.001$ each).

Table (3): Obesity indices among different study groups.

	Controls (n=17)	**Overweight (n=17)**	**Moderate Obese (n=17)**	**Severe Obese (n=17)**	**Morbid Obese (n=17)**
BMI (Kg/m^2)					
Range	21.2 - 25.7	26.3 - 31	30 - 52.02	35.2 - 40	40.4 - 60.2
Mean±SD	23.56±1.44	28.48±1.32	33.32±5.00	37.72±1.75	46.08±4.74
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001
WHR(W/H)					
Range	0.72 - 0.82	0.79 - 0.85	0.79 - 0.86	0.82 - 0.9	0.86 - 0.96
Mean±SD	0.77±0.03	0.82±0.02	0.83±0.02	0.86±0.02	0.90±0.04
P1		<0.001	<0.001	<0.001	<0.001
P2			NS	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.01
BFP(BF%)					
Range	25.33 - 35.55	33.06 - 40.76	37.1 - 66.43	45.28 - 51.52	52.97 - 74.89
Mean±SD	30.09±3.19	36.76±2.16	41.81±6.78	48.89±2.09	58.58±5.09
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001
FMI (kg/m^2)					
Range	5.38 - 9.1	8.69 - 12.58	11.28 - 34.56	16.17 - 20.26	21.4 - 45.54
Mean±SD	7.14±1.16	10.58±1.10	14.26±5.38	18.82±1.23	27.25±5.57
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001

P1: comparison between control & other groups. **P2**: comparison between overweight & other groups. **P3**: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Table (4): Biochemical & clinical data of different study groups.

	Controls (n=17)	Overweight (n=17)	Moderate Obese (n=17)	Severe Obese (n=17)	Morbid Obese (n=17)
AST (IU/L)					
Mean±SD	12.59 ± 2.24	15.29 ± 1.76	14.94 ± 1.25	12.94 ± 1.71	14.53 ± 1.12
P1		< 0.01	< 0.01	NS	< 0.01
P2			NS	< 0.001	NS
P3				< 0.001	NS
P4					< 0.01
ALT (IU/L)					
Mean±SD	8.53 ± 2.12	18.47 ± 1.81	14.06 ± 0.83	23.53 ± 3.02	21.88 ±2.39
P1		< 0.001	<0.001	< 0.001	< 0.001
P2			<0.001	< 0.001	< 0.001
P3				< 0.001	< 0.001
P4					NS
Serum Creatinine (umol/L)					
Mean±SD	63.64 ± 2.28	51.02 ± 9.60	46.12 ± 14.08	48.59 ± 6.72	50.22 ± 8.78
P1		<0.001	< 0.001	< 0.001	<0.001
P2			NS	NS	NS
P3				NS	NS
P4					NS
BUN (mmol/L)					
Mean±SD	3.38 ± 0.65	2.92 ± 0.84	3.15 ± 1.15	2.44 ± 0.74	3.08 ± 1.27
P1		NS	NS	<0.001	NS
P2			NS	NS	NS
P3				<0.05	NS
P4					NS
Serum Cholesterol (mg/dl)					
Mean±SD	145.31±16.64	157.01 ± 16.76	176.90 ± 16.23	182.35 ± 15.62	227.05 ± 23.98
P1		NS	< 0.001	< 0.001	< 0.001
P2			< 0.01	< 0.001	< 0.001
P3				NS	< 0.001
P4					< 0.001
HDL-C (mg/dl)					
Mean±SD	52.44 ± 2.74	42.11± 3.11	33.26 ± 3.76	27.76 ± 1.66	24.21 ± 5.01
P1		< 0.001	< 0.001	< 0.001	< 0.001
P2			< 0.001	< 0.001	< 0.001
P3				< 0.001	< 0.001
P4					< 0.05
LDL-C (mg/dl)					
Mean±SD	75.23 ± 15.14	90.96 ± 14.43	112.87 ± 16.86	115.65 ± 16.86	159.08 ± 24.82
P1		< 0.05	< 0.001	< 0.001	< 0.001
P2			< 0.01	< 0.001	< 0.001
P3				NS	< 0.001
P4					< 0.001
Serum TAG (mg/dl)					
Mean±SD	88.24 ± 8.83	119.71 ± 9.60	153.82 ± 9.93	194.71 ± 8.19	218.82 ± 11.53
P1		< 0.001	< 0.001	< 0.001	< 0.001
P2			< 0.001	< 0.001	< 0.001
P3				< 0.001	< 0.001
P4					< 0.001

P1: Comparison between control & other groups. **P2**: comparison between overweight & other groups. **P3**: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Evaluation of autophagy in obesity

Table 5 shows the mRNA expression levels of autophagy marker ATG5; (a marker of autophagosome formation from the phagophore). The results demonstrated higher expressions of ATG5 in a stepwise fashion in obese subgroups in comparison to controls. Moreover, the significant peak upregulations were exhibited by the very severe obese group (morbid) in comparison to controls with a mean fold change of 2.68 vs 1.12. Regarding P70S6 kinase-1 (the significant downstream effector of mTOR levels), it was noticed that its levels were significantly increased in all patient groups in relation to that of control group (P<0.001), where also the highest value was exhibited by the very severe group.

ROC curve analysis was used to evaluate both the diagnostic and prognostic performance of the two autophagic parameters, to identify obese patients and discriminating their different groups (**table 6**). Both p70S6 kinase-1 and ATG5 were sensitive for prediction of overweight women with P70S6 kinase-1 presenting as a better specific and diagnostic parameter (AUC = 1).

Table (5): Autophagy parameters in different study groups.

	Controls (n=17)	**Overweigh t (n=17)**	**Moderate Obese (n=17)**	**Severe Obese (n=17)**	**Morbid Obese (n=17)**
ATG5 expression (fold change)					
Range	0.26 - 1.76	0.26 - 2.65	0.3 - 9.35	0.58 - 6.34	0.7 - 5.84
Mean±SD	1.12±0.48	1.24±0.84	1.95±2.61	2.43±1.72	2.68±1.35
P1		NS	NS	<0.01	<0.001
P2			NS	<0.05	<0.001
P3				<0.05	<0.05
P4					NS
P70S6 kinase-1 (ng/ml)					
Range	41.32 - 48.05	51.67 - 63.92	54.83 - 73.13	74.72 - 88.54	89.26 - 119.7
Mean±SD	44.34±2.23	58.09±3.37	65.34±5.81	82.39±3.69	99.89±8.32
P1		<0.001	<0.001	<0.001	<0.001
P2			<0.001	<0.001	<0.001
P3				<0.001	<0.001
P4					<0.001

P1: Comparison between control & other groups. **P2**: comparison between overweight & other groups. **P3**: comparison between moderate obese & other groups. **P4**: comparison between severe obese & morbid obese.

Table (6): ROC curve analysis (AUC, Cutoff value, Sensitivity, Specificity, PPV, NPV and Accuracy)

In over weight group							
	AUC	**Cutoff value**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
P70S6 kinase-1 (ng/ml)	1.00	> 48.05	100	100	100	100	100
ATG5 expression (fold change)	0.51	≤ 1.76	76.47	0	43.30	0	76.50
In moderate obese group							
	AUC	**Cutoff value**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
P70S6 kinase-1 (ng/ml)	0.84	> 63.92	70.59	100	100	77.30	85.30
ATG5 expression (fold change)	0.50	> 0.77	41.18	41.18	41.20	41.20	41.20
In severe obese group							
	AUC	**Cutoff value**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
P70S6 kinase-1 (ng/ml)	1.00	> 73.13	100	100	100	100	100
ATG5 expression (fold change)	0.72	> 0.77	94.12	58.82	69.60	90.90	76.50
In morbid obese group							
	AUC	**Cutoff value**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
P70S6 kinase-1 (ng/ml)	1.00	> 88.54	100	100	100	100	100
ATG5 expression (fold change)	0.57	> 2.26	64.71	64.71	64.70	64.70	64.50

To test for the prognostic performance of autophagic parameters, ROC curve (AUC) of the autophagy parameters was performed in moderate obese women in relation to overweight. P70S6 kinase-1 was demonstrated as a sensitive and accurate parameter in discriminating the moderate obese from overweight women (AUC = 0.84).

In severe obese in relation to moderate obese group, both P70S6 kinase-1 and ATG5 were sensitive and accurate in discriminating the severe obese from moderate obese women (AUC = 1, 0.72 respectively). In morbid obese in relation to severe obese group, P70S6 kinase-1 confirmed its prognostic validity in discriminating morbid obese from severe obese women, (AUC = 1).

Correlation analyses

Significant positive correlations were observed between the autophagy marker: P70S6 kinase-1 and obesity indices in addition to lipid profile except for HDL-C which showed a negative correlation. Furthermore, ATG5 exhibited a significant positive correlation with serum TAG and a negative correlation with HDL-C levels (table 7).

Table (7): Correlation analyses of autophagy parameters, obesity indices and lipid profiles.

	P70S6 kinase-1		ATG5 (fold change)	
	r	**p**	**r**	**p**
BMI (Kg/m²)	**0.795****	0.000	0.226	0.064
Waist Circum (cm).	**0.589****	0.000	0.201	0.101
Hip Circum.	**0.436****	0.000	0.158	0.198
WHR	**0.796****	0.000	0.231	0.058
BFP %	**0.811****	0.000	0.212	0.083
FMI (kg/m²)	**0.769****	0.000	0.204	0.096
Serum T.Ch (mg/dl)	**0.737****	0.000	0.213	0.081
HDL-Ch (mg/dl)	**-0.766-****	0.000	**-0.269***	0.027
LDL-Ch (mg/dl)	**0.714****	0.000	0.200	0.103
Serum TAG (mg/dl)	**0.892****	0.000	**0.329****	0.006

Significant correlations are bold.

Discussion

Obesity is a global pandemic of the present century[1-4]. It is a risk factor for several diseases as type 2 diabetes, hypertension, cardiovascular diseases, hepatic diseases, and cancer[5] which leads to a reduced life quality and increases the overall risk of morbidity and mortality[13]. Hence the necessity to study the role of potential agents for obesity prevention and management[14].

Autophagy is a highly conserved lysosomal degradation process that maintains cellular homeostasis. At basal level, it is cytoprotective, constantly operating at low levels in most cell types to regulate the intracellular conditions through cytoplasmic turnover of long-lived misfolded proteins, lipids, and injured organelles[15-17]**.** Autophagy is triggered by different stimuli including oxidative stress, hypoxia, protein-aggregates, and toxic molecules[18&19]**.** Altered autophagy (enhanced or suppressed) has been reported to play a destructive role and is linked to various diseases[20]**.**

Autophagy dysregulation has been reported in obese subjects and animal models [21]**.** Previous studies showed obesity-induced defects at the autophagosome-lysosome fusion step that lead to inhibition of the autophagic flux[6]. Insulin resistance and hyperinsulinemia associated with obesity and its related pathologies were suggested to be causes for autophagy inhibition during obesity[22]**.** On the contrary, Kovsan et al., Jansen et al., Nunez et al., and Zhang et al.[23-26] reported enhanced autophagy in response to obesity in multiple tissues including liver and adipose tissues. ER-stress, lipotoxicity, inflammation and oxidative stress were reported to upregulate autophagy in obesity as a part of a cellular defense mechanism[6&27&28.]

ATG5 is involved in phagophore expansion and elongation to form autophagosomes[16&29]**.** It is a ubiquitin-like modifier that covalently binds to both ATG12 and ATG16LI generating a large multimeric complex that lipidates ATG8 to form ATG8-phosphadidyl ethanolamine (ATG8-PE), which in turn mediates membrane fusion and elongates the phagophore to complete autophagosome formation[30].

The results of the current study revealed higher mRNA expression levels of ATG5 in a stepwise fashion in obese subgroups in comparison to control levels where the peak level was exhibited by the morbid obese group. In line with our results, Xu,Q et al.[31] found increased expression of ATG5 in overweight/obese subjects compared to lean subjects that seems to be mainly related to glucose tolerance. In addition, Haim, Y. et al. and Kosacka, J. et al.[32&33] reported up-regulated ATG5 in adipose tissue AT of obese subjects. Moreover, Kovsan, J. et al.[23] have shown that

ATG5 mRNA is upregulated in human subcutaneous and visceral AT in both nondiabetic and diabetic obese subjects. Conversely, Yang et al.[34] studies on obese mice observed down-regulation of ATG5 expression and subsequent inhibition of autophagosome formation.

The ribosomal protein P70S6 kinase-1 is the most well established significant downstream effector of the mammalian target of rapamycin complex 1 (mTORC1)[35&36]. Klionsky, DJ [37] suggested that, P70S6 kinase-1 is a positive regulatory factor for autophagy and its activation plays a more important role in the increased autophagy. Activation of ribosomal S6kinase-1 depends on its multiple site phosphorylation events and the conformational changes in response to diverse extra-cellular stimuli, including nutrients and growth factors which cooperate with each other to fully activate it[38&39]. S6kinase-1 is directly phosphorylated by mTOR1 upon diverse stimuli, such as amino acids and insulin stimulation[40.]

S6kinase -l has also been implicated in regulation of cell cycle control, cell size, cell differentiation, cell motility and also coordinating other downstream cellular progression and metabolic reactions to nutrients and energy inputs as lipid homeostasis[41&42&43]. Previous studies showed that, S6kinase-1 plays an important role in glucose homeostasis via feedback regulation of IRS-1 to improve glucose tolerance and insulin sensitivity[44].

It was noticed in the present study that, the mean serum levels of P70S6 kinase-1 were significantly increased in all obese subgroups, where, the morbid group possessed the highest value. This finding is consistent with those of Beals, JW et al.[45] who reported a 1.6-fold greater in total P70S6 kinase-1 at basal state in obese group compared to normal weight group. Kosacka, J. et al., and Saha et al.,[18&33] suggested that, the elevated autophagy in obesity may represent a protective mechanism against obesity-related AT dysfunction and insulin resistance. S6kinase-1 is also reported to heterodimerize with AMPKa2, phosphorylating it, which increases food intake and weight gain[46].

Our ROC curve studies showed that the P70S6 kinase-1 is the most specific and best diagnostic parameter for identifying overweight women (AUC=1) besides its confirmed prognostic validity in discriminating the three obese classes (moderate , severe and morbid) from each other (AUC = 0.84; 1; 1 respectively). In contrast, ATG5 ROC curve was not an accurate diagnostic parameter for identifying overweight women or discriminating them from moderate obese or differentiating severe obese from morbid one.

Highly significant positive correlations were found in the current study between P70S6 kinase-1 level and all obesity indices (BMI, WHR, FMI and BFP), LDL-C and TAG. These results are in harmony with those of Kovsan, J., et al. [23] who reported a correlation of autophagy related genes with the degree of obesity, visceral fat mass and adipocyte hypertrophy. It is also noteworthy to mention that a significant positive correlation was observed between P70S6 kinase-1 and ATG5 in the present study, which means their interrelation in obese women.

Nevertheless, some researchers showed that, absence of S6kinase-1 protects against diet induced obesity[39]. Recently, Zhang N and Ma SH; Lluch et al.,[35&36] showed that, the inhibition of the function of P70S6 kinase-1 can reduce the risk of obesity and helps to treat dyslipidemia, enhance insulin sensitivity and extend life span.

In conclusion, these results of the present study demonstrated autophagy dysregulation associated with obesity in Egyptian women. These result highlight autophagy inhibition as a possible potential strategy for protection against obesity and alleviation of its future complications.

Limitations

More studies on a larger sample size are required to fully understand the autophagy role in obesity and to extend these data. Moreover, other autophagy-related marker proteins, molecular events, mechanisms, and cross talks among these signaling pathways are required to be explored. Extra studies are needed to reveal the precise role of autophagic modulating drugs targeting obesity that may represent a promising therapeutic pathway for its management.

Acknowledgments

None

Declarations

Conflicts of interest

The authors report no conflict of interest.

Ethics approval

All study procedures were approved by the Medical Ethics Committee, Faculty of Medicine, Assiut University (IRB no: 17200758).

Consent to participate

A written informed consent was obtained from each participant.

Consent for publication

Participants have consented to the submission of data.

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Bull. Pharm. Sci., Assiut University, Vol. 46, Issue 1, 2023, pp. 483-496.

نشرة العلوم الصيدلية
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مصاحبة السمنة لاضطراب الالتهام الذاتي عند النساء المصريات

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تسبب السمنة انخفاض في جودة الحياة وعبئا اقتصاديا على المصريين. لذلك من الأهمية بمكان بحث عوامل الخطر لوضع استراتيجيات وقائية محتملة لها ولمضاعفاتها المستقبلية.

تهدف هذه الدراسة المقطعية إلى بحث الخلل في عملية الالتهام الذاتي في النساء المصريات البدينات.

شملت الدراسة خمس وثمانون امرأة تم تقسيمهم إلى ٥ مجموعات (١٧ شخصًا لكل منها):

١- أصحاء ، ٢- وزن زائد ، ٣- متوسطي السمنة ، ٤- شديدة السمنة ، ٥- سمنة مفرطة.

تم تقديرمستويات التعبير عن الجين المرتبط بالالتهام الذاتي (ATG5) بواسطة تفاعل البلمرة المسلسل QRT-PCR ، بينما تم تقييم نشاط انزيم الفوسفو ٧٠ كيناز P70S6-kinase في مصل الدم بواسطة طريقة الإليزا.

كشفت نتائج الدراسة عن مستويات مرتفعة من تعبير الرنا المرسال لـ ATG5 مع مستويات مرتفعة بشكل ملحوظ في انزيم P70s 6 كيناز بطريقة تدريجية في المجموعات الفرعية للسمنة.

وتخلص الدراسة من خلال تحليل منحنى ال ROC أن انزيم p70s6 كيناز أثبت فعاليته كمؤشر حيوي مفيد لتشخيص مجموعات السمنة الفرعية والتنبؤ بها.