Medical Journal of Babylon
Vol. 12- No. 4:1037 -1043 , 2015
http://www.medicaljb.com
ISSN 2312-6760©2015 University of Babylon

Original Research Article

Evaluation of Oxidative Stress Status and Some Biochemical Change in Adult Obese Individuals in Hilla City

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Accepted 7 September, 2015

Abstract

Obesity is a rapidly growing epidemic worldwide, influenced by both genetic and environmental factors. The onset of obesity is due mainly to low energy expenditure (such as from exercise) combined with high caloric intake. The study was conducted on fifty obese individuals and fifty apparently healthy control individuals; the age was between (18-60) years. Blood samples obtained from Marjan Medical City in Babylon Province. The aim of this study to evaluate the differences of oxidant malondialdehyde (MDA), antioxidantenzyme superoxide dismutase (SOD),catalase (CAT), glutathione peroxidase (GPx), insulin resistance (IR) and lipid profile in sera of adult obese individuals and the control group. The results of present study revealed a significant increase in MDA ($p<0.001$), blood glucose ($p<0.001$), insulin ($p<0.001$), insulin resistance ($p<0.001$), total cholesterol ($p<0.05$), TG ($p<0.001$), VLDL-cholesterol ($p<0.001$) and LDL-cholesterol ($p<0.001$) concentration in sera of obese individuals whencompared to those of the control group. Also this study show significant decrease in SOD ($p<0.001$), CAT ($p<0.001$), GPx ($p<0.001$) and HDL-C ($p<0.05$) concentration in sera of obese individualswhen compared to those of the control group. The study concluded that obesity is associated with increase oxidative stress. The increase of MDA concentration and decrease of SOD, CAT and GPxconcentration may contribute in the development of complications of obesity.

Key words: Obesity, MDA, SOD, CAT, GPx, Insulin resistance and Lipid profile.

الخلاصة

السمنة هي الوباء المتنامي بسرعه كبيره حول العالم يتأثر بعوامل وراثية وبيئية .بداية السمنة تعود بصوره رئيسيه الى نقصان الطاقة المفقودة بالمقارنة مع تناول سعرات حرارية عاليه. أجريت هذه الدراسة لتقييم حاله الجهد التاكسدي وبعض التغيرات البايوكيميائية لدى الاشخاص البدناء . ولتحقيق هذا الهدف اختير خمسون شخصا يعانون من السمنه المفرطة بأعمار تتراوح بين(١٨-٦٠) في مقارنه مع خمسين شخصا أصحاء ظاهريا بنفس اعمار المجموعة الاولى كمجموعة سيطرة.تم جمع العينات من مدينه مرجان الطبيه في محافظه بابل.و لتحقيق هذا الهدف تم تقدير الاختلاف بين تركيز المالون ثنائي الألدهايد و مضادات الاكسده (انزيم السوبر اوكسيد دسميوتيز, كاتاليز والكلوتاثايونبيروكسيديز) لدى مجموعه البدناء ومجموعه السيطرة ,و كذلك دراسة المقاومة للانسولين و حاله ايض الدهون في كل من مجموعه البدناء و مجموعه السيطرة.قد اوضحت النتائج وجود زيادة ملحوظة في تركيز هرمون الانسولين ($p < 0.001$) ,المقاومة للانسولين($p < 0.001$),المالون ثنائي الألدي‌هايد ($p < 0.001$), الكلوكوز في الدم ($p < 0.001$), الكولسترول الكلي($p<0.05$),الكليسيريدات الثلاثية ($p < 0.01$), البروتينات الدهنية واطئة الكثافة جدا ($p < 0.001$) و البروتينات الدهنية واطئة الكثافة($p<0.001$) كذلك شوهد نقصان ملحوظ في تركيز سوبر اوكسيد دسميوتيز($p<0.001$) الكلوتاثايونبيروكسيديز ($p<0.001$) ,الكاتاليز ($p<0.001$) والبروتينات الدهنية عالية الكثافة($p<0.05$)في مصول الاشخاص البدناء عند مقارنتها مع مجموعة السيطرة. استنتجت هذه الدراسة إن السمنة لها دورواضح في زيادة حالة الإجهاد التأكسدي و تساهم في تطور مضاعفات السمنة.

Introduction

Obesity is worldwide disease among children and adolescents especially in developed and high resource countries[1],it is a complex disease that involves the interaction between genetic and environmental factors [2]. According to theWorld Health Organization (WHO), there were one billon adults overweight and about 400 million obese people in 2005 while in 2015 the number will become 700 million people. The incidence of obesity in the Arab world (mostly Arab gulf countries) was approximately similar to that found in developed countries[3]. Obesity classification depend on the body mass index (BMI), that equal to weight (in kilograms) divided by the square of height (in meters) [4]. Disequilibrium between energy intake and energy expenditure result inthe accumulation of abnormal and excessive fat stored in adipose tissue[5].

The development of obesity is stimulated by oxidative stress which motivates white fat deposition and changing diet ingestion. Cell culture and animal studies expressed that oxidative stress lead to hypertrophy and hyperplasia of adipose tissue[6].High blood sugar, high circulating free fatty acid (FFA), decreased antioxidant defenses, chronic inflammation and hyperleptinemia might be the linkage between obesity and oxidative stress[7].In obesity high level of metabolism produce increment production of reactive oxygen species (ROS) [8].

Lipid peroxidation is an indicator of oxidative stress in cells and tissues, and is a well-established mechanism of cellular injury in human. Lipid peroxides, derived from oxidation of polyunsaturated fatty acid, are unstable and decompose to form different complex compounds. These include reactive carbonyl compounds such as Malondialdehyde (MDA),which is very reactive bifunctional molecule and has been exposed to cross connection erythrocyte phospholipids and protein. Therefore MDA measurement is widely used as pointer of oxidative stress [9].

Antioxidants define as any molecules able to reduce or avoid the oxidation of other molecules[10]. Antioxidant molecules can be divided into two categories, Endogenous antioxidants: include enzymes that destroy ROSat different stages and in different compartments including intra and inter cellular, such as glutathione peroxidases (GPXs), catalase (CAT) and superoxide dismutases (SODs). Exogenous antioxidants: include some vitamins, carotenoids, polyphenols and some trace elements[11].The antioxidant process can be classified into two types, the first type: Antioxidant enzymes like SOD,CAT and GPx which prevent the formation of ROS by reducing the rate of series beginning; by scavenging initiating radicals[12], and the second type: by Chain-breaking; a free radical releases or steals an electron, a second radical is formed, this molecule then turns around and third radical is formed, continuing to generate more unstable products. The process continues until termination occurs either the radical is stabilized by a chain-breaking antioxidant such as B-carotene, vitamins C and E, or it is simply decayed into a harmless product [13].

Obesity is the important cause of insulin resistance, and obese personshave a tendency to high level of plasma FFAs as a result of reduced suppression of lipolysis by insulin resistance. It is also hypothetical that a reduced ability of adipocytes to store additional calories as triglycerides also contributes to increased accumulation of lipids and their metabolites in other tissues that are not essentially improved to lipid storage such as muscle and liver. As a result, the increase of lipid metabolicintermediates stimulates a variety of cellular abnormalities such as apoptosis, oxidative stress, and endoplasmic reticulum stress, which damages cellular function [14].

The aims of this study are to evaluate the differences of oxidant (malonyldialdehyde), antioxidantenzymes (superoxide dismutase, catalase and glutathione peroxidase), insulin resistance and lipid profile concentration in the sera of obese individuals and control.

Materials and Methods

Subjects

The study included two groups (obese and control group), the age was between(18-60) years. All samples were collected from November 2014 till February 2015. The practical side of the study was performed at the laboratory of Biochemistry Department inCollege of Medicine / University of Babylon . The study was performed on 50 adult obese individuals. All samples ofthis group were classified according to the BMI. They were collected from Marjan Medical City in Babylon Province. The control group includes 50 apparently healthy individuals were collected from medical staff and relatives. They were free from symptoms and signs of any diseases. Any subject (obese and control group) suffered from disease such as, diabetes, circulating diseases(including coronary artery disease,peripheral vascular disease),stroke, hypertension and malignancy which affect oxidation state were excluded.

Blood Sampling

Blood samples were collected from all participants in fasting status using disposable syringes (five mL) at rest.Vein of cubital fossa was punctured and blood drawn slowly then put in plain disposable tube. Blood was allowed to clot at 37˚C for 10-15 minutes and then centrifuged at 2000 Xg for approximately 10-15 minutes, obtained sera stored in five eppindorfs at -20˚C until analysis.

Methods

Serum SOD, CAT and Insulin concentration were determined bycreative diagnostics (USA) ELISA kit. Serum MDA concentration are determined by Guidet B. and Shah S.method **[15]**. Serum glucose concentration was determined by plasmatic (France) spectrophotometric kit. Serum GPx concentration was determined according to the procedure of Rotruck et al with some modification**[16]**. Serum total cholesterol, TGs and HDL-cholesterol concentration were determined by Biolabo SA (France) spectrophotometric kit.VLDL-cholesterol concentration was calculated by dividing triglycerides value by 2.22 **[17]**. LDL-cholesterolconcentration was calculated by using Friedewald equation**[18]**.

Statistical analysis

The results were expressed as mean ± SD. Student's t- test were used for the evaluation of data. Statistical analysis were performed with SPSS version 18.0 software.A p value of < 0.05 was considered to be statistically significant.

Results

The demographic and clinical characteristics of the present study show no significant differences ($p>0.05$) in age, sex and residence in obese group when compared to those of the control group while there was significant increase($p<0.001$) in BMI in obese group when compared to control group, as shown in table (1).

Table 1 : Demographic and Clinical Characteristics of obese and Control Group.

Characteristic	Control group Mean ±SD	Obese group Mean ±SD	p-value
No.	50	50	
Age (years)	32.08 ± 13.44	30.8 ±11.7	P > 0.05
Sex M/F	19/31	20/30	P > 0.05
Residence Urban/Rural	33/17	31/19	P > 0.05
BMI	22.79 ± 1.45	38.36 ± 4.7	P < 0.001

Table (2) showed a significant increase ($p < 0.001$) in fasting serum glucose, fasting serum insulin , HOMA-IR, TGs, VLDL-cholesterol LDL- cholesterol and significant increase ($p < 0.05$) in total cholesterol concentration were found in sera of obese groupwhen compared to those of the control group. In contrast to theHDL-cholesterol concentration which significantly decrease ($p< 0.05$) in sera of obese groupwhen compared to those of the control group.

Table 2 : Mean Fasting Serum Glucose, fasting serum insulin, HOMA-IR, Total Cholesterol, HDL- Cholesterol, TGs, VLDL-Cholesterol and LDL Cholesterol Concentrationin obese and Control Groups.

Parameter	Subjects	Mean ± SD	p-value
Glucose (mmol/l)	Control Obese	4.97 ± 0.56 8.06 ± 1.43	< 0.001
Insulin (µIU/ml)	Control obese	10.85 ±1.84 21.69± 4.24	< 0.001
HOMA-IR	Control obese	2.21± 0.84 7.53 ± 2.44	< 0.001
T-cholesterol (mmol/l)	Control obese	4.1 ± 0.75 5.73 ± 0.27	< 0.05
HDL-cholesterol (mmol/l)	Control Obese	1.32 ± 0.3 1 ± 0.26	<0.05
Triglycerides (mmol/l)	Control Obese	1.27 ± 0.43 2.58 ± 0.84	< 0.001
LDL-cholesterol (mmol/l)	Control Obese	2.12 ± 0.32 3.65 ± 0.41	< 0.001
VLDL-cholesterol (mmol/l)	Control obese	0.52 ± 0.19 1.07 ± 0.42	< 0.001

MDA concentration was found to be significantly increased ($p< 0.001$) in sera of obese group when compared to those of the control group. Also this study showed na significant decrease ($p <0.001$) in SOD,CAT and GPx concentration in sera of obese group when compared to those of the control group as shown intable (3).

Table 3 : Mean serum Super Oxide Dismutase (SOD) , catalase (CAT), glutathioneperoxidase (GPx) and Malondialdehyde (MDA),Concentration in sera of obese and Control Group.

Parameters	Subjects	Mean ± SD	p-value
SOD(ng/ml)	Control Obese	2.35±0.47 0.9 ±0.6	< 0.001
CAT (pg/ml)	Control Obese	321.3 ±78.3 144.2 ± 77.2	< 0.001
GPx (µIU/ml)	Control Obese	731.4 ± 211.4 510.5 ± 208.3	< 0.001
MDA(µmol/l)	Control Obese	2.78 ±0.32 5.29 ± 0.39	< 0.001

Discussion

The increase of insulin resistance and change in lipid profile in obese individuals of the present study might be attributed to the increases in lipolysis by obesity not only increases local extracellular lipid concentrations but also derives accumulation of macrophages in adipose tissue **[19]**, which is associated with systemic hyperinsulinemia and insulin resistance in obese subjects **[20]**.Furthermore, the binding of Insulin to its receptors on the cell membrane necessary to influencethe hormonal actions, therefore, the characters of the insulin receptor were influenced by the structure and functional integrity of the cell membrane.

The fluidity of the cell membrane was dependent upon the fatty acid composition. Increased saturated fatty acids, in hyperinsulinemia were led to diminution the affinity and quantity of insulin receptors which may cause insulin resistance linked with hyperinsulinemia **[21]**. Also, adipose tissue,especially visceral adipose tissue release fatty acid through lipolysis, which causes higher transport of fatty acids to the liver and production of very-low-density lipoprotein (VLDL). High concentration of free fatty acids lead to decrease expression of mRNA and so decline lipoprotein lipase activity(LPL) in adipose tissue and skeletal muscle, and elevated synthesis of VLDL in the liver can prevent lipolysis of chylomicrons, which stimulates hypertriglyceridemia **[22]**.

The outcome of this studywas in agreement with Francesco Perticone *et al.***[23]** who found, that there was a significant increase in fasting serum insulin fasting glucose and HOMA-IR in obese people when compares to the control group and the study conducted by Waleed Mohamed **[24]** who found that obesity is associated with several deleterious modifications in lipid metabolism.

In the current study,the antioxidant enzyme concentration was significantly decreased in obese group. The decrease of antioxidant concentration might be attributed to the increment of ROSin adipocytes which complemented by increasem RNA expression levelsof subunits of NADPH oxidase, an enzyme complex thatcreates ROS, in addition to low levels of mRNA expression and activities of antioxidant enzymes such as glutathione peroxidase (GPX), Cu/Zn superoxide dismutase (Cu/Zn SOD),and catalase(CAT), which are required for balance of redox state and are activated to eliminate ROS when cells are exposed to oxidative stress in other organs . Thus, dysfunction of adipocytes due to dysregulation of antioxidant enzymes **[25]**.The results of the present study were similar to those of Moor de Burgos **[26]**, whose found decreased antioxidant levels in obese adults when compared with the control group.

In the present study obese group show statistically significant increase in serum

MDA level. The most probable causes for the increase MDA level in obese group were increases the mechanical and metabolic load on the myocardium, thus elevated myocardial oxygen consumption. A negative result of the increase in myocardial oxygen intake is the production of ROS such as $OH^{\bullet}$, $O_2^{\bullet-}$ and H_2O_2 from the elevated mitochondrial respiration**[27]**.Also, obesity can cause improved oxidative stress by advanced and accumulative cell injury resulting from pressure from the fat body mass. The release of cytokines by cell injury, especially TNF-α which generates ROS and RNS from the tissues which in turn causes lipid peroxidation**[28]**. The result of the present study were in agreement with *SO Olusi***[29]** who found that sever obesity is associated with lipid peroxidation.

Conclusion

The study concluded that obesity is associated with oxidative stress and insulin resistance which might be contributed to complication of obesity.

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