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ANTIOXIDANT AND OXIDATIVE STRESS IN IRAQI YOUNG OBESE MEN

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Abstract
The aims of the current study was to assess the lipid abnormalities and its effect on the oxidative stress by measuring malonyldehyde MDA level and some antioxidants including superoxide dismutase SOD, catalase CAT and glutathione GSH in one hundred Iraqi obese men in addition to fifty aged matched normal weight young men. The results showed that the MDA level was significantly higher ($p< 0.001$) in obese men 140±16.19 ng/ml than in normal weight 67.6±4.64 ng/ml. The mean SOD concentration in obese men was 1.28±0.46 ng/ml and it was significantly higher ($p< 0.001$) than in normal weight 0.55±0.13 ng/ml . Serum Catalass levels in obese and normal weight were 10.98±6.9 pg/ml and 18.54±2.89 pg/ml respectively and it was significantly ($p< 0.001$) lower in obese group in comparison to normal weight group . The mean serum glutathione concentration was significantly lower ($p< 0.001$) in obese group 5.07±2.43 ug/ml in comparison to normal weight 16.25±5.85ug/ml, the results also showed a distinctive lipid abnormalities represented by the significant increase in cholesterol, triglycerides, LDL and VLDL plus a significant decrease in HDL in obese men than in normal weight.
Key words: Obesity, Oxidative stress, Malondialdehyde, Catalase, Antioxidant

Introduction

Obesity is a medical problem when additional body fat has accumulative to the point that it might be dangerous to health. The body mass index (BMI), which is detected by dividing a one's weight in kilograms by the square of the height in meters, is used by the National Institutes of Health to describe obesity. An individual is deemed obese if their BMI is greater than 30, and obesity is characterized by an increase in the size and number of adipocyte cells (hypertrophy and hyperplasia) [1,2]. In humans, adipose tissue, a big and active endocrine organ that is involved in energy storage, accounts for between 2 and 70% of total body weight. [3]. Adipocytes in obese people extent non-physiological limitations and are not capable to serve as an organ for storing energy. As a result, fat incorrectly accumulates in the heart, muscle, liver, and pancreas, where it can lead to organ malfunction. Particularly, the malfunction of adipose tissue accelerates the start of oxidative stress by enhancing macrophage infiltration, leading to an excess of ROS and inflammatory cytokines. [4,5]. The metabolic balance, which includes lipid metabolism, inflammation, hormonal processes, endoplasmic reticulum stress, and insulin resistance, is dysregulated in obesity. [6,7]. Oxidative stress, which is defined as an inequality between antioxidant defenses and free radical generation, is a frequent mechanism that connects obesity with other related complication which caused tissues damage including endoplasmic reticulum stress, extracellular matrix overproduction, autophagic flux disruption and inflammation . [8,9]. In oxidative stress the production of ROS are partially decreased, some

of the oxygen-containing metabolites are free radicals, which are greatly reactive and have the capacity to damage DNA, fats and proteins, they are produced because of regular cellular metabolism and environmental influences, contrarily, enzymatic such us superoxide dismutase, glutathione peroxidase and catalase plus non-enzymatic such us glutathione, vitamin C plus E. Antioxidants counteract the effects of extremely reactive ROS by converting them into less reactive species and removing oxidation by-products, shielding cells from oxidative damage. [10] Malondialdehyde (MDA), also known as advanced lipid peroxidation end products, is a polyunsaturated lipid peroxidation pathway end product that can bind to proteins and create persistent adducts [11]. Lipid abnormalities are the most frequently feature of obese patients, about 60-70% of obese people have dyslipidemia. In obese patients, higher blood apolipoprotein B, non HDL-C ,triglyceride and VLDL, levels are among the lipid abnormalities. [12]. Oxidative stress is the leading cause of serious obesity complications so the aim of this study was to evaluate some antioxidant comprising superoxide dismutase (SOD). Catalase (CAT), glutathione and the concentration of lipid oxidation product malondialdehyde (MDA) in addition to evaluate the concentration of lipid profile including (cholesterol, triglycerides, HDL,LDL, VLDL) in a group of young obese Iraqi men.

Materials and Methods:

Subjects:

One hundred obese young men aged 15- 30 years and fifty age and sex matched normal weight were comprised in this study. The protocol of the current study was approved via the Clinical Research Committee of university of Anbar- Ramadi- Iraq. Those with severe chronic disease such us diabetes mellitus, hypertension and other diseases related to endocrine disorders, men who taking lipids lowering drugs and those with history of drug use were excluded. Anthropometric information comprising body weight, height and body mass index BMI were measured. The BMI was designed via the equation of weight (kilograms) divided by height (squared meters) as an indicator of general obesity.

Blood samples

Fasting blood samples were drawn into white tubes and putted at room heat for about 20 minutes , the tubes were centrifuged for 10 minute at 3000 RPM, serum was collected and stored at -40°C for future determinations of biochemical outcome measures. commercially available enzyme-linked immunosorbent assay (ELISA) teqneque to determine the concentration of MDA, CAT, GSH, and SOD via using kits from Wuhan Fine Biotech Co.,Ltd. (Wuhan,China) . Determination of Serum Cholesterol, Triglycerides plus HDL By Using DRI-CHEM NX700 Fujifilm using the manufacturer's instructions Fujifilm- Japan. LDL plus VLDL was measured via calculation using Friedewald equation .

Statistics: The statistical analyses were down via the computer programmer SPSS version 20 (Statistical Package for Social Sciences). All studied parameters were stated as mean ± standard deviation (SD). T test was done to indicate the significance of differences among the sets. P value <0.05 was determined as significant.

Result:. The results of BMI and lipid profile were illustrated in table 1 (Table 1), The MDA concentration was significantly higher ($p< 0.001$) in obese men 140±16.19 ng/ml than in normal weight 67.6±4.64 ng/ml (Fig.1). The mean SOD concentration in obese men was 1.28±0.46 ng/ml and it was significantly higher ($p< 0.001$) than in normal weight 0.55±0.13 ng/ml (Fig.2). Serum Catalass levels in obese and normal weight were 10.98±6.9 pg/ml and 18.54±2.89 pg/ml respectively and it was significantly ($p< 0.001$) lower in obese group in comparison to normal weight group (Fig.3). The mean serum glutathione concentration was significantly lower ($p< 0.001$) in obese group 5.07±2.43 ug/ml in comparison to normal weight 16.25±5.85ug/ml (Fig.4).

Table:1 Comparison of BMI and Lipid profile status between obese and normal weight young men

Variables	Obese young men N=100	Normal weight young men N=50
BMI	37.22±3.049**	23.09±1.26
Total cholesterol (mg/ dl)	253.4±33.8**	157.5±29.7
Triglycerides (mg/ dl)	244±71.95**	110.8±27.96
HDL (mg/ dl)	30.38±5.79**	49.44±6.21
LDL (mg/ dl)	87.9±23.63**	32.29±7.15
VLDL (mg/ dl)	48.88±14.44**	22.16±5.59

** Mean there is significant difference at $p \leq 0.001$

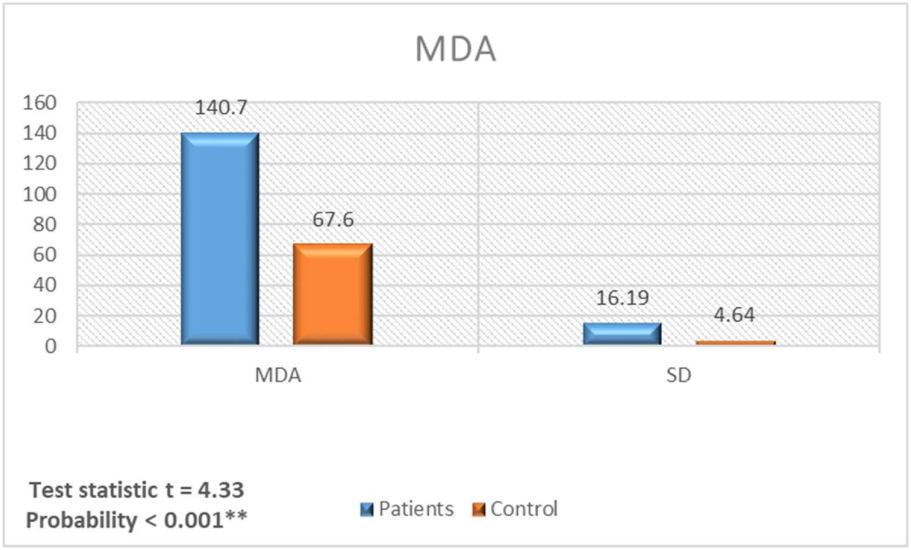

Figure (1): The mean of MDA(ng/ml) in obese patients and normal weight control group

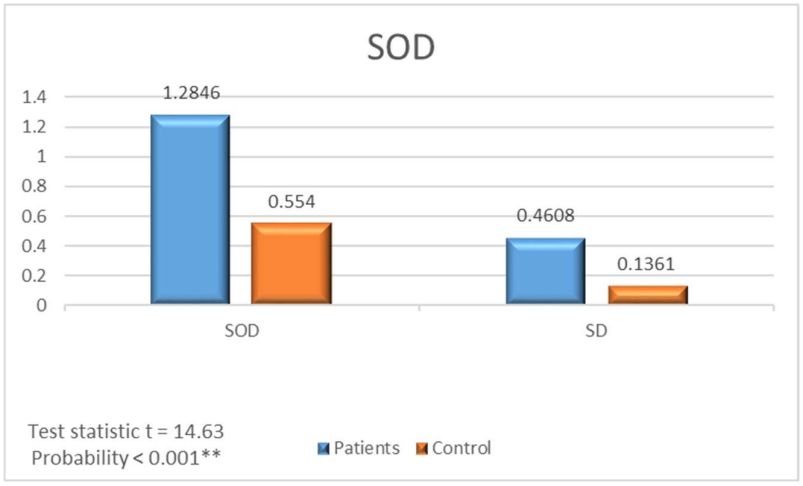

Figure (2): The mean of SOD (ng/ml) in obese patients and normal weight control group

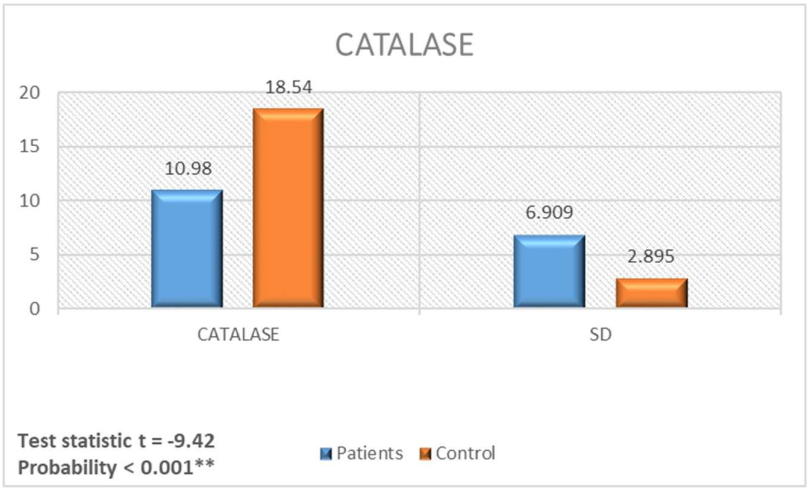

Figure (3): The mean of CAT (pg/ml) in obese patients and normal weight control group

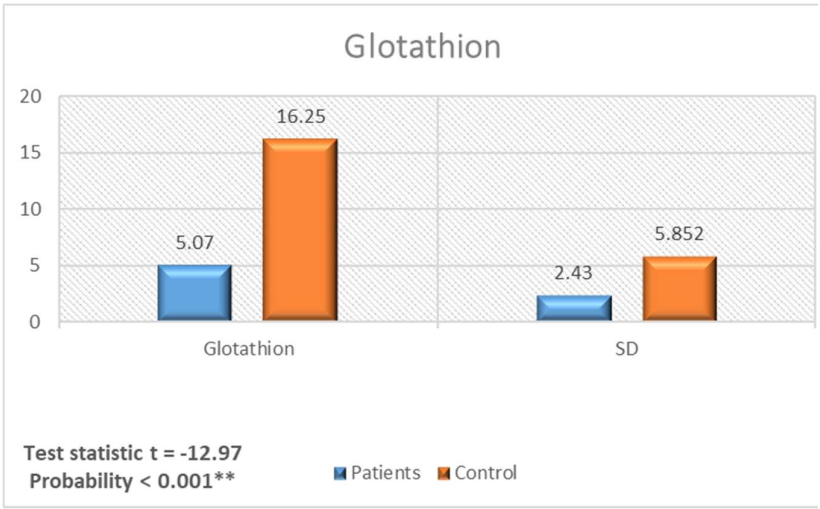

Figure (4): The mean of GSH (ug/ml) in obese patients and normal weight control group

Discussion:

The dyslipidemia seen in obese patients can be attributed to a variety of various disorders [13,14], these anomalies are determined by two condition the first was the larger supply of free fatty acids FFAs into the liver cells from enlarged total plus visceral adiposity, the second condition was a pro-inflammatory status which made by the macrophages which infiltrate to adipose tissue [15]. In obese patients a reduction in the activity of insulin owing to insulin resistance cause an increase in triglyceride lipolysis plus a rise in triglyceride degrade in adipose tissues results in enlarged fatty acid supply into the liver cells [16,17]

Obesity is considered as a pro-inflammatory condition owing to the macrophages which permeate to adipose tissue, Adipokines released by fat cells and macrophage-derived cytokines both modify lipid metabolism [18,19]. The cytokines that cause inflammation such us IL-1 and TNF trigger lipolysis in adipocytes and enlarge the free fatty acid concentrations in the blood, which will offer substrate for the formation of hepatic triglyceride, inside the liver these cytokines activate de novo fatty acid plus triglyceride formation [20], as a results of these changes the generation plus secretion of VLDL will rise, at greater concentrations the cytokines enlarged the angiopoietin like protein 4 expression, the compound that cause an inhibition of lipoprotein lipase and reduce the expression of lipoprotein lipase [21], these changes reduce the activity of lipoprotein lipase, thus delaying the triglyceride rich lipoproteins clearance. The increases in the levels of cytokines which cause inflammation will activate the formation of triglyceride rich lipoproteins and postponement the triglyceride rich lipoproteins clearance, which leading to the rise in serum triglycerides levels that happens in obesity people, pro-inflammatory cytokines also have a potent influence on the metabolism of HDL [22], the first effect, by decreasing the formation of Apo A-I, the chief protein constitutive of HDL, the second effect was that the pro-inflammatory cytokines in macrophages reduce the expression of both ABCA1 plus ABCG1 leading to a reduction in the passing of cholesterol plus phospholipids from the cells to HDL, the third effect was that the pro-inflammatory cytokines reduce the creation and the action of LCAT, causing delay in the alteration of cholesterol to cholesterol esters in HDL molecule, The step which necessary for the creation of a typical globular HDL molecule and simplifies the capacity of HDL to carry cholesterol, in addition to this the pro-inflammatory cytokines reduce CETP concentration, leading to reduce the move of cholesterol from HDL to Apo B enclosing lipoproteins, the other effect of the pro-inflammatory cytokines was the decreasing in the SR-B1 expression in the hepatic cells, the SR-B1 acting a major effect in the acceptance of cholesterol from HDL molecules into liver cells, Cytokines also reduces other vital tasks of HDL, such as its capacity for preventing the oxidation of LDL [18]. The raise in the amount of triglyceride rich lipoproteins in sequence has influences on other lipoproteins definitely cholesterol ester transfer protein CETP the , protein which simplifies the movement of cholesteryl esters plus triglycerides among the lipoproteins and collect triglycerides from VLDL or Chylomicrons and convert them for cholesteryl esters [23]. The raise in triglyceride rich lipoproteins results in an raise in CETP mediated exchange and increase the triglyceride contented and reducing the cholesterol contented of together LDL plus HDL. Obesity as well rises both the action and the quantity of CETP [23]. The triglyceride in both LDL plus HDL molecules is subsequent disassociation via hepatic lipase plus

lipoprotein lipase results in the formation of small opaque LDL plus small HDL molecules [14]. On the other hand the hepatic lipase activity is increased in obese patients with increased visceral adiposity, the increase of the activity of this enzyme simplify the elimination of triglyceride from LDL and HDL forming small lipoprotein particles [14]. The attraction of Apo A-I to small HDL molecules is decreased results in the dismantling of Apo A-I plus the clearance and breakage of Apo A-I via the kidneys [16]. These alterations cause decreas concentrations of Apo A-I plus HDL-C in obese people.

Abnormal metabolism plus metabolites in adipose tissue could produce and stimulate the releasing of extra quantity of pro-inflammatory and inflammatory cytokines in addition the irregular metabolism of the other biochemical components may promote formation and releasing of huge quantity of O_2, OH, H_2O_2 and extra ROS species that rise both oxidative stress plus lipid peroxidation [24]. The current results indicate that the activity of SOD which consider the initial enzyme in the antioxidant defense was significantly higher ($p<0.001$) in obese young men than in normal weight, while the activity of glutathione and CAT were significantly lower ($p <0.001$) in obese young men. A considerably higher oxidative state in obese people may be related to the pathophysiology of obesity [25,26]. Obesity can stimulate a systemic oxidative status via many biochemical mechanisms, including the stimulation of peroxide formation via NADPH oxidases, glycerylaldehyde autoxidation, oxidative phosphorylation, protein kinase C activation and the stimulation of the polyol plus hexosamine paths [27] Additional causes that also leading to the raise of oxidative stress in obese people was hyperleptinemia a state found potentially in obese patients [27]. According to some research, obese individuals have reduced blood antioxidant enzyme activities that depend on having more body fat and central obesity that is directly linked to having more visceral fat [28–33] the results of the current study indicate a significant drop in GSH and CAT levels in obese young men than in normal weight. Adipocyte size increases as a person gains weight, causing adipose tissues to enlarge and become dysfunctional. These tissues also attract macrophages, which alter to a pro-inflammatory state, inflated adipose cells produce extra FFAs, extra ROS, plus extra pro-inflammatory cytokine, the extra FFAs plus diet fats pass into the cells of the muscle, pancreas plus liver causing lipotoxicity, this toxic lipids impaired cellular organelles especially mitochondria, lysosomes and endoplasmic reticulum, these impairment organelles generate extra ROS plus pro-inflammation status, leading to systemic inflammation [8]. The mitochondrial features of obese people are differ from that of lean people. In obese people, the morphology of mitochondria is changed, the biogenesis of mitochondria is defected, the lipid peroxides of mitochondria are enlarged, plus imperfect oxidation of fatty acid , all these changes will generates diacylglycerol, acetyl CoA particles, plus ceramides [34] . The hydrogen peroxide may suffer the Fenton reaction in obese people and form extra hydroxyl radicals [35-37], because of the excess quantities of lipid and glucose which found in obese subjects , mitochondria yield additional quantities of ROS, while in lean people the mitochondria generate less quantities of ROS, the huge quantity of ROS which produce in obese patients will damage the protein, lipid membranes, DNA, plus enzymes involved in the respiratory chain which occur in the mitochondria [38]. The mitochondria of white adipose tissue in obese people are a major origin of ROS, the generation of ROS is accompanies by a rising in the expression

of NADPH oxidase plus a decreasing in the expression of antioxidant enzymes, in addition to this the adipocytes release inflammatory cytokines which are strong activator of ROS formation by monocytes plus macrophages, the raised cytokine concentrations may be also the cause of the rises in oxidative stress in obese patients [39] when the lipids react with ROS the result was lipid peroxides like lipid hydroperoxide, which is hydrolyzed to a mix of mixtures including aldehyde as a major particle, extra MDA formation has poisonous influence on antioxidant enzyme thus MDA can cause a modification in amino acid side chains and oxidize the thiol groups of antioxidant enzyme; these changes frequently leading to partial or complete loss of antioxidant enzymes activity [20].

Conclusion:

Obesity can associate with severe life-threatening effects which can occurs in all people and in different age categories, there is a significant increase in cholesterol. Triglycerides, LDL, and VLDL levels in obese Iraqi young men. The increasing BMI enhanced lipid peroxidation as indicated via increasing MDA plus SOD activity, oxidative status may stimulated by low-grade systemic inflammation state which induced free radical formation and subsequent increase in lipid peroxidation.

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