



EVALUATION OF SERUM REACTIVE OXYGEN SPECIES AND GLUTATHIONE PEROXIDASE IN IRAQI OBESE/OBESE-HYPERTENSION FEMALES

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Abstract

Obesity is a chronic disease of multifactorial origin that associated strongly with the development of hypertension. Our study designed to estimate the oxidative stress in both healthy obese and those with hypertension by measuring the serum level of ROS and GPx. the study comprises 90 subject, divided as 30 healthy control, 30 healthy obese, and 30 obese with hypertension. The age of individuals was (18-72) years old with no significant differences in means, $P>0.05$. ROS mean of healthy obese (154.62 ± 36.02 IU/mL) was significantly higher than controls (85.17 ± 18.71 IU/mL) and the highest value obtained for obese with hypertension (195.93 ± 36.78 IU/mL) $p<0.001$. GPx on the contrary showed a significant decreasing in the obese (33.18 ± 19.58 ng/mL) compared with controls (69.23 ± 17.73 ng/mL) and the lowest value obtained for obese with hypertension (14.91 ± 7.01 ng/mL), $p<0.001$. Thus, obesity induces oxidative stress in females on its own, and the incidence of hypertension leads to raise the level of oxidants.

Keywords : Obesity, oxidative stress, ROS, GPx.

Introduction

Obesity is a disease describe a case of excessive accumulation of body fats (Park *et al.*, 2014). Basically obesity arise from the imbalance between intake and expended energy (Bluher, 2019). Besides the behavioral factor obesity also arise from the interaction of psychological, cellular, metabolic, molecular, and social factors (Fernández-Sánchez *et al.*, 2011). The prevalence of obesity has become worldwide in a rates increases in all ages, ethnicities, and both sexes, obesity along with overweight, extended to almost a third of the population of the world nowadays (Chooi, Ding, & Magkos, 2019). According to world health organization (WHO) obesity defined as a body mass index (BMI) of 30 kg.m^{-2} or greater (Williams, Mesidor, Winters, Dubbert, & Wyatt, 2015). Another way for indicates obesity, central obesity, is waist circumference, and waist to hip ratio (McCarthy, Ellis, & Cole, 2003; Mushtaq *et al.*, 2011). They gave a detection for fat distribution unlike BMI which indicates the overall obesity (Chan, Rimm, Colditz, Stampfer, & Willett, 1994; Kobo, Leiba, Avizohar, & Karban, 2019; Lazarus, Sparrow, & Weiss, 1997).

Obesity is linked to the morbidity of human health, by developing cardiovascular, and metabolic diseases, including type 2 diabetes mellitus, insulin resistance, hypertension, hyperlipidemia, heart diseases, and various cancers (Amirkhizi *et al.*, 2010; Calle & Kaaks, 2004; Verkouter *et al.*, 2019). Obese people have shown an increasing of the likelihood of having hypertension by 3.5 folds, on the other hand, it has been estimated that 60-70 percent of hypertension could be attributed to obesity (Shibao, 2012).

Reactive oxygen species (ROS) is a term comprises all oxygen radicals (e.g., hydroxyl radicals, superoxide radicals, peroxy radicals, singlet oxygen, etc.) and certain non-radical oxygen centered molecules that easily converted into oxygen radicals (e.g., hypochlorous acid, hydrogen peroxide and ozone) (Ghosh, Das, Chaffee, Roy, & Sen, 2018). ROS considered as a main mediators of redox signaling, also regarding to their reactivity, ROS can react with other cellular components such as lipid and DNA, hence causing a

damage (de Lucca Camargo & Touyz, 2019). Excessive production of ROS disturbs the redox homeostasis and leads to oxidative stress (He *et al.*, 2017). Against these oxidants, the cells have evolved an antioxidant defense system which help to neutralize the oxidants in a physiological conditions (Bondia-Pons, Ryan, & Martinez, 2012). Endogenous antioxidants divided into enzymatic such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (Liguori *et al.*, 2018) and non-enzymatic antioxidants such as reduced glutathione (GSH), bilirubin, uric acid, etc. (Rizzo *et al.*, 2010). Any disturbance in the favor of oxidants (increasing in the oxidants or decreasing in the antioxidants) leads to the appearance of oxidative stress (de Barboza, Guizzardi, Moine, & de Talamoni, 2017).

Materials and Methods

Subjects

The study included three groups control (A), healthy obese (B), and obese with hypertension (C). Thirty healthy females were collected as controls for the study from Mustansiriyah University, as well as thirty females of each of the remaining groups were collected from AL-Kindy Obesity Research and Therapy Unit. The ages of the samples were between (18-72) years old. The laboratory side of the study was performed at the laboratory of biochemistry research in the department of chemistry science, Mustansiriyah University.

Sample collection

Blood samples were collected from the individuals in a period of 12 hour fasting. A plastic disposable 5mL syringe was used for venipunctures and blood drawn slowly. Then the blood was translocated into gel tube and left for 10 min at room temperature to clot. Blood samples then centrifuged at 3000g for 10 min and the obtained serum stored in three Eppendorf tubes at $-70 \text{ }^{\circ}\text{C}$ until analysis. Anthropometric measurements was obtained also from the participants including height, weight, and waist circumference.

Methods

Serum ROS and GPx concentration was determined by the ELISA kit from MELSIN (China) and Bioassay Technology Laboratory (China) respectively. BioTech ELISA microplate washer ELX50 and microplate reader ELX800 devices were used.

Results

Results data expressed in the form of (Mean \pm SD) and considered significant at $p \leq 0.5$. The age is non-significant among the three groups. Anthropometric measurements are listed in Table 1, there was significant increase in the BMI, waist circumference (W.C.), and waist to hip ratio (WHpR), in group B and C comparing with controls, $P < 0.001$, the highest was for group C.

Table 1 : Age and anthropometric outcomes in control, healthy obese, and obese with hypertension.

	Group A	Group B	Group C	P-value
Age (year)	32.63 \pm 16.03	34.73 \pm 11.33	39.97 \pm 14.07	0.117
BMI (Kg.m ⁻²)	22.63 \pm 1.78	41.29 \pm 10.85	40.02 \pm 9.97	<0.001
W.C. (cm)	79.57 \pm 5.64	119.43 \pm 16.30	118.60 \pm 15.75	
WHpR	0.77 \pm 0.05	0.95 \pm 0.12	0.96 \pm 0.08	

Serum ROS for obese with hypertension was significantly the highest, where healthy obese females was in the middle with a significant change from the controls $P < 0.001$, (figure 1). the GPx concentration in the serum of

obese females lowered than control and the changes is significant $P < 0.001$, the lowest mean was obtained for obese with hypertension group (figure 2), data listed in table 2.

Table 2 : The differences in means of reactive oxygen species and glutathione peroxidase among the three females groups controls, healthy obese, and obese with hypertension.

	ROS (IU/mL)	GPx (ng/mL)
Group A	85.17 \pm 18.71	69.23 \pm 17.73
Group B	154.62 \pm 36.02	33.18 \pm 19.58
Group C	195.93 \pm 36.78	14.91 \pm 7.01
P-value	<0.001	

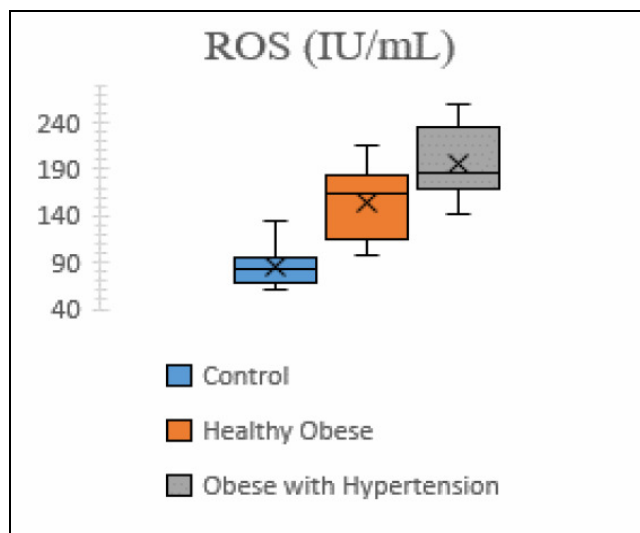


Fig. 1 : Serum reactive oxygen species ROS significant differences among females groups in the study.

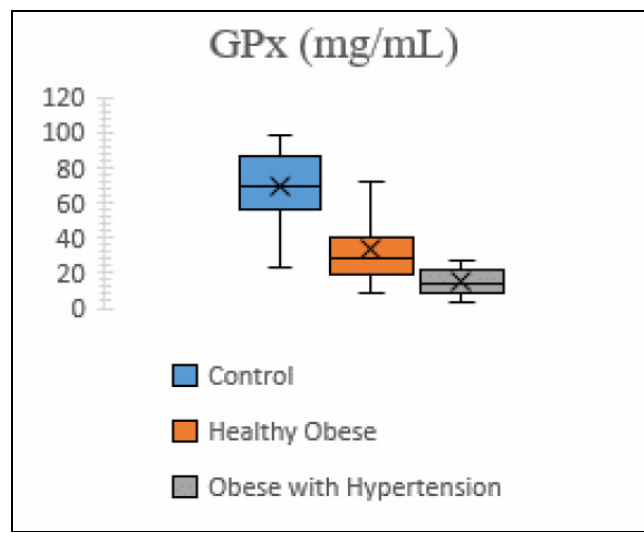


Fig. 2 : Serum GPx significant differences among females groups in the study.

Table 3 : Pearson correlation coefficients (r) among ROS, GPx, BMI, W.C., and WHpR in obese females, (group B+C, n=60).

	BMI	W.C.	WHpR	GPx
ROS	0.372**	0.352**	0.14	-0.449**
GPx	-0.189	-0.157	-0.016	1
** $P < 0.01$				

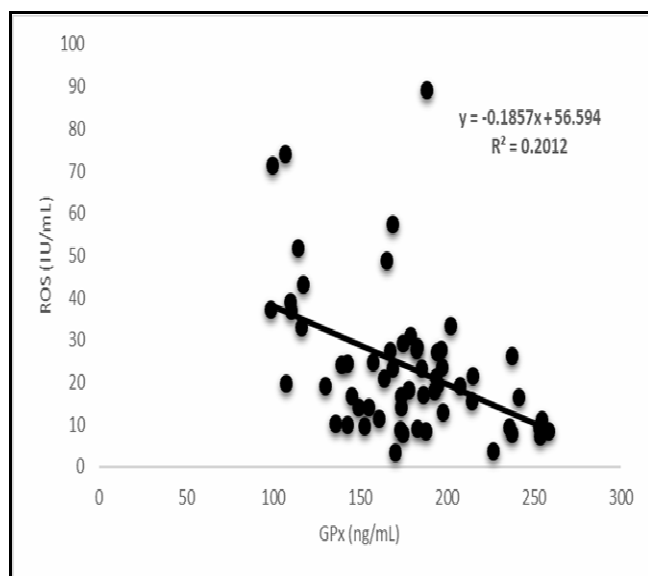


Fig. 3 : Correlation between serum ROS and GPx in obese females.

Serum ROS negatively correlate with GPx ($r=-0.449$, $P<0.001$) (fig. 3), while it correlates in a positive manner with BMI ($r=0.352$, $P<0.005$), and W.C. ($r=0.352$, $P<0.01$). Serum ROS shows no significant correlation with WHpR ($r=0.14$, $P>0.05$). Serum GPx doesn't show a significant correlation with BMI, W.C., and WHpR. Data are listed in Table 3.

Discussion

Oxidative stress status are linked with obesity (Bonnefont-Rousselot, 2014). ROS level increases due to high calories intake or inflammation (Nijhawan, Arora, & Behl, 2019). The generation of hydrogen peroxide and lipid peroxide are elevated in adipose tissue, thus, adipose tissue could be a major source of ROS in obesity (Matsuda & Shimomura, 2013) also, an increasing of the oxidative stress markers were observed in obese humans (Matsuzawa-Nagata *et al.*, 2008).

Our data supports the hypothesis that obesity inducing oxidative stress and similar to research approaches of few researchers. Furukawa *et al* (2004) study on obese humans reveals a strong positive correlation between BMI from one side and the oxidative stress marker, MDA from the other side. Also, H_2O_2 level in the adipose tissue of obese mice were increased, thus, allowed them to presume that the majority of elevated plasma ROS belongs to adipose tissue (Furukawa *et al.*, 2017). Amirkhizi *et al.* (2007), reported that plasma MDA were elevated in obese women as the BMI increases in a positive correlation, and it was significantly higher than healthy women. They also pointed to the activity of the erythrocyte enzymes Cu/Zn-SOD and GPx were significantly higher in healthy women than obese women. Thus, a systemic oxidative stress could enhance by obesity (Amirkhizi *et al.*, 2010). Olusi (2002) observations on oxidative stress markers was the same (Olushi, 2002).

Furthermore, obesity is associated with hypertension, and the relationship between them are well established (Jiang, Lu, Zong, Ruan, & Liu, 2016). There are few suggested mechanisms for the development of hypertension from obesity. The activation of sympathetic system, and renin-angiotensin-aldosterone over activation in obesity, as

well as inflammation and oxidative stress all lead to the incidence of hypertension (Dorresteijn, Visseren, & Spiering, 2012). The data of our study (Table 2) indicates the highly elevation of systemic oxidative stress in obese females who suffering from hypertension, in the comparison with healthy obese, which allow us to presume that oxidative stress is a serious pathway to the development of hypertension in obese individuals.

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