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Relationship Between Obesity and 8-hydroxy-2-deoxy Guanosine as an Oxidative Marker in Obese Adolescents of Giza

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This study was conducted to assess the relationship between obesity markers (Body mass index (BMI), fat percentage) and DNA oxidation marker 8-hydroxy-guanosine (OHG) , as a predictor for future clinical problems in obese adolescents of Giza. The study was conducted on 103 adolescents aged 13-18 years (22 boy, 81 girl). BMI was calculated as body weight (kg) divided by height (m^2) squared and obesity was defined as BMI of 95 percentile. Fat percentage was determined by using Biological impedance technique. Oxidative stress markers as 8- hydroxyl guanosine, superoxide and glutathione were measured. The adolescents were divided according to BMI into two groups. Group I with BMI >95 percentile and less than 97 percentile (obese) and Group II with BMI >97 percentile (severely obese). Significant differences were detected between the two groups (Group I and II) of the study as regard obesity markers (BMI, fat%) and oxidative stress markers (lipid oxidation, superoxide dismutase enzyme activity, glutathione peroxidase enzyme activity, 8-hydroxyl guanosine) ($p<0.0005$). Significant positive correlations were detected between obesity markers and oxidative stress markers among adolescent severely obese cases (group II). Obesity is highly associated with states of oxidative stress in adolescents, with elevated levels of oxidative stress markers, with a positive relation with 8-hydroxy-guanosine and obesity markers and other oxidative markers, suggesting that this marker might play an important role in the prediction of future development of some clinical diseases.

Key words: Obesity, oxidative stress, body mass index, hydroxyl-guanosine, glutathione

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INTRODUCTION

Oxidative stress has been defined as an elevation in the steady state concentration of various Reactive Oxygen State species (ROS) on a cellular level, such as the hydroxyl radical (OH), super oxide anion radical and the nitric acid radicals (Guilder *et al*., 2006). Antioxidants defense mechanisms begin to work by preventing ROS formation and their induced damage through a number of enzymatic and non-enzymatic systems. Under normal physiological conditions, there is a balance maintained between endogenous oxidants and antioxidants, when imbalance occurs, through the excessive generation of oxidants or a decrease of antioxidants, this abnormal oxidant system then enters what is called oxidative stress (Di Renzo *et al*., 2010). Various markers of oxidative damage have been identified (Keaney *et al*., 2003), the most popular markers designed for lipid peroxidation, were malondialdehyde (MDH) and oxidized low density lipoprotein (ox LDL). Recently 8-hydroxy-2-deoxy guanosine (8-oH-2-deoxy Guanosine) (8-OH-dG) emerged as a marker for oxidative stress and acts as a reliable biomarker for DNA oxidative damage (Taylor *et al*., 2010). Excess weight has a great impact on the health and quality of life of individuals. Obesity is reported to be associated with hypertension and elevated serum levels of total and -LDL-cholesterol. It is also a risk factor for diabetes mellitus (Paek and Chun, 2010), cardiovascular diseases, (Guilder *et al*., 2006) and certain neoplasm such as colorectal cancer (Furukawa *et al*., 2004). The exact mechanisms are not well understood, some studies have provided evidence that obesity could contribute to oxidative stress (Keaney *et al*., 2003). In the present study we aim to determine the relation between the degree of obesity and 8- hydroxy-2- deoxy guanosine as a marker of oxidative stress in obese adolescents of Giza.

MATERIALS AND METHODS

This study was conducted by a team from the National Research Center, Egypt, to estimate the prevalence of obesity and metabolic syndrome among school children and adolescents and the potential risk factors for these diseases. It was a cross-sectional survey. Four local public schools situated in Giza governorate were included in the study). The study was performed during the period of October 2007 to April 2009. Permission to perform the study was granted by the Ministry of Education and the protocol was approved by the Ethical Committee of the National Research Centre. Of the total sample, one hundred and three obese adolescents meet the inclusion criteria (22 boys and 81 girls), were included in the current research after obtaining written informed consent from their parents. Adolescents assent was also obtained.

The participated adolescents must meet the following inclusion criteria: age from 13-18 years and Body Mass Index (BMI) greater than the 95 percentile for age and gender based on the CDC Charts (Uhegbu *et al*., 2012).

Adolescents were excluded if they had major illness, specially, type 1 or 2 diabetes, under medications or had a condition known to influence body composition, or insulin action (e.g., glucocorticoid therapy, hypothyroidism and Cushings disease).

Each adolescent underwent a complete physical examination, including anthropometric measures. Height was measured to the nearest 0.5 cm on a Holstein portable anthropometry and weight was determined to the nearest 0.1 kg on a Seca Balance with the subject dressed minimum clothes and no shoes.

Body Mass Index (BMI) was calculated as weight (in kilograms) divided by height (in meters squared). Fat% was measured using Holten Body composition analyzer.

Each measurement was taken as the mean of three consecutive readings following the recommendations of the International Biological program Hiernaux and (Hiernaux and Tanner, 1969). Human Cu/Zn SOD activity was estimated in serum by using Enzyme-linked immuno-sorbent assay ELISA kit produced by Bender Med system GmbH, Austria, Europe, the limit of detection (sensitivity) was determined to be 0.04 mg mL^{-1}.

Glutathione peroxidase activity was estimated in erythrocyte lysate by using ELISA kit produced by Bender Med system GmbH, Austria, Europe, the limit of detection (sensitivity) was determined to be 0.04 mg mL^{-1}.

8-hydroxy-2-deoxyguanosine (8-oH-2-dG) was measured by using Enzyme-linked immuno-sorbent assay ELISA kit, which is fast and sensitive competitive immunoassay for the detection and quantitation of 8-oH-2-dG in serum from Biovender. The inter-assay coefficient of variation of stressgen 8-oH-2-dG ELISA has been determined to be <10%, where the intra-assay coefficient of variation was determined to be <10%.

Lipid peroxidation was determined by using Lipid Peroxide (LPO) assay kit catalogue number 705003 from cayman chemical company. The dynamic range of the kit was 0.25-5 nmol hydroperoxide per assay tube.

The participated adolescents were divided into two groups according to BMI percentile: Group I: with BMI >95 percentile and less than 97 percentile which include 6 boys and 33 girls, GroupII: with BMI >97 percentile which include 16 boys and 48 girls (Uhegbu *et al*., 2012).

Statistical analysis: All subjects values were recorded and tabulated in excel sheets for Microsoft offices xp. SPSS 9.0 was used for analysis of the variables. Quantitative variables were expressed by mean and Standard Deviation (SD). Comparison between group I and group II was done using t-student test. In all tests p-value was considered significant when less than 0.05 and highly significant when less than 0.01. Pearson correlation well used for correlation between different variables.

RESULTS

Body Mass Index percentile (BMI) 97.92±0.64 and fat percentage (fat %) 44.38±6.76 (markers of obesity), were increased in group II versus group I with a high significant difference (p>0.0005) (Table 1). As regard oxidative stress markers, all oxidative stress markers used in this study were highly increased in group II over group I with highly significant difference (p<0.0005) (Table 2). Correlations between obesity markers (BMI and Fat %) and oxidative stress markers (GPx, SOD, 8-oH-2-dG) among group II (severely obese adolescents) were discussed in Table 3. A highly positive correlation between BMI and 8-oH-2-dG (r = 0.886), SOD (r = 0.939) and GPx (r = 0.931), respectively were detected. Fat % as one of the obesity markers also, showed a high positive correlation with 8-oH-2-dG (r = 0.788), SOD (r = 0.788) and GPx, (r = 0.889).

The fourth parameter of oxidative stress, lipid per oxidation level was significantly correlated to 8-hydroxyl guanosine (r = 0.891), to super oxide dismutase activity (r = 0.874) and to glutathione peroxidase activity (r = 0.784), respectively (Table 3). Our data revealed l6 out of 22 (88%) obese male adolescent with mean level of serum 8-oH-2-dG (8.83±2.36) compared to 48 (60%) out of obese female adolescent with 8-oH-2-dG (6.93±0.11) with p<0.05 (Table 4).

Table 1: Markers of obesity in the two groups

Parameters	Mean ±SD	Significance p
BMI percentile Group I	95.93±0.55	p<0.0005
BMI percentile Group II	97.92±0.64	p<0.0005
Fat%I group I (>95 and <97 percentile)	34.96±4.60	p<0.0005
Fat %II group II (>97 percentile)	44.3867±6.7669	p<0.0005

Results are expressed as Mean±SD

Table 2: Markers of oxidative stress in the study groups

Parameters	Mean ±SD	Significance p
OHG I (ng mL^{-1})	1.727±1.12	p<0.0005
OHG II (ng mL^{-1})	10.93±2.36	p<0.0005
Superoxide I (ng mL^{-1})	36.033±19.25	p<0.0005
Superoxide II (ng mL^{-1})	99.93±12.125	p<0.0005
Glutathione I (nmol mL^{-1})	26.500±15.60	p<0.0005
Glutathione II (nmol mL^{-1})	49.03±32.70	p<0.0005
Lipid peroxide I (nmol mL^{-1})	11.13±4.97	p<0.0005
Lipid peroxide II (nmol mL^{-1})	25.36±10.47	p<0.0005

Results are expressed as Mean±SD

Table 3: Correlations between obesity and oxidative stress markers among group II, with referring to correlation between lipid per oxidation and other stress markers

Parameters	BMI II	Fat%	Lipid peroxidation
OHG (ng mL^{-1})			
r	0.886	0.788	0.891
Significance p	<0.001	<0.001	<0.001
Superoxide dismutase activity(ng mL^{-1})			
r	0.939	0.788	0.874
Significance p	<0.001	<0.001	<0.001
Glutathione peroxidase activity (nmol mL^{-1})			
r	0.931	0.889	0.784
Significance p	<0.001	<0.001	<0.001

Table 4: Effects of Gender on levels of 8-hydroxyl Guanosine

Data	Males	Females	p-value
Total number	22	81	
No of positive 8-OHG	16	48	
Percent	88%	60%	
Mean±SD	8.83±2.36	6.93±0.11	p<0.05

DISCUSSION

Obesity is defined by the Body Mass Index (BMI) and Fat % was used for the measure of the degree of body fatness (Wang *et al*., 2008). Various markers of oxidative damage have been identified. The most popular markers were designed for lipid peroxidation, such as Malondialaldehyde (MDA), oxidized LDL (ox LDL) (Channon and Guzik, 2002). Recently 8-hydroxy-2-deoxy guanosine has been emerged as a marker for oxidative DNA damage (Fruebis *et al*., 2001). The latter results from reaction between hydroxyl radical and guanine and acts as reliable biomarker for oxidative damage of DNA (Matsuzawa *et al*., 2002).

The estimation of the degree of oxidative damage and antioxidant status, in obese patients, by appropriate techniques appears to be of interest. Increased oxidative stress and activated antioxidant defense mechanisms, were clearly seen in this study, where the markers of oxidative stress represented by 8-hydroxy-2-deoxy guanosine, showed significant increase in the higher obese (BMI>97 percentile) group compared to the obese (BMI<97 percentile) one. These findings support the results of the study of Karaouzene *et al*. (2011), as they concluded that the oxidative stress markers levels are elevated in human obesity as well as, the markers of antioxidant defense mechanisms represented by the activity of Glutathione Peroxidase (GPx) and the Superoxide Dismutase (SOD) which showed the same significance. Our results clearly showed systemic

oxidative damage of DNA associated with sufficient or increased defense mechanisms against ROS which has been already present in obese children and adolescents. In the current study, group II where found to have significantly higher mean of serum 8-hydroxy -2- deoxy guanosine level compared to group I. This finding, was consistent with that reported by Wiegand *et al.* (2010) who stated that elevated 8 -hydroxy-2-deoxy guanosine among obese. Our finding confirmed that measuring 8-OHG is a novel convenient method for evaluating oxidative DNA damage which may contribute to development of future diseases.

In addition, our result revealed high level of 8-hydroxy-2-deoxy guanosine in boys more that in girls obese adolescent, this gender effect was in reverse to that of Keaney *et al.* (2003) who found high prevalence of oxidative stress markers among females more than in males and also of that reported by Paek and Chun (2010) While this finding supports the result of Taylor *et al.* (2010) on the gender effect on oxidative stress markers. Our findings confirmed that, the 8-hydroxy-2-deoxy guanosine is a reliable method for evaluating oxidative DNA damage in obese person.

Association between obesity markers and oxidative stress markers have been reported in this work as significant positive correlations among obese cases (Table 3) these results are identical to those of Ostrow *et al.* (2011) in which, they concluded that oxidative stress is correlated with adiposity. The mechanism of such relations between obesity and oxidative stress are unclear. Even if several theories have been proposed (Furukawa *et al.*, 2004) it has been suggested that oxidative stress in obesity may result, partially, from the accumulation of triglycerides (Ostrow *et al.*, 2011). Specifically, intra cellular triglycerides are supposed to elevate super oxide radical generation within the electron transport chain by inhibiting the mitochondria adenosine nucleotide transporter (Bakker *et al.*, 2000). The inhibition of this transporter leads to a diminution in intra-mitochondrial Adenosine Diphosphate (ADP), that in turn, reduces the proton flux through the adenosine triphosphate-synthetics reaction (Berg *et al.*, 2002).

As a conclusion present findings confirmed that measuring 8- OHG is a novel convenient method for evaluating oxidative DNA damage which may contribute to development of future diseases. Biochemical investigations aimed at promptly detecting the danger of an oxidative imbalance and restoring antioxidant reservoir for triggering the irreversible damage cascade and thus mandatory, they should be performed in the future as routine and repeated along the treatment plan to monitor the efficacy of antioxidants compounds. We can't exclude, in the future, that an oxidative status check-up followed by immediate targeted treatment will achieve better social health perspectives, especially preventing major illness and heavier social cost burden.

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