Original Article

Association of serum paraoxonase enzyme activity and oxidative stress markers with dyslipidemia in obese adolescents

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ABSTRACT

Objectives: The aim of the present study was to investigate the serum paraoxonase 1 (PON1) concentration and oxidative stress markers and assess its relations with the biochemical parameters in obese adolescents. **Materials and Methods**: One hundred and fifty obese adolescents (range 16-18 years) and 150 healthy age- and sex-matched controls were enrolled in the study. The data were extracted from a project entitled "Obesity among Youth: Lifestyle and Genetic Factors" funded by the Science and Technology Development Fund, Egypt. Serum paraoxonase 1 (PON1), nitric oxide (NO), and malonaldehyde were measured. Anthropometry, fasting glucose, insulin concentrations, total cholesterol, high density lipoprotein–cholesterol, low density lipoprotein–cholesterol, triglycerides, systolic and diastolic blood pressure (BP) were measured. Insulin resistance was determined by Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Diagnostic accuracy of oxidative markers to identify dyslipidemia was calculated with ROC analysis.**Results:** The study showed that PON1 activity was significantly lower in obese adolescents than controls. Obese adolescents had significant lower NO level and significant increased MA values as compared to controls. PON1 was negatively correlated with MAD and body mass index in obese subjects. Obese adolescents showed dyslipidemia and increased blood pressure and HOMA-IR values. PON1 had high area under the curve in ROC analysis for identifying dyslipidemia in obese subjects. **Conclusions:** Our results indicate that obese subjects have increased oxidative stress and decreased PON1 activity. The lower paraoxonase level might contribute to the greater risk of dyslipidemia, insulin resistance, high blood pressure that are considered as important components in the pathogenesis of the metabolic syndrome in obese adolescents.

Key words: Dyslipidemia, obesity, oxidative stress, paraoxonase activity

Introduction

Obesity is associated with several alterations in the lipid metabolism, leading to changes in lipoprotein levels and composition. The relative risk values for developing diabetes, hypertension, dyslipidemia, insulin resistance, dyspnea, and apnea for obese individuals are more than three. Several studies have demonstrated an increase in oxidative stress in obese subjects, with a higher susceptibility to lipid peroxidation of LDL isolated from obese subjects compared with healthy subjects.[1-4] Oxidative stress has emerged as one of the principal causes of atherogenic modifications in low-density lipoproteins (LDL) and, consequently, of atherosclerotic disease.[5] High-density lipoproteins (HDL) have a well-established inverse correlation with the incidence of coronary disease. Several studies have shown that paraoxonase-1(PON1) protects LDL and HDL against oxidative modification. It has been demonstrated that PON1 deficiency is related to increased susceptibility to LDL oxidation and development of atherosclerosis.[6,7] Obesity is associated with enhanced lipid peroxidation and malondialdehyde (MDA), one of several by-products of lipid peroxidation process, is a biomarker that provides an indication of lipid peroxidation level.[8] Moreover,

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endothelium-dependent vasodilation of NO is impaired under conditions of overweight and obesity, which is observed equally in the presence of hypercholesterolemia.[9] It has been reported that obesity may induce systemic oxidative stress (OS) and, in turn, OS is associated with an irregular production of adipokines, which contributes to the development of the metabolic syndrome (MS).[10]

Nitric oxide (NO) is a physiological regulator of diverse functions in several tissues including cardiovascular, neuromuscular, neurological, genitourinary, gastrointestinal, and renal. Inhibitors of nitric oxide synthase (INO) reduce NO production and prevent the decrease in insulin secretion caused by free fatty acids.[11] NO is an important anti-atherogenic agent and it inhibits platelet activation and aggregation, leukocyte chemotaxis, and endothelial adhesion.[12]

The aim of this study was to evaluate PON1 activity and the oxidative status in obese adolescents. This study investigate the paraoxonase activity in obese adolescents group and in healthy controls group, evaluating nitric oxide (NO), malonaldehyde (MDA), clinical characteristics, and biochemical data. We also aimed to examine the association between serum PON1 activity, MAD, body mass index (BMI), and the biochemical parameters.

Materials and Methods

The study included 150 obese adolescents and 150 controls matched by age and sex. Adolescents were defined obese when their body mass index (BMI) above the 95th percentile for gender and chronological age according to Egyptian growth curves.[13] The data were collected from June 2011 to December 2012 and were extracted from a project entitled "Obesity among Youth: Lifestyle and Genetic Factors" funded by the Science and Technology Development Fund (STDF), Egypt. This study protocol was approved by the ethical committee board of the National Research Center of Egypt (No. 10/223). An informed written consent was obtained from all participants. All individuals were clinically evaluated, and anthropometric data were collected. The anthropometric measurements and instruments followed the International Biological Program (IBP) included body weight, height, and waist circumferences. Measurements were taken three times, and the mean values used in the analysis. Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 1 mm. Waist circumference (WC) held at a level midway between the lower rib margin and iliac crest using a non-stretchable tape. All circumferences were measured to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by the square of height in meters. Inclusion criteria were body mass index (BMI) above the 95th percentile for gender and chronological age according to Egyptian growth curves, absence of kidney disease, hypothyroidism, hypertension, or medical problems other than obesity, using various blood tests. Blood pressure was measured 3 times with a standard mercury sphygmomanometer and appropriately sized adult cuffs on the right arm of each subject after a 10-minute rest in a sitting position, and the mean values were used for analysis

All obese subjects and controls underwent standard physical examination; blood samples were obtained following overnight fasting. Serum total cholesterol, HDL-cholesterol and LDL, triglycerides (TG), insulin, and glucose were analyzed by routine biochemical procedures. Insulin resistance was assessed at baseline by using the homeostasis model assessment (HOMA). The HOMA-IR was derived as estimates of insulin sensitivity. HOMA-IR was calculated using the formula fasting insulin (U/mL) X fasting glucose (mmol/L)/22.5.

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the tissue homogenates. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea *et al*.,[14] in which the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red-colored complex having a peak absorbance at 532 nm (UV-VI8 Recording Spectrophotometer, Shimadzu, Kyto, Japan). Nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage *et al*.,[15] where nitrite, stable end product of the nitric oxide radical, is mostly used as an indicator for the production of nitric oxide. Determination of paraoxonase activity was carried out by measuring arylesterase activity of paraoxonase spectrophotometrically in serum following the procedure described by Higashino *et al*.[16] and Watson *et al*.[17] using phenyl acetate (Sigma) as substrate.

SPSS 16.0 software was used for statistical analysis. Quantitative variables were given as mean and standard deviation. Receiver operating characteristic (ROC) curve analyses was used to calculate the area under ROC curves between impaired lipid profile and PON 1. Optimal cutoff value was denoted by Youden index, which is the value that had the highest sum of sensitivity and specificity. All results are presented as mean ± SD. Student's *t*-test was used for the analysis of data with a Gausian distribution. The data with non-Gausian distribution were compared with Mann-Whitney U-test. $P < 0.05$ value was considered to be statistically significant. The Pearson's product moment and Spearman's correlation coefficients were used to determine the relationships between the studied parameters. Receiver operating characteristic (ROC) curve analyses was used to

calculate the area under ROC curves between impaired lipid profile and paraoxonase activity.

The MS was diagnosed by the occurrence of 3 or more of the following risk factors according to the 2007 International Diabetes Federation (IDF): WC greater than 90th percentile for age and gender, Tg ≥ 150 mg/dL, HDL < 40 mg/dL, BP ≥ 130/85, basal blood glucose ≥ 100 mg/dL.

Results

Table 1 summarizes the clinical and biochemical in obese and healthy adolescents. The mean of body weight, BMI, and WC were significantly higher in obese subjects than controls. Serum cholesterol, LDL-C, TG, insulin, glucose and HOMA-IR levels were significantly increased in obese adolescents as compared to controls, while HDL-C was significantly decreased. In addition, SBP and DPB levels were significantly elevated in obese adolescents than controls. Prevalence of MS was significantly higher in obese group compared to controls.

Table 2 presents oxidative parameters and serum paraoxonase level in obese subjects and healthy controls. The paraoxonase and NO levels in the obese patients were significantly lower than controls, while MAD was significantly increased.

Table 1: Clinical characteristics and biochemical parameters in obese adolescents and controls

	Obese adolescents	Controls	*P*
Age (y)	18.17±3.771	19.67±2.679	0.34
Weight (kg)	89.25±8.92	56.26±7.24	<0.05
BMI (kg/m2)	34.01±7.04	21.48±3.161	<0.05
Waist circumference (cm)	99±7.33	86±6.55	<0.05
SBP (mmHg)	119.14±11.81	103.06±11.01	<0.05
DBP (mmHg)	77.24±10.48	68.89±6.07	<0.05
Glucose (mg/dl)	96.71±10.28	89.83±9.07	<0.05
Insulin (μU/mL))	11.61±6.98	10.61±6.98	<0.05
HOMA-IR	3.92±1.59	2.32±1.55	<0.05
Cholesterol (mg/dl)	187.18±27.56	145.00±25.46	<0.05
TG (mg/dl)	95.57±47.97	59.61±23.38	<0.05
HDL-C (mg/dl)	50.21±16.02	64.94±9.61	<0.05
LDL-C (mg/dl)	120.07±32.27	103.59±34.66	<0.05
Cases with MS: *n* (%)	50 (33.3%)	10 (6.6%)	<0.001

BMI: Body mass index, MUAC: Mid upper arm circumference, HDL: High density lipoprotein-cholesterol, LDL: Low density lipoprotein-cholesterol, TG: Triglycerides, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Table 2: Oxidative parameters and paraoxonase level in obese adolescents and controls

	Obese adolescents	Controls	*P*
Paraoxonase (U/L)	129.84±30.75	175.11±9.81	<0.05
MDA (nmol/ml)	2.021±0.57	1.08±0.14	<0.05
Nitric oxide (μM)	36.10±8.90	40.61±2.33	<0.05

MDA: Malondialdehyde

There was a significant negative correlation between PON1 and MAD levels and between PON1 and BMI in obese adolescents [Figures 1 and 2].

Figure 3 shows ROC plot of serum PON level to identify dyslipidemia in obese adolescents. Area under receiver operating characteristic curve (and 95% confidence interval) for PON was 0.98 (.95-1.01) in obese subjects to identify dyslipidemia (high levels of TG and LDL and low levels of HDL). These results demonstrates that serum PON was closely associated risk factor with dyslipidemia and it was a sensitive predictor for dyslipidemia

Discussion

The present study showed that body weight, BMI, and WC were significantly higher in obese children as compared to the controls. As it was expected, the obese group had significantly higher fasting cholesterol LDL-c, TG, insulin,

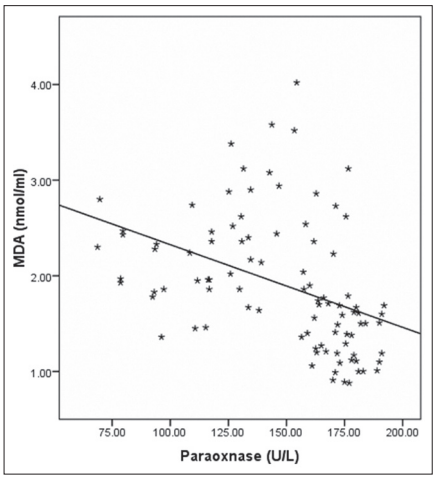

Figure 1: Correlation between the serum levels of paraoxonase and MDA in obese adolescents

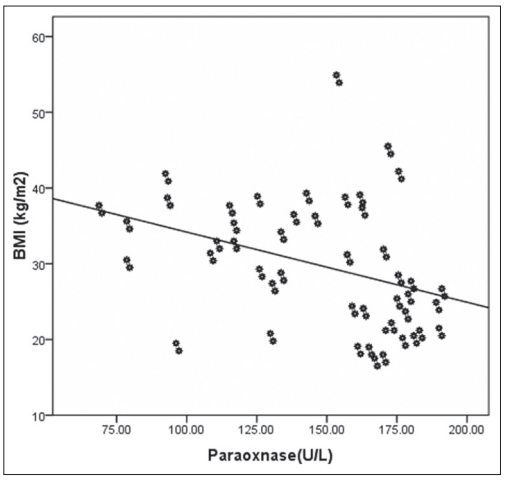

Figure 2: Correlation between the serum levels of paraoxonase and BMI in obese adolescents

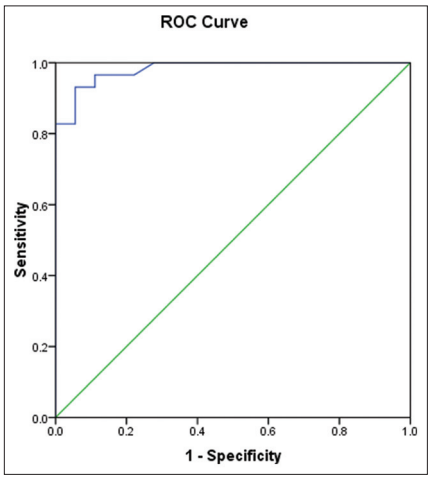

Figure 3: Receiver operating characteristic curves plot of serum PON1 level for identify dyslipidemia in obese adolescents

glucose, and increased HOMA-IR and blood pressure levels as compared to controls. The relationship between insulin resistance and fasting lipids can be explained through the effect of insulin on lipoprotein metabolism. Insulin plays a central role in determining triglyceride clearance from the blood via activation of lipoprotein lipase and triglyceride output through effects on the synthesis and secretion of VLDL by the liver. It is thought that in the insulin-resistant state, triglyceride-rich lipoproteins accumulate in the circulation due to decreased activity of lipoprotein lipase, increased lipolysis in adipose tissue, and increased output of VLDL particles from the liver.[18,19]

Increased oxidative stress and inflammatory biomarkers are known to play an important role in the initiation and progression of atherosclerotic vascular disease.

PON1 is an antioxidant enzyme that inhibits oxidative modification of LDL and contributes to most of the antioxidative activity that has been attributed to HDL. We observed that PON1 activity was significantly lower in obese subjects than in controls. Moreover, our results showed significant negative correlations between PON1 level and BMI as well as with MAD level. Several studies have suggested that there is an association between increased oxidative stress and BMI in obese subjects.[20,21]A number of studies have also suggested that there is a negative correlation between PON1 activity and BMI. However, Rector *et al*.[22] described lower serum PON1 activity in patients with reduced body weight. PON activity has been evaluated in several diseases associated with alterations of plasma lipid levels. The present study showed dyslipidemia in obese adolescents *(*high cholesterol, LDL-c, TG, and low HDL-c levels). The lower PON activity in HDL of obese patients could be due to the presence of circulating inhibitors such as lipid peroxidation products. This hypothesis is supported by previous studies by Aviram *et al*.[23] In this study, serum lipid peroxidation was evaluated by measuring MDA level in obese adolescents. The significant negative correlation found between PON1 and MAD in obese cases demonstrates that subjects with lower PON activity are more exposed to oxidative damage than subjects with high PON activity. A higher susceptibility to lipid peroxidation of LDL related to a decrease in the level of antioxidant molecules has been previously observed in obese patients.[1,4] Oxidative stress of lipoproteins is implicated in the development of coronary heart disease and atherosclerosis.[24] Modifications of serum PON activity have been demonstrated in patients affected by diseases associated with alterations of lipoprotein metabolism, such as diabetes mellitus, familial hypercholesterolemia, and metabolic syndrome.[25,26] Previous studies have shown that the mean MDA levels are higher in obese individuals compared to non-obese healthy controls.[27-30] Moreover, the present study shows that area under the curve (AUC) for identifying the dyslipidemia was high for PON. Oxidative stress has been reported to be involved in the pathogenesis of various diseases such as hyperlipidemia, diabetes, hypertension, which are also associated with obesity and atherosclerosis.[30] The present study showed that NO level was significantly decreased in obese adolescents compared to normal weight controls. Our findings are in the line with other studies, which implicate an association between obesity and decreased bioavailability of NO.[11,31]

As obesity is characterized by excessive storage of adipose tissue, adipokine secretion is increased; therefore, the effects produced in the body are altered, and resistance to its effect can be generated, as in the case of leptin. In addition to adipokines, an overproduction of reactive oxygen species (ROS), which damage cellular structures and trigger, together with underproduction of NO, progressive accumulation of fat and, eventually, the development of other pathologies. Inhibitors of nitric oxide synthase (INO) reduce NO production and prevent the decrease in insulin secretion caused by free fatty acids.[32] The amino acids of NO synthesis, arginine and citrulline reveal no clear correlation with NO. However, both correlate with obesity and with parameters of the glucose metabolism, suggesting an involvement in obesity-related insulin resistance.[33]

Insulin resistance is thought to be the core,[34] dyslipidemia, abdominal obesity, high blood pressure, and thrombotic and inflammatory states are considered as important components in the pathogenesis of the metabolic syndrome.

In conclusion, the present study suggests that obesity is an important factor for enhanced oxidative stress and it is associated with lower antioxidant PON1 enzymatic

capacity. The interaction of oxidative stress with obesity complication, dyslipidemia, hyperinsulinemia, and insulin resistance could contribute to the greater risk of metabolic syndrome in obese adolescents.

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