Study of Myeloperoxidase (MPO) as an Early Indicator of Metabolic Risks in Obese Children


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ABSTRACT
Childhood obesity is associated with a state of chronic low-grade inflammation. It is a major risk factor for chronic diseases and metabolic syndrome risks. Myeloperoxidase (MPO) plays an important role in the initiation and progression of acute and chronic inflammatory diseases, suggesting a positive correlation between activation of MPO and metabolic disorders in obese children. The current study goal to estimate serum MPO levels in obese children and to assess whether MPO is an early indicator for insulin resistance and consequently a predictor for metabolic syndrome in these children. Case-control study included 32 obese children aged 7–15 years compared to 32 normal weight matched age and sex children as control. Clinical examination for all children was done. Anthropometric as well as dietary intake were evaluated for all children. In addition, MPO, glucose, insulin, metabolic lipid parameters, Hb and C-reactive protein (CRP) were determined. MPO was significantly elevated in obese children and positively associated with obesity indices (BMI z-score) and metabolic lipid parameters. Also, MPO showed positive correlation with such proinflammatory and cardiovascular risk biomarkers as CRP. Moreover, higher MPO levels were associated with higher SBP and DBP in obese children. In conclusion, myeloperoxidase (MPO) is an early biomarker and indicator for inflammation associated with cardiovascular and metabolic risks in obese children.

Key words: Childhood obesity, MPO, metabolic risks, inflammation, CVD.

INTRODUCTION
Childhood obesity is increasing in prevalence worldwide, and it is often a precursor to adult obesity. Thus, childhood obesity is considered as a major health problem. Metabolic risks (mainly cardiovascular and diabetes risks) leading to adult comorbidities have become very common and very serious. This increases the need to justify the search for early biomarkers and indicators for those metabolic risks. Endothelial dysfunction is present in atherosclerosis early stages; it can be assessed non-invasively by accepted and standardized techniques at the level of macrocirculation. Correlation between childhood obesity with endothelial dysfunction, inflammation and oxidative stress markers has been reported in many studies, yet more investigations still needed to explore this correlation and relationship. However, some studies provided essential data as regard evolution of endothelial dysfunction in obese children at stage of pro-inflammatory and pro-oxidative changes, and relative insulin resistance. Childhood obesity is associated with a state of low-grade inflammation and obese children usually develop many inflammatory and atherogenic changes. These are associated with vascular diseases in adults and characterized by high serum levels of CRP, tumor necrosis factor-a (TNF-a) and interleukin, whereas some other substances like adiponectin are decreased. Myeloperoxidase (MPO) is an enzyme expressed abundantly in monocytes and neutrophils. MPO is usually associated with oxidative stress and inflammation and it is linked to progression and initiation of chronic and acute inflammatory diseases. MPO-derived substances are thought to be an important cause of endogenous cells damage, particularly in the arterial wall, in which MPO promotes oxidation of low-density lipoprotein (LDL) to contribute in the process of atherogenesis.

MPO serum level has been proved to be high in obese adults with high blood pressure (BP) independently of other cardiovascular risk factors. So it has been widely used to predict cardiovascular risk. It also plays a role in nitration and chlorination of high-density lipoprotein (HDL) in atherosclerotic tissue, which deprive HDL from its cardioprotective effect. Also, high MPO serum levels are observed in adults with chronic kidney disease (CKD), but relation of MPO circulating levels and loss of residual renal function in children is still controversial. Little evidence has been found on association of MPO with cardiovascular risk in children, as high serum MPO was reported in a sample of obese prepubertal children in association with other metabolic and cardiovascular risk factors.
Oxidative stress has been proved to be strongly associated with insulin resistance (IR) in obese children. Recent studies suggest that elevated mitochondrial reactive oxygen species plays a causative role in many forms of muscle insulin resistance. The protective effect of the antioxidant defense system against insulin resistance has been previously demonstrated. Nutritional or behavioral factors, assedentary lifestyle and high-fat diet may lead to insulin resistance and oxidative stress in obese subjects. However, it also has been found that some enzymes, such as MPO, play a role in the development of insulin resistance and oxidative stress; therefore, these enzymes and substances needs to be more investigated in obese children having insulin resistance.

In the current study, we sought to determine whether MPO activation is implicated in the development of obesity-associated insulin resistance and in turn metabatc risks in Egyptian children.

**SUBJECTS AND METHODS**

**Subjects**

This case-control study included 32 simple obese children (Obese) compared to 32 healthy normal weight children (Control) with age range of 7-15 years attending Nutrition Outpatient Clinic of the Medical Research Centre of Excellence-National Research Centre (MRCE-NRC), Giza, Egypt. Medical Ethical Committee of the National Research Centre approved the present protocol. All children were apparently and clinically healthy, without acute infection, and they did not take drugs known to induce weight changes.

**Methods**

All children were subjected to full personal, past history for systemic diseases, and family history of chronic non-communicable diseases (obesity, diabetes, cardiovascular diseases and hypertension).

Detailed medical history and assessment, physical examination and anthropometric evaluations were performed to all children; including measurement of blood

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Table 1: Anthropometric, clinical and laboratory data of obese children and control.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Obese (N=32) Mean±SD</th>
<th>Control (N=32) Mean±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI z-score (kg/m²)</td>
<td>4.33±1.24</td>
<td>0.23±0.17</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.8±2.0</td>
<td>11.1±2.4</td>
<td>^0.634</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 15 (46.9%)</td>
<td>Female 11 (34.4%)</td>
<td>#0.309</td>
</tr>
<tr>
<td>Waist circumference (WC) (cm)</td>
<td>112.0±26.7</td>
<td>61.8±3.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>108.3±10.0</td>
<td>87.7±4.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.0±10.5</td>
<td>58.4±2.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12.2±0.9</td>
<td>12.1±1.0</td>
<td>0.527</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>83.6±16.1</td>
<td>81.0±6.3</td>
<td>0.400</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>164.7±37.4</td>
<td>148.6±19.1</td>
<td>0.034*</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>93.5±34.2</td>
<td>77.9±16.2</td>
<td>0.023*</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>107.8±36.9</td>
<td>86.1±18.5</td>
<td>0.004*</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>38.4±7.1</td>
<td>46.5±5.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MPO (ng/ml)</td>
<td>32.0±13.5</td>
<td>6.3±2.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>5.4±3.3</td>
<td>2.9±0.8</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*p<0.05, *p<0.001, ^t-test, #Chi square test

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Table 2: Correlation between MPO and anthropometric, clinical and laboratory data in obese children

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.026</td>
<td>0.888</td>
</tr>
<tr>
<td>BMI z score</td>
<td>0.492</td>
<td>0.004*</td>
</tr>
<tr>
<td>SBP</td>
<td>0.357</td>
<td>0.045*</td>
</tr>
<tr>
<td>DBP</td>
<td>0.354</td>
<td>0.047*</td>
</tr>
<tr>
<td>Hb</td>
<td>0.132</td>
<td>0.470</td>
</tr>
<tr>
<td>FBG</td>
<td>0.150</td>
<td>0.411</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.357</td>
<td>0.045*</td>
</tr>
<tr>
<td>TG</td>
<td>0.362</td>
<td>0.041*</td>
</tr>
<tr>
<td>LDL</td>
<td>0.373</td>
<td>0.035*</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.384</td>
<td>0.030*</td>
</tr>
<tr>
<td>CRP</td>
<td>0.656</td>
<td>&lt;0.001*</td>
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One study on animals described the association between neutrophils infiltrate in adipose tissue during early stage of high fat diet administration and release of some various substances, including reactive oxygen species, TNF-α, and MPO. These modulators are well known inducers for inflammation. Studies on humans showed that neutrophil counts in peripheral blood are increased with obesity and type 2 diabetes mellitus. Patients with either one of these diseases have high serum MPO levels, suggesting a positive correlation between development of metabolic diseases and serum MPO. Thus, MPO is considered as cardiovascular risk factor and early biomarker of inflammation in obesity. However, functional role of high MPO levels in the pathogenesis of insulin resistance still needs further studies.

Because MPO is predominantly derived from activated monocytes and neutrophils, high serum MPO levels in obese children is likely to reflect activation and influx of these cells. Chronic low-grade inflammation and the activation of the immune system recognized to be involved in the pathogenesis of insulin resistance associated with obesity and type 2 diabetes. Adipose tissue, liver, muscle and pancreas are common sites of low-grade inflammation in obesity. An infiltration of immune cells and macrophages is observed in those tissues and associated with a cell shift from anti-inflammatory profile to pro-inflammatory profile.

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Table 3: Regression models for factors affecting MPO in obese children

<table>
<thead>
<tr>
<th>Factors</th>
<th>β</th>
<th>SE</th>
<th>P</th>
<th>95% CI</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI z-score</td>
<td>4.489</td>
<td>2.484</td>
<td>0.081</td>
<td>-0.584–9.562</td>
<td>0.098</td>
</tr>
<tr>
<td>CRP</td>
<td>1.737</td>
<td>0.232</td>
<td>&lt;0.001</td>
<td>1.264–2.211</td>
<td></td>
</tr>
</tbody>
</table>

β: Regression Coefficient, SE: Standard Error, CI: Confidence Interval, R²: Coefficient of determination, *Significant

pressure and comparing it to age specific blood pressure percentiles reported by Falkner and Daniels.

All anthropometric measurements have been obtained using standardized equipment, and following the recommendations of the International Biological Program. Children were weighed (in kg) using a calibrated Seca scale to the nearest 0.1kg while height (in cm) was measured using a Harpenden Stadiometer to the nearest 0.1 cm. Body mass index for age was recorded according to World Health Organization (WHO) standards. Calculation of BMI according to the following known formula: BMI = Weight (kg) / Height (m)². The Z-score for BMI (BMIz) was based on reference standards published by Cole et al.

Obesity is defined as a BMI at or above 95th percentile for children of the same age and sex. Waist circumference was measured to the nearest 0.1 cm. Assessment of waist circumference was done using categories reported by Fernández et al. Systolic and diastolic BP were measured three times by the same examiner using a mercury sphygmomanometer and following International Recommendations. The mean of the three measurements was considered the current value.

Laboratory assessment

Blood samples were withdrawn from children after overnight fasting. Each blood sample was divided into two portions, the first small portion was taken on EDTA coated tube for determination of hemoglobin concentration. The other large portion was left to clot and serum was separated by cooling centrifugation (4°C) at 1800 xg for 10 min, then stored immediately at -20°C in clean plastic eppendorf until analysis. Hemoglobin concentration was determined according to the colorimetric method of Drabkin and Austin. Fasting blood glucose was determined using Stanbio Enzymatic glucose procedure. Fasting serum insulin was estimated using BioSource INS-ELIZA following the method of Temple et al. Cholesterol was measured using an enzymatic method. Triglycerides level was quantified by using an enzymatic method. Serum HDL-cholesterol concentration was measured after precipitation of the very low density lipoprotein (VLDL), and low density lipoprotein (LDL) using BioSystems kit, according to the method of Grove. Then, HDL-cholesterol was spectrophotometrically estimated from the supernatant using the cholesterol Spinreact kit, according to the method of Naito. Serum level of LDL-cholesterol was measured from the difference between serum total cholesterol concentration and cholesterol concentration in the supernatant of serum sample which obtained as a result from precipitation of LDL-c using BioSystem kit according to the method of Assmann et al. CRP was measured by CRP-Latex test using CRP-Latex kit purchased from CHEMICALS S.L. CRP Latex test is based on a modification of the latex fixation method of Singer and Plotz developed for the direct detection and semi-quantitation of CRP in serum. Serum myeloperoxidase (MPO) was estimated using Quantikine ELIZA kit of R&D systems catalog number DMYE00B for the quantitative determination of human MPO concentration in serum according to the manufacturer’s instructions.

Statistical analysis

Collected data were coded, tabulated, and statistically analyzed using IBM SPSS VS. 22.0, IBM Corp., Chicago, USA, 2013. Descriptive statistics were done for quantitative data as minimum and maximum of range as well as mean±SD (standard deviation) for quantitative parametric data, while number and percentage were done for qualitative data.

Inferential analyses were done for quantitative variables using 95% confidence interval (CI) independent t-test in cases of two independent groups with parametric data. In qualitative data, inferential analyses for independent variables were done using Chi square test for differences between proportions. Correlations were done using Pearson correlation for numerical parametric data, and partial correlation test in cases of controlling certain variables. Linear regression model used to find out independent factors affecting certain conditions. Level of significance was taken at P value < 0.05, otherwise is insignificant.

RESULTS

Table (1) shows the anthropometric, clinical and laboratory data of the children, confirming the similar age and sex distribution between normal weight (Control group) and obese children (Obese group). The mean values of BMI z-score, WC, SBP, DBP, Cholesterol, TG, LDL, MPO and CRP were all significantly higher in obese children in comparison with the controls. Meanwhile, the mean value of HDL was significantly lower in obese children versus the controls. No significant differences were found in fasting blood glucose and Hb concentrations between the two groups.

The data in Table (2) show that in obese group there are significant positive correlations between MPO and BMI z-score, SBP, DBP, Cholesterol, TG, LDL, and CRP. On the other side, there is significant negative correlation between MPO and HDL in obese group as well. Figure (1) shows significant positive correlation between MPO and CRP. On the other hand, there is significant negative correlation between MPO and HDL (Figure 2).

Multiple linear regression analysis of MPO with associated risk factors was carried out to evaluate whether these factors (BMI z-score, Age, Sex, WC, SBP, DBP, Hb, FBG, Cholesterol, TG, LDL, HDL and CRP) were dependent on MPO or not. This analysis showed that only...
BMI z-score ($\beta = 4.489; P= 0.081$), and CRP ($\beta = 1.737; P < 0.001^*$), were dependently associated with MPO in obese children (Table 3).

DISCUSSION
Although there is backbone of literature tackling MPO relation with metabolic risks and inflammation in obese adults; yet there are no enough studies emphasizing this relation in children and adolescents age group. Furthermore, some recent prospective studies in children proved that childhood obesity-associated cardiometabolic risk factors can negatively affect overall health condition in adulthood.

The current study investigates the relation of circulating MPO levels and metabolic as well as cardiovascular risk factors in a group of obese children in comparison to normal weight children as controls. Obesity is specifically related to some anthropometric, physiological, and biochemical abnormalities, which predispose to metabolic risks and insulin resistance in children. Although waist circumference in children is considered as good predictor of visceral adiposity and related risks$^4$, it may not be useful to detect differences among variations in ethnic populations and puberty-related body proportions. The present study demonstrates correlation of BMI z-score with blood pressure and
dyslipidemia in obese children which proves better findings than waist circumference in this situation. By choosing BMI z-score for detection of metabolic risk in obese children, we found that it strongly correlated with the lipid profile in obese children versus the normal weight control group. Results also proved elevation in metabolic risk factors in obese children than normal weight control group such as: BMI z-score, WC, SBP, DBP, Cholesterol, TG and LDL. These results come in line with those previously published by Shen24 and Shao et al25. High serum cholesterol level in obese children suggests an increased incidence of myocardial infarction in adults. Moreover, these children will suffer from hypercholesterolemia in adulthood2.

Acute inflammation alters the concentration of HDL-associated enzymes as a growing body of evidence indicated that inflammation converts HDL into a dysfunctional form and myeloperoxidase may contribute to these aberrant functions of HDL with impaired levels in patients with atherosclerosis. Thus, circulating HDL might be useful indicators of the risk of cardiovascular disease.

The present work proved negative correlation between MPO and HDL in obese children indicating that altered HDL in obese children with high MPO level is more prone to cardiovascular diseases in their adulthood. These results match those in the recent studies performed by Shao et al25, and Marin et al26, who mentioned that altered HDL and LDL concentrations along with high MPO in obese children with T2DM reflect potentially increased cardiovascular risk. Our results revealed an elevated level of MPO and significant positive correlation between MPO and cholesterol in obese hypercholesterolemic children, suggesting the increased risk of endothelial dysfunction in adulthood among obese children. These results fit similar findings reported by Pignatelli et al27, and Olza et al28. who considered elevated MPO plasma levels in hypercholesterolemic prepubertal obese as direct metabolic and cardiovascular risk.

Myeloperoxidase is proved to be an active modulator of endothelial dysfunction in animal models, adult humans and cell culture29 and there is a strong relation between MPO and endothelial dysfunction in overweight adults. In addition, high levels of MPO have been associated with increased metabolic and cardiovascular risks in healthy adults29. That’s why MPO serves as an independent biomarker in assessment of cardiometabolic risk of future coronary arteriosclerosis3.

Our study didn’t prove correlation between MPO and fasting serum glucose level in obese children or significant statistical difference between obese and normal weight controls, which fit results reported by Olza et al3. This indicates that MPO could be considered as better indicator for cardiovascular risk and endothelial damage than indicator for insulin resistance and related health risks. MPO and its derived reactive substances are thought to be associated with arterial wall injury leading to elevated blood pressure. Blood pressure tracks through childhood into adult life and represents one of the foremost vital predictors of cardiovascular risks later in life3. The fact that obese children with high serum MPO levels are having high blood pressure raises the probability of having cardiovascular diseases later in their life.

According to the present study, high MPO levels were detected with high systolic blood pressure and diastolic blood pressure in obese children for their ages. Moreover, MPO was correlated significantly to BP for age in obese children group. Circumstantial evidence supports that, even in children, blood pressure measurements are accurate method to predict target-organ damage and CV risk in obese children3, which is concordant with the findings of Van der Zwan et al25 and Özgen et al2.

C-reactive protein (CRP), a well-known biomarker of inflammation, associated with adiposity in children and adolescents has been proposed to be metabolic risk in obese children29. Mechanisms of action of MPO in metabolic and cardiovascular risks associated with obesity involve modulation of inflammation and the immune system response, suppression of nitric oxide production, endothelial dysfunction and lipoprotein function impairment. MPO is taken into account as a typical link between inflammation and aerobic stress systems and it is closely related to the vascular wall25.29. On the opposite hand, endothelial dysfunction with no inflammation may go through more benign prognosis3, unfortunately suggesting that inflammation could act as a catalyst for initiation of cardiovascular disease.

Tabulated results revealed that serum levels of CRP as proinflammatory factors are significantly increased in obese children relative to normal weight counterparts. Furthermore, strongest linear positive correlation and multiple linear regression analysis of serum MPO are observed with CRP in obese children. Therefore, the role of MPO as a biomarker, predictor and relevant indicator of cardiovascular risks in obese children is reinforced, by showing that obese children already have a state low grade inflammation and pro-oxidation which is contributing in the process of endothelial impairment. Thus, our results add more support to the theory that childhood obesity may lead to metabolic and cardiovascular risk later in life2.

Mechanisms and associations involved continue to be hardly understood. Further studies are still needed to highlight the role of MPO and its related oxidative stress and inflammation in the pathophysiology of obesity associated metabolic and cardiovascular risks especially in children. Fortunately, compared to adults, youngsters are better educated to decrease the risks of cardiometabolic disorders.

In conclusion, the aforementioned data provide a clear evidence favoring a direct role of MPO in inflammation associated with cardiovascular and metabolic risks in obese Egyptian children. Thus, MPO could be considered as a good predictor for cardiometabolic complications of obesity.

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