

Serum levels of monocyte chemoattractant protein-1, interleukin-6, and paraoxonase-1 in childhood obesity

Enas R. Abd El Hamid^a, Azza A. Abdel-Shaheed^a, Rania N. Sabry^a, Eman R. Youness^b, Mones M. Abu Shady^a, Amr Said Megawer^c, Nadia A. Mohamed^b

Departments of ^aChild Health ^bMedical Biochemistry, National Research Center
^cDepartment of Pediatrics, Research Institute of Ophthalmology, Cairo, Egypt

Correspondence to Rania N. Sabry, PhD,
Department of Child Health, National Research
Centre, El Bouhouth Street, 12311 Dokki,
Cairo, Egypt
Tel: +20 100 512 9866; fax: 002 22697702;
e-mail: rania.n.sabry@gmail.com

Received 18 November 2014

Accepted 18 January 2015

**Journal of the Arab Society for Medical
Research** 2015, 10:27–31

Background/aim

Obesity is becoming an epidemic health problem. Elevated cytokines and chemokines are prominent features in obesity, which play a main role in the development of other chronic diseases. The aim of this study was to determine the serum levels of monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), and serum paraoxonase-1 (PON1) in childhood obesity.

Materials and methods

The present study included 40 obese school-aged children (5–15 years) and 40 healthy children as controls. The patients were presented to the outpatient clinic in National Institute of Nutrition. MCP-1, IL-6, PON1, total cholesterol, and triglycerides were measured in all participants.

Results

The mean serum levels of MCP-1, IL-6, and total cholesterol were significantly higher in obese participants than in controls ($P < 0.0001$), whereas the PON1 was significantly lower in obese participants than in controls ($P < 0.0001$). MCP-1, IL-6, and serum cholesterol levels showed significant positive correlation with BMI ($P < 0.05$), whereas PON1 showed a significant negative correlation with BMI ($P < 0.05$). Multiple regression analysis showed a strong association between PON1 activity and BMI ($P < 0.0001$).

Conclusion

Childhood obesity is associated with increased serum MCP-1 and IL-6 and decreased PON1 and hypercholesterolemia suggesting an increase in adulthood disease risk. Measuring serum MCP-1, IL-6, PON1 activity in obese children may be a good predictor for future chronic disease development and complications.

Keywords:

interleukin-6, monocyte chemoattractant protein-1, obesity, paraoxonase-1

J Arab Soc Med Res 10:27–31
© 2015 The Arab Society for Medical Research
1687-4293

Introduction

Obesity is becoming an epidemic health problem. Worldwide, the prevalence of obesity in children has steadily increased over the last 20 years, especially in developing countries [1]. Being overweight or obese is determined on the basis of BMI, with varying measurements for the different age groups and sexes.

Systemic inflammation, characterized by elevated circulating cytokines and chemokines, is a prominent feature of obesity [2]. Research in humans has demonstrated a mediating role for systemic inflammation in the development of chronic diseases, such as cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2) [3]. Peripheral blood monocytes play a role in cytokine production, migration into inflamed adipose tissue, and vascular adhesion [4], all of which contribute to the development of obesity comorbidities. Cytokines and chemokines, including monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6), interact with monocytes, contributing to the inflamed state [5].

MCP-1 is a monomeric polypeptide that belongs to the chemokine family, which plays a crucial role in monocyte recruitment. IL-6 is a circulating cytokine with various functions such as inflammation, host defense, and tissue injury. It is produced by many cell types and tissues, including immune cells, fibroblasts, endothelial cells, skeletal muscle, and adipose tissue. Adipose cells contribute 15–30% of circulating IL-6 levels in the absence of acute inflammation. IL-6 production is significantly enhanced by adipose tissue in obesity. The two major adverse effects of increased IL-6 in obesity are insulin resistance and increased risk for cardiovascular complications [6]. In adults, monocyte concentration and activation state are elevated with increasing body fat [7]. There are several causes of obesity, including metabolic, hormonal, and genetic factors. The main factor contributing to excessive weight gain in children is the imbalance between energy intake and loss. Most of the known genetic causes of obesity primarily play role by increasing energy intake. Increasing evidence suggests that numerous cytokines, which are secreted

mostly from adipose tissue play a role in energy balance, metabolism, and inflammatory response in humans [8].

Human serum paraoxonase-1 (PON1) is an enzyme with esterase activity and is physically bound to high-density lipoproteins (HDLs). It plays a key role in the action of HDL toward protection of lipoprotein and biological membrane against oxidative damage. It may have a protective role against atherosclerosis by virtue of its action on hydrolyzing lipid peroxides and preventing accumulation of phospholipids in oxidized low-density lipoprotein [9]. Early signs and predictors of CVD and DM2 are now present in young children, including hypertension and dyslipidemia [10]. Obesity during childhood is strongly correlated to adult obesity, furthering the risk for chronic diseases and posing a challenge to both individual quality of life and economic and healthcare costs [11].

Few studies have searched the relationship between monocytes, systemic inflammation, and disease risk markers in overweight and obese children. The aim of this study was to evaluate the relationship between obesity status and systemic inflammation markers including circulating cytokines and chemokines such as MCP-1 and IL-6, as well as markers of disease risk, including total cholesterol and triglycerides, in school-aged Egyptian children. This study reviews the changes in serum PON1 activity in obese children.

Materials and methods

This case-control cross-sectional study included 40 obese school-aged children (age range 5–15 years). Their mean age was 8.98 ± 2.87 years; 16 of them were boys and 24 were girls. No sex difference was found between male and female samples as regards studied parameters ($P < 0.05$). Forty healthy age-matched and sex-matched children served as controls. All patients were from Cairo, Egypt. The study was approved by the local ethical committee of the National Research Center. Written consent was obtained from the children's parents for participation in the study. BMI was calculated using height and weight (weight in kg/height² in meter). Participants were classified on the basis of BMI using age-specific BMI percentile of National Egyptian Growth Curves for children: obese children (BMI percentile ≥ 95 for age and sex) and healthy children (BMI percentile < 85 for age and sex). Patients included in the study did not have hepatic or renal dysfunction, chronic inflammation, DM, or clinically significant infectious diseases. None of the patients was taking any medications that interfere with the study. The patients were recruited from the

outpatient clinic, National Institute of Nutrition and subjected to full history taking, general examination including anthropometric measurements, and laboratory investigations including blood chemistry and cytokine analysis. MCP-1, IL-6, PON1, total cholesterol, and triglycerides were measured in all patients.

Venous blood sample was drawn after a 12 h overnight fast. Samples were separated by means of centrifugation and stored at -80°C until analysis. Serum levels of MCP-1 were measured using Human Monocyte Chemotactic Protein-1 ELISA Kit (Life Technologies, Chicago, USA). IL-6 was measured using a commercially available IL-6 ELISA Kit (R&D System, USA). Total cholesterol and triglycerides were measured using Abbott-Aeroset (Life Technologies, Chicago, Illinois, USA) autoanalyzer with original kits. Arylesterase activity of PON1 was measured spectrophotometrically in supernatants using phenylacetate as a substrate by the method of Watson *et al.* [12].

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS), version 16, for Windows (SPSS Inc., Chicago, Illinois, USA). Continuous data were expressed as mean $\pm$ SD and were compared using Student's *t*-test. Pearson's correlation analysis was conducted to evaluate the association between continuous exposure and continuous covariates. Multiple linear regression analysis was carried out to identify the influence of multiple variables (age, sex, MCP-1, paraoxonase, IL-6, serum cholesterol, and triglyceride) on a dependent variable (BMI). A *P* value less than 0.05 was accepted as statistically significant.

Results

The current study included two groups: obese children (40 children) and control (40 healthy children).

The anthropometric and laboratory data of the obese and control children are shown in Table 1. There were statistically significant differences in the means of BMI, MCP-1, IL-6, PON1, and serum cholesterol levels between obese and control participants ($P < 0.001$). The mean BMI, MCP-1, IL-6, and cholesterol was significantly increased in obese children compared with the control group, but the mean PON1 activity was significantly decreased in obese children compared with control children. No statistically significant difference was found between obese and control participants as regards serum triglyceride ($P = 0.623$).

Table 1 Comparison of anthropometric and laboratory data between the obese and control participants

Variable	Obese (mean $\pm$ SD)	Control (mean $\pm$ SD)	t-Test	P
Age (years)	8.98 $\pm$ 2.87	9.40 $\pm$ 3.39	-0.61	0.547
BMI	25.12 $\pm$ 6.22	15.24 $\pm$ 2.55	9.30	<0.0001*
MCP-1 (pg/ml)	272.29 $\pm$ 30.40	82.06 $\pm$ 14.13	11.53	<0.0001*
IL-6 (pg/ml)	3.84 $\pm$ 0.99	2.72 $\pm$ 0.69	5.82	<0.0001*
PON1 activity (U/ml)	117.32 $\pm$ 41.90	231.60 $\pm$ 51.96	-10.83	<0.0001*
Cholesterol (mg/dl)	166.95 $\pm$ 22.79	130.50 $\pm$ 15.24	8.41	<0.0001*
Triglyceride (mg/dl)	66.43 $\pm$ 14.06	64.90 $\pm$ 13.57	0.49	0.623

IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PON1, paraoxonase-1; * $P < 0.001$ is highly significant.

Table 2 shows the correlation between BMI and different laboratory data in all participants. MCP-1, IL-6, and serum cholesterol showed significant positive correlation with BMI ($P < 0.05$), whereas PON1 showed a significant negative correlation with BMI ($P < 0.05$).

To test whether the associations of BMI with the different laboratory data seen in the univariate correlation analysis were independent of other parameters, we carried out multiple regression analysis (Table 3) after adjustment for age and sex. In this model, paraoxonase showed a strong association with BMI ($\beta = -0.524$, $P = 0.000$); however, MCP-1, IL-6, serum cholesterol, and triglyceride were not proven to be strong predictors. In this way we assumed that according to this analysis 1 U increase in paraoxonase was associated with 0.04 decrease in BMI (unstandardized coefficient = -0.04).

Discussion

The prevalence of obesity is especially high among Egyptian children, and it has steadily increased in recent years. The leading causes of childhood obesity in developing countries are cheap foods with high content of sugar and fat, sedentary lifestyle, socioeconomic state, urbanization, mechanization, and rural-to-urban migration [13]. Obesity is an independent risk factor for chronic diseases. Childhood obesity increases the risk for development of adulthood dyslipidemia, DM2, and early CVDs.

Our study investigated the relationship between obesity status and markers of systemic inflammation, as well as markers of disease risk, in a sample of school-aged children.

There was a statistical significance between the two studied groups as regards BMI ($P < 0.0001$).

This study identified statistically significant differences in cytokine and chemokine serum levels (MCP-1, IL-6) between obese and healthy participants. The

Table 2 Correlation between BMI and different laboratory data in all participants

Variable	BMI	
	R	P
MCP-1 (pg/ml)	0.44	<0.0001*
IL-6 (pg/ml)	0.37	<0.0001**
PON1 activity (U/ml)	-0.62	<0.0001**
Cholesterol (mg/dl)	0.413	<0.0001**
Triglyceride (mg/dl)	0.15	0.198

IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PON1, paraoxonase-1; * $P < 0.05$ is significant ** $P < 0.001$ is highly significant.

Table 3 Multiple regression analyses for BMI and different independent variables

Variables	BMI ($R^2 = 0.467$)		
	Standardized β coefficient	t-Test	P
Age	0.045	0.510	0.611
Sex	-0.018	-0.203	0.840
MCP-1	-0.234	-1.813	0.074
IL-6	0.240	1.939	0.056
PON1 activity	-0.524	-4.967	<0.0001**
Cholesterol	0.210	1.971	0.053
Triglyceride	0.160	1.831	0.071

IL-6: interleukin-6; MCP-1: monocyte chemoattractant protein-1; PON1: paraoxonase-1; ** $P < 0.001$ is highly significant.

mean MCP-1 serum level was significantly elevated in obese participants as compared with controls. This is in agreement with that reported by Breslin *et al.* [14], who stated that the obese group had 22% greater circulating levels of MCP-1 compared with the control group ($P = 0.015$). This finding was also reported by Utsal *et al.* [16]. MCP-1 is an inflammatory marker and acts as a chemoattractant, recruiting monocytes to inflamed adipose tissue and completing the inflammatory cycle; therefore, elevated levels of MCP-1 in obesity may lead to increased risk of comorbidities such as DM2 and CVD.

Present study revealed that the mean IL-6 serum level is significantly elevated in obese participants as compared with controls ($P < 0.0001$). Similarly, Arslan *et al.* [6], and Utsal *et al.* [16], stated the same results as regards IL-6. Some previous studies explored

that high levels of IL-6 is a predictor of DM2 and myocardial infarction. This effect is thought to come from resistance to insulin action [15]. Others proved that it was an independent risk factor for CVD if its serum levels elevated [17], that is why we decided to analyze IL-6 levels in obesity to explore its relation with development of comorbidities.

Current study found that serum PON1 in obese participants was significantly decreased compared with controls ($P < 0.0001$). This is in agreement with that reported by Kota *et al.* [9], and Rupérez *et al.* [18], and Koncsos *et al.* [19], who reported significantly lower activity of paraoxonase in obese children compared with that before the program, thus supporting our results. PON1 plays a significant role in inhibiting the oxidation of low-density lipoprotein and HDL particles [20]. Therefore, decrease in its serum level leads to accumulation of lipid peroxides and in turn increase the risk of development of CVD. In another study in which PON1 activity was evaluated after a 2-week-long supervised diet and aerobic exercise program, obese children had significantly higher levels of paraoxonase compared with that before the program, and this validates our results [21].

The result concerning PON1 activity in obesity was in agreement with that reported by Ferré *et al.* [22], who reported that there was decreased PON1 activity in obese compared with nonobese children.

This study reported that the mean serum cholesterol level was significantly higher in obese participants than in controls ($P < 0.0001$). Total serum cholesterol is a CVD risk marker; its elevated levels in obese children put them at a greater risk of developing obesity-related chronic diseases during adulthood.

No statistically significant difference was found between obese participants and controls as regards serum triglyceride ($P = 0.623$). These results are in disagreement with those of Breslin and colleagues, who found that obese children exhibited symptoms of dyslipidemia, including elevated triglycerides. Other authors have observed no significant difference in total cholesterol levels between obese and nonobese children [14,23]. This discrepancy may be due to the difference in obesity degree.

We made correlations between BMI and different laboratory data in all participants. It revealed that MCP-1, IL-6, and serum cholesterol showed significant positive correlation with BMI ($P < 0.05$), whereas PON1 showed a significant negative correlation with BMI ($P < 0.05$). Both MCP-1 and IL-6 are markers of systemic inflammation, which is

an underlying mechanism connecting obesity and its disease complications. Total serum cholesterol is a CVD risk marker; its elevated levels in obese children put them at a greater risk of developing obesity-related chronic diseases during adulthood. The lower serum PON1 activity in obesity confirms that it is associated with oxidative damage of lipoproteins and may explain the increased cardiovascular risk in obese children. Another study concluded that obesity is associated with 50–60% reduction in PON1 activity; this reflects a higher atherosclerotic potential [10]. Bajnok *et al.* [24] reported that BMI is an independent predictor of PON1 activity. Studies have not yet analyzed the corresponding fall in PON1 activity with increased BMI.

To test whether the associations of BMI with the different laboratory data seen in the univariate correlation analysis were independent of other parameters, we carried out multiple regression analysis after adjustment for age and sex. In this model, paraoxonase showed strong association with BMI ($\beta = -0.524$, $P = 0.000$); however, MCP-1, IL-6, serum cholesterol and triglyceride levels were not proven to be strong predictors. In this way we could conclude that according to this analysis 1 U increase in PON1 activity was associated with 0.04 decrease in BMI (unstandardized coefficient = -0.04).

Conclusion

There are increased serum levels of inflammatory markers (MCP-1, IL-6) associated with childhood obesity. Moreover, serum PON1 level decreases in cases of obesity, and this in turn put children at higher risk of developing obesity-related chronic diseases during adulthood, such as atherosclerosis and CVD. Childhood obesity is associated with hypercholesterolemia suggesting an increase in adulthood disease risk.

Acknowledgements

Conflicts of interest

None declared.

References

- 1 Kamal NN, Ragy MM. The effects of exercise on C-reactive protein, insulin, leptin and some cardiometabolic risk factors in Egyptian children with or without metabolic syndrome. *Diabetol Metab Syndr* 2012; 4:27.
- 2 Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. *Nat Rev Cardiol* 2009; 6:399–409.
- 3 Rana JS, Nieuwdorp M, Jukema JW, Kastelein JJ. Cardiovascular metabolic syndrome – an interplay of, obesity, inflammation, diabetes and coronary heart disease. *Diabetes Obes Metab* 2007; 9:218–232.
- 4 Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; 112:1796–1808.

- 5 Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA Jr, *et al.* MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature* 1999; 398:718–723.
- 6 Arslan N, Erdur B, Aydin A. Hormones and cytokines in childhood obesity. *Indian Pediatr* 2010; 47:829–839.
- 7 Poitou C, Dalmás E, Renovato M, Benhamo V, Hajduch F, Abdenour M, *et al.* CD14^{dim}CD16⁺ and CD14⁺CD16⁺ monocytes in obesity and during weight loss: relationships with fat mass and subclinical atherosclerosis. *Arterioscler Thromb Vasc Biol* 2011; 31:2322–2330.
- 8 Skinner AC, Steiner MJ, Henderson FW, Perrin EM. Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood. *Pediatrics* 2010; 125:e801–e809.
- 9 Kota SK, Meher LK, Kota SK, Jammula S, Krishna SV, Modi KD. Implications of serum paraoxonase activity in obesity, diabetes mellitus, and dyslipidemia. *Indian J Endocrinol Metab* 2013; 17:402–412.
- 10 Thompson DR, Obarzanek E, Franko DL, Barton BA, Morrison J, Biro FM, *et al.* Childhood overweight and cardiovascular disease risk factors: the National Heart, Lung, and Blood Institute Growth and Health Study. *J Pediatr* 2007; 150:18–25.
- 11 Singh GK, Kogan MD, Van Dyck PC, Siahpush M. Racial/ethnic, socioeconomic, and behavioral determinants of childhood and adolescent obesity in the United States: analyzing independent and joint associations. *Ann Epidemiol* 2008; 18:682–695.
- 12 Watson AL, *et al.* Evidence of paraoxonase independent inhibition of low density lipoproteins oxidation by high density lipoproteins. *J Clin Invest* 1995; 96:2882.
- 13 Aziz S, Noorulain W, Zaidi UE, Hossain K, Siddiqui IA. Prevalence of overweight and obesity among children and adolescents of affluent schools in Karachi. *J Pak Med Assoc* 2009; 59:35–38.
- 14 Breslin WL, Johnston CA, Strohacker K, *et al.* Obese Mexican American children have elevated MCP-1, TNF, monocyte concentration and dyslipidemia. *Pediatrics* 2012; 129:1180–1186.
- 15 Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* 2001; 9:414–417.
- 16 Utsal L, Tillmann V, Zilmer M, Mäestu J, Purge P, Jürimäe J, *et al.* Elevated serum IL-6, IL-8, MCP-1, CRP, and IFN- γ levels in 10- to 11-year-old boys with increased BMI. *Horm Res Paediatr* 2012; 78:31–39.
- 17 Sachek J. Pediatric obesity: an inflammatory condition? *JPEN J Parenter Enteral Nutr* 2008; 32:633–637.
- 18 Rupérez AI, López-Guarnido O, Gil F, Olza J, Gil-Campos M, Leis R, *et al.* Paraoxonase 1 activities and genetic variation in childhood obesity. *Br J Nutr* 2013; 110: 1639–1647.
- 19 Koncsos P, Seres I, Harangi M, Illyés I, Józsa L, Gönczi F, *et al.* Human paraoxonase-1 activity in childhood obesity and its relation to leptin and adiponectin levels. *Pediatr Res* 2010; 67:309–313.
- 20 Draganov DI, La Du BN. Pharmacogenetics of paraoxonase: a brief view. *Arch Pharmacol* 2004; 369:79–88.
- 21 Koncsos P, Seres I, Harangi M, Páll D, Józsa L, Bajnok L, *et al.* Favorable effect of short-term lifestyle intervention on human paraoxonase-1 activity and adipokine levels in childhood obesity. *J Am Coll Nutr* 2011; 30:333–339.
- 22 Ferré N, Feliu A, García-Heredia A, Marsillach J, París N, Zaragoza-Jordana M, *et al.* Impaired paraoxonase-1 status in obese children. Relationships with insulin resistance and metabolic syndrome. *Clin Biochem* 2013; 46:1830–1836.
- 23 Thompson DR, Obarzanek E, Franko DL, Barton BA, Morrison J, Biro FM, *et al.* Childhood overweight and cardiovascular disease risk factors: the National Heart, Lung, and Blood Institute Growth and Health Study. *J Pediatr* 2007; 150:18–25.
- 24 Bajnok L, Seres I, Varga Z, Jeges S, Peti A, Karanyi Z, *et al.* Relationship of serum resistin level to traits of metabolic syndrome and serum paraoxonase 1 activity in a population with a broad range of body mass index. *Exp Clin Endocrinol Diabetes* 2008; 116:592–599.