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Research Article

Comparison of Serum Levels of Osteoprotegerin, Insulin, Triglyceride, and Cholesterol in Obese and Non-obese Children

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Abstract

Background: Childhood obesity is associated with cardiovascular risk factors including dyslipidemia, insulin resistance, hypertension, and atherosclerosis. Some studies have also shown that mortality in obese people is higher than in other non-obese people. Due to the prevalence of obesity and the need to investigate the causes of atherosclerosis, insulin resistance and other related disorders, we tried to assess the serum levels of osteoprotein, insulin, triglycerides and cholesterol in obese and non-obese children.
Methods: In this case-control study, we randomly selected 76 students aged 12-18 years in Birjand schools in 2019 and stratified them into obese and overweight groups as the case arm and non-obese groups as the control arm. We used a "Secca" height-weight scale with an accuracy of 50 grams and 0.1 cm to measure the children's height and weight. The children's venous blood samples were collected and the sera were isolated. The following kits were licensed in Germany and made in China: The Roche kit for cholesterol, triglyceride, and glucose, the Diametra kit for insulin, and the Eastbiopharm kit for osteoprotegerin. For analyses of data, independent t-test, Mann-Whitney and Chi-square test were used. The significance level for all the test was less than 5%.
Results: Both case and control groups were similar in terms of age and sex (P>0.05). The mean serum levels of triglycerides, cholesterol, insulin, and glucose in the case group were significantly higher than in the control group (P<0.05). The mean osteoprotegerin level was significantly lower in the case group than in the control group (P<0.05).
Conclusion: Obese children have a higher risk of developing atherosclerosis, insulin resistance, and other metabolic disorders.

Keywords: Obesity, Osteoprotegerin, Insulin, Triglyceride, Cholesterol

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1. Introduction

With the change in living conditions in recent decades, the incidence of obesity is increasing alarmingly in most human societies and children and adolescents are no exception to this phenomenon (1). In 2013, 42 million infants and young children worldwide were overweight and obese, and if we continue this trend by 2025, we will have 70 million overweight and obese children. Childhood obesity is associated with cardiovascular risk factors including dyslipidemia, insulin resistance, hypertension, and evidence of atherosclerosis (2). Over the past 30-40 years, obesity has become a serious problem in childhood and adolescence. Metabolic syndrome in Iranian obese children has been reported in 31.9% and 34.22% in studies (3).

Obesity is the excessive accumulation of fat in the body, which is associated with various complications (4). It is related to type 2 diabetes, dyslipidemia, hypertension, cardiovascular disease, and cognitive disorders (5, 6). Obesity is the result of an improper unhealthy lifestyle as well as poor eating habits (7). Several studies showed that childhood obesity is a major public health issue associated with many complications in children (8). Obesity in association with insulin resistance leads to the transformation of vascular smooth muscle cells into

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osteoblast-like cells (9). Finally, hydroxyapatite is produced and causes vascular calcification (10), resulting in atherosclerosis, coronary artery disease, and an increased risk of cardiovascular disease (8).

Obesity-induced dyslipidemia is associated with elevated levels of triglycerides (TG) and free fatty acids, decreased and impaired levels of HDL, and increased LDL and total cholesterol (Chol) (11, 12). Lipid peroxidation initiates the oxidation of other compounds such as protein and apolipoprotein, which leads to the production of free radicals. Macrophages and smooth muscle cells remove oxidized LDL to form atherosclerotic plaques (13). On the other hand, serum levels of lipids and lipoproteins in childhood are predictors of these factors in adulthood (14).

Osteoprotegerin (OPG), a glycoprotein, is a Tumor Necrosis Factor-alpha (TNF-α) receptor that inhibits osteoclast cells via binding to the Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) receptor (15, 16). The OPG/RANKL system plays an essential role in angiogenesis, pathological inflammation (17), and cell survival and promotes calcification (18). Different types of tissues produce OPG, including the cardiovascular, lungs, kidneys, intestines, and bones. (19). Increased levels of OPG indicate the onset of different diseases, such as coronary heart disease (20). The results of several studies are contradictory, representing the unclear role of this factor in the association with childhood and adolescence obesity and related diseases (8).

Atherosclerosis is a chronic inflammatory process caused by the deposition of peptides and fatty streaks in the intima of arteries, leading to atherosclerotic plaque formation (21). It is one of the main risk factors of ischemic stroke. The manifestations of atherosclerosis occur in childhood and adolescence, especially in people with risk factors such as dyslipidemia, hypertension, obesity, and diabetes (22-25). Atherosclerosis is thought to begin in childhood and grow silently for decades before events occur (26).

Given the above and due to high prevalence of obesity, atherosclerosis, and the need to investigate the main factors involved in the development of atherosclerosis, insulin resistance, dyslipidemia, and diabetes, we aimed to evaluate and compare the serum blood levels of osteoprotegerin, insulin, triglycerides, and cholesterol in obese and non-obese children.

2. Methods

In this case-control study, the population were students aged 12-18 years from Birjand City schools in 2019. The obese and overweight groups (with a Body Mass Index (BMI) above the 85th percentile) stratified as the case group and the non-obese children (with a BMI below the 85th percentile) as the control group. We excluded the children who had a chronic illness or did not agree to participate, and we randomly selected a total of 38 students from the classes for each group. Both two groups were similar in terms of age and sex. We held a briefing at the schools for parents of eligible children, and after obtaining the consent to participate in this study, we gave them a referral form to the lab. While referring to the lab, children›s height and weight were measured with a «Secca» electronic height-weight scale with an accuracy of 50 grams for the weight (without shoes with minimal clothing) and 0.1 cm for the height (standing without shoes). We obtain their BMI by dividing weight by height squared in meter (kg/m2). Children with a BMI above the 85th percentile stratified as the case group and children with a BMI below the 85th percentile as the control group.

6 CCs of blood were drawn from the vein of each participant and collected in non-containing anticoagulant tubes. Blood samples were centrifuged at 3000 rpm for 20 minutes. Parameters such as serum insulin, blood sugar, lipid profile, and osteoprotegerin were measured. “Euroimmun Analyzer I-2P” was used to measure the serum levels of cholesterol, triglyceride, and glucose using Roche kit, insulin with Diametra kit, and osteoprotegerin with Eastbiopharm kit made in China.

We entered the collected data into SPSS version 22. Descriptive data were reported using central statistical indices and dispersion. Kolmogorov-Smirnov test was also used to evaluate the normality of the data. If the data distribution was normal, the independent t-test was used, and if the non-parametric Mann-Whitney test was abnormal, the Chi-square test was used to compare the qualitative variables at a significance level less than 5%.

3. Results

There were 38 students in the normal-weighted group and 38 students in the overweight and obese group. The mean age and sex frequency distribution in the two

Table 1: Comparison of Body Mass Index, serum levels of Triglycerides, Cholesterol, Insulin, and Glucose in case and control groups

Groups parameters	Control Mean ± SD	Case Mean ± SD	Independent t-test
BMI(Kg/m^2)	18.91 ± 3.4	28.4 ± 3.6	P<0.001
TG (mg/dl)	82.6 ± 31.3	126.4 ± 4.03	P<0.001
Chol (mg/dl)	145.3 ±23.2	162.9 ± 32.5	P= 0.002
Insulin (Iu/dl)	8.9 ± 4.2	24.9 ± 10.8	P<0.001
Glucose (mg/dl)	87.3 ± 7.9	94.9 ± 10.7	P= 0.002

Table 2: Comparison of osteoprotegerin in case and control groups

Groups parameters	Mean ± SD	(q1-q3) Median	Mann Whitney
Control	3.19 ± 2.1	(2.29 – 3.48) 2.82	P=0.005
Case	2.5 ± 2.5	(1.03- 2.86) 2.08	

groups were not statistically significant (P>0.05). Otherwise, both two groups were similar in terms of age and sex.

The results of this study (shown in Table 1) show that the mean serum levels of triglyceride, cholesterol, insulin, and glucose parameters in the case group are significantly higher than the control group (P<0.05).

Based on the data shown in the table 2, the mean serum level of osteoprotegerin in the case group was significantly lower than the control group (P=0.005).

Table 3 shows no statistically significant difference between the frequency distribution of abnormal glucose levels in the normal-weighted and the obese groups (P=0.24). There was a significant difference between the frequency distribution of abnormal cholesterol (P<0.001), triglyceride (P=0.014), and insulin (P<0.001) in the case and control groups.

It was estimated that the probability of abnormal cholesterol levels in the case group was 3.76 times greater than the case group, the probability of abnormal TG levels in the case group was 9.45 times greater than the control group, and the probability of abnormal insulin levels in the case group was 148.7 times greater than the control group.

4. Discussion

The mean serum levels of triglycerides, cholesterol, insulin and glucose in the case group were significantly higher than the control group, which is associated with increased production of free radicals and leads to inflammation and atherosclerotic plaque formation. The mean of osteoprotein was significantly lower in the case group than the control group.

Some studies showed decreased OPG levels in obese people compared to non-obese people, while some studies found no association between osteoprotegerin and obesity (27). In a study of the general population, OPG, a risk factor in the progression of atherosclerosis, was associated with the severity of coronary

Table 3: Comparison of the frequency distribution of serum Glucose, Cholesterol, Triglycerides, and Insulin levels in case and control groups

Groups Parameters		Control Frequency (percentage)	Case Frequency (percentage)	Chi-square	OR (CI,95%)
Glucose (mg/dl)	Normal	37 (100)	37 (97.4)	P*= 0.24	1
	Abnormal	0 (0)	1 (2.6)		
Triglycerides (mg/dl)	Normal	31 (83.8)	22 (57.9)	P=0.014	3.76 (1.11-3.1)
	Abnormal	6 (16.2)	16 (42.1)		
Cholesterol (mg/dl)	Normal	24 (64.9)	6 (15.8)	P<0.001	9.45 (3.3-29.7)
	Abnormal	13 (35.1)	32 (84.2)		
Insulin (Iu/dl)	Normal	35 (94.6)	4 (1.05)	P<0.001	148.7(25.2-866)
	Abnormal	2 (5.4)	34 (89.5)		

*Fisher's exact test

atherosclerosis (28-31). This paradoxical increase in OPG level was interpreted as a protective regulatory response to atherosclerosis. Despite the above findings, the association of OPG with obesity, lipid profile, and insulin sensitivity is controversial.

In this study, the mean serum levels of TG, Chol, insulin, and glucose in the obese group were significantly higher than in the non-obese group. Oh and colleagues (32), Çelik and co-workers (33), and Jose and colleagues (34) showed that the mean serum levels of glucose and insulin in obese people were significantly higher than the normal-weighted group, which was consistent with our study. In a study by Gannagé-Yared and co-workers (35), Serum cholesterol, triglyceride, and HDL levels were not significantly different between obese and non-obese groups, which was not consistent with our study. Abnormal fasting blood sugar and insulin levels are associated with metabolic syndrome and insulin resistance. The mean level of osteoprotegerin in the obese group was significantly lower than the non-obese group.

Sulibursk a and colleagues (36) found that serum OPG level in the obese group was significantly lower than in the non-obese group. In a study by Gannagé-Yared and colleagues (37), there was a positive correlation between OPG and (Homeostatic Model Assessment for Insulin Resistance) HOMA-IR. The study demonstrated that OPG has beneficial effects on vascular tissue because obesity is associated with endothelial dysfunction and insulin resistance. In a study by Erol and colleagues (38), the mean OPG serum level in obese children was significantly lower than in the non-obese group, consistent with our study. The diagnostic OPG level for obesity was less than 46.19 pg/mL. Irene Lambrinoudaki and colleagues (27) showed that patients with long-term diabetes had higher carotid Intima-Media Thickness (cIMT) markers and higher triglyceride levels than other patients. BMI was the only factor associated with cIMT. BMI was associated with OPG in both groups, while sRANKL level was not associated with any factor. As a result, BMI was the strongest predictor independent from cIMT in the general population, especially in diabetics, indicating a possible correlation between diabetes and obesity in association with atherosclerosis. Body mass index is generally associated with circulatory OPG, but the source of this association is still unknown. The studies by Gannagé-Yared and co-workers (35) and Kotanidou and co-workers (8) found that OPG levels were not significantly different between the obese and non-obese groups, which was not consistent with our study. It was also suggested that OPG had a significant relationship with the HOMA-IR index and mean SGPT level in obese people. OPG was associated with BMI in obese people. However, novel relationships were described between OPG and both HOMA and CRP indices.

A study by Ugur-Altun and co-workers (39) found that obese people with lower insulin resistance had lower OPG levels than other non-obese groups, consistent with our study. They also observed a significant negative correlation between OPGc and fasting glucose, fasting insulin, and HOMA-IR. Insulin resistance in obesity was associated with decreased serum level of OPG.

A study by Venuraju and colleagues (40) presented that the mean increased OPG levels were consistently associated with the incidence and prevalence of coronary artery disease, consistent with our study. Some duality in the role of OPG, RANKL, and ligand-induced apoptosis is associated with infertility in atherosclerosis and plaque stability.

Stanik and co-workers (41) suggested that the levels of sclerotin, osteoprotegerin, and bone alkaline phosphatase in the fasting state and after the Oral Glucose Tolerant Test (OGGT) are associated with bone growth and insulin resistance. Obesity with HOMA-IR appears to play a crucial role in increasing osteoprotegerin.

The present study estimates that the probability of having abnormal levels of cholesterol, TG, and insulin in obese people, respectively, are 3.76, 9.45, and 148.7 times higher than non-obese people. Erol and colleagues (42) found a significant relationship between the mean serum level of fasting insulin with obesity, which was consistent with our study. The limitations of this study is the poor cooperation of some parents of students. In order to increase the level of cooperation in the study, it is suggested that blood sampling of students be provided in schools.

5. Conclusion

Triglyceride, cholesterol, insulin, and glucose serum levels in the case group were significantly higher than in the control group. Besides, the mean osteoprotegerin level in the case group was significantly lower than in the

healthy group. There was no statistically significant difference in the prevalence of abnormal glucose levels in both case and control groups. Moreover, the prevalence of abnormal levels of cholesterol, triglyceride, and insulin in the case group was significantly higher than in the control group.

Conflict of interests: None declared.

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Ethical Approval: The Ethics Committee of Birjand University of Medical Sciences approved the present study with the code of Ir.bums.REC.1398.234. We referred to the Department of Education of Birjand City and got permission to go to the schools for data collection.

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