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Association of osteoprotegerin and rankl levels with insulin resistance in pubertal obese children

Research Article

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Abstract: Osteoprotegerin (OPG)/"receptor activator of nuclear factor kappa B-ligand" (RANKL) system has an important role in the remodeling of bone through regulation of osteoclastogenesis. We aimed to detect OPG and RANKL levels, particularly in obese children in the pubertal period and to investigate whether these parameters correlate with insulin resistance in childhood. Our study included 66 obese children ranging in age from 9.1 to 16 years, and 22 non-obese children ranging in age from 10.5 to 16 years. Blood glucose, insulin, total cholesterol, HDL cholesterol, and LDL cholesterol levels were measured for all cases; HOMA-IR, Quicki index and atherogenic index were calculated. Serum OPG and RANKL levels were also measured. OPG and RANKL levels did not show any difference between obese and non-obese children (P>.05). No difference in these 2 parameters were observed among the children with and without insulin resistance (P>.05). No correlation could be established between the OPG, the HOMA-IR, Quick and atherogenic indices. Obesity and insulin resistance are believed to show their effect in the later period of life to become able to change some of the parameters.

Keywords: Henoch-Schönlein purpura • Nephrotic syndrome • Adulthood

1. Introduction

Prevalence of obesity among children has increased worldwide. Obesity, insulin resistance, type 2 diabetes, and hyperlipidemia in childhood may particularly predispose to atherosclerosis. Nevertheless, bone mineral density (BMD) increases with the fat content of the body, and obesity is believed to have a protective effect against osteoporosis [1,2]. Although this protective effect has been attributed to a combination of hormonal (peripheral aromatization of androgens to estrogens in adipose tissue) and mechanical factors (weight-bearing bone), it has not been clearly elucidated in the literature. Recently, serum insulin levels have been reported to be associated with BMD, and hyperinsulinemia has been chosen as a potential explanation for the association between obesity and BMD; this effect is probably due to direct mitogenic effects of insulin on osteoblasts [3,4].

Recent studies on the biology of bone have shown that activation and regulation of osteoclasts is performed through locally active factors synthesized by osteoblasts or stromal cells. It has been also discovered that intercellular connections and osteoclastogenesis are regulated by the OPG/RANKL system. OPG is a component of the recently described cytokine system that plays a role in osteoclast maturation. In this process, a RANKL-ligand present on the osteoblasts binds to RANK on osteoclast precursors to provoke their differentiation and inhibit osteoclast apoptosis [5,6]. Although OPG's mechanism of action has been described by in vitro and animal studies; human studies on OPG that investigated its association with BMD and other variables have yielded variable results and mostly failed to demonstrate a correlation between OPG and BMD [6-12]. Additionally, several studies on adults have suggested that OPG may have some effect on angiogenesis other than its association with vascular injury and atherosclerosis [12,13].

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This study was conducted to detect OPG and RANKL levels in obese children during the pubertal period and to investigate whether these parameters are correlated with insulin resistance and atherogenic index in these children.

2. Material and Methods

This study included 66 obese children (33 female, 33 male) with the age ranging from 9.1 to 16 years and 22 non-obese children (11 female, 11 male) with the age ranging from 10.5 to 16 years. All the patients were pubertal (Tanner stage II-IV). The study group consisted of obese children followed in our pediatric endocrinology clinic that did not have diabetes, hypertension, hyperlipidemia, hypothyroidism, Cushing's syndrome, severe chronic disease, or acute illness. Non-obese children without any chronic disease or infection formed the control group.

All anthropometric measurements were performed in the morning while children were wearing only underclothes without shoes. Height and weight were measured at 2 different occasions throughout the interview, and averages of these 2 values were used for analyses. Height was measured using a Harpender stadiometer and approximated to the nearest 0.1 cm. Children were weighed twice by a portable digital scale, and these values were also approximated to nearest 0.1 kg; measurement was repeated if the first 2 measurements differed by >0.2 kg. Body mass index (BMI) was calculated by dividing weight (kilogram) by square of height (meter). All subjects had a BMI above 95 percentile for their age and gender; and therefore were classified as obese [14]. Detailed family histories of all subjects were obtained and physical examinations were performed. Daily calcium and phosphorus intake were calculated by a 3-day diet history and all patients were found to have adequate intake according to recommendations of daily allowance.

Samples of venous blood were collected from each subject into tubes without anticoagulant (for preparation of serum). The tubes were centrifuged (4000 rpm) at room temperature for 10 min. The serum samples were stored at –80 °C until time of analysis.

Insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) were measured with chemiluminessence assays using autoanalyser (Abbott laboratories, Chicago, IL, USA). A plasma testosterone (T) concentration was analyzed using a commercially available RIA kit (Diagnostic System Laboratories, USA). The serum calcium, magnesium, and phosphate, were determined by spectrofluorometric analysis.

Serum 25-OHD levels were performed using a liquid chromatographic system equipped with a Thermo Finnigan High Performance Liquid Chromatography (HPLC) system connected to an Ultraviolet (UV-1000) detector. Detection was carried out at 264 nm. A C_{18} column was used as an analytical column to provide further separation of analyses from other endogenous plasma components before detection. Serum PTH concentrations were measured with DSL-8000 radioimmunoassay kits from DSL with a sensitivity of 12 pg/mL and intra- and interassay CV of 5.7 and 4.5%, respectively. Serum ALP concentrations were measured spectrophotometrically with DAX Technicon 48 using American Associated Clinical Chemistry (AACC) and International Federation of Clinical Chemistry (IFCC) methods. Fasting glucose (FG), total cholesterol, LDL-cholesterol, and HDL-cholesterol levels were measured by spectrophotometric technique using commercial kits of Olympus AU-2700 auto analyzer (Olympus, Hamburg, Germany).

Homeostasis model assessment for insulin-resistance (HOMA-IR) was used to estimate the insulin resistance in our population [15]. Formula for calculating HOMA-IR index was: [fasting plasma glucose (mmol/L) × fasting serum insulin (mU/mL)] / 22.5. Insulin sensitivity was determined by the Quantitative insulin-sensitivity check index (QUICKI). Formula of QUICKI: 1 / [Log (Fasting insulin µU/mL) + Log (Fasting Blood Glucose mg/dL)]. Atherogenic index was calculated from the following formula: (Total Cholesterol – HDL Cholesterol) / HDL Cholesterol. Obese patients enrolled into the study were divided into 2 groups, either insulin-resistant [IR(+)] if their HOMA-IR values were above 3.16 or non-insulin resistant [IR(-)] if their HOMA-IR values were lower than 3.16 [15].

OPG and RANKL levels in serum were measured in duplicate. ELISA kits supplied from Biomedica (Vienna, Austria) were used for measurements. Intra- and inter-assay coefficients of variation (CV) for RANKL were less than 5% and less than 9%, respectively. Intra- and inter-assay CV for OPG were less than 4% and less than 8%, respectively. Limit of detection were 0.08 pmol/L for RANKL and 0.14 pmol/L for OPG.

Bone mineral density was measured at the L2-4 level of lumbar spine and femoral neck using dual energy x-ray absorptiometry by a Norland ER-35 analyzer.

Approval for the study was obtained from the Gazi University Faculty of Medicine Ethics Committee. Informed consent was taken from the parents.

Table 1. Anthropometric measurements of obese and non-obese children.

	Obese		Control	p value
	IR (+)	IR (-)		
	(n:33)	(n:33)	(n:22)	
Year (age)	13.4 ± 1.8	13.7 ± 2.3	14.1 ± 2.8	>0.05
Height (cm)	162.6 ± 10.5	157.6 ± 12.3	154.6 ± 13.1	>0.05
Weight (kg)	79.9 ± 14.9 *	73.1 ± 16.6 ▲	50.0 ± 11.6 *, ▲	<0.001
BMI (kg/m²)	30.4 ± 3.2 *	29.8 ± 3.3 ▲	21.1 ± 2.9 *, ▲	<0.001
BMI SDS	2.2 (2.0-3.5) *	2.1 (2.0-3.8) ▲	-0.1 (-1.8 -(1.9)) *, ▲	<0.001

Data are presented with the mean ± SD or median (min-max)
*: p<0.05 : Between Group IR (+) and control
▲: p<0.05 : Between Group IR (-) and control
Abbreviations:
BMI   : Body mass index
BMI SDS   : Body mass index standart deviation scores

2.1. Statistical analysis

Data analysis was performed by SPSS software for Windows, version 11.5. Shapiro-Wilk test was used to detect whether the distribution of continuous variables were normal or not. Data were shown as mean ± standard deviation for continuous variables and percentages for categorical ones. Student's t test or Mann Whitney U test were used to compare groups. The statistical significance of differences between groups was assessed by One Way Analysis of Variance (One-Way ANOVA) or Kruskal Wallis test, where appropriate. If the *P*-value of One-Way ANOVA or Kruskal Wallis test showed statistical significance, post hoc Tukey, Bonferroni adjusted Mann-Whitney U tests were used respectively to determine which group differed from the others. For categorical comparisons Chi-square or Fisher's Exact test were used, where appropriate. Degree of association between BMD, OPG, RANKL and other continuous variables were calculated by Pearson's correlation coefficient. If the *P*-value of Pearson's correlation coefficient was statistically significant, a partial correlation test was used to adjust for body mass index (BMI). A *P*<.05 was considered statistically significant.

3. Results

In the obese group, 16 of the IR (+) cases and 16 of the IR (-) cases were females, whereas control group included 11 females. Anthropometric characteristics of all patients are presented in Table 1. Obese patients were matched with members of the control group for age and height. Weight, weight SDS, BMI and BMI SDS of the IR (+) children in the obese group were higher compared to IR (-) children; however this difference did not reach the level of statistical significance. IR (-) obese children and control group had no difference in terms of anthropometric characteristics.

Blood glucose and insulin levels; insulin resistance indexes and insulin sensitivity, lipid profile, and atherogenic index values are presented in Table 2. IR (-) patients in the study group were found to have no difference compared to control group for parameters such as glucose, insulin, and insulin resistance. Assessment of lipid profiles and atherogenic indexes demonstrated no difference between either the obese group and the control group or between IR (+) and IR (-) obese patients. Additionally E and T levels of the obese and control group showed no significant difference (data not shown).

There was no determinable difference between the serum calcium, magnesium, phosphate, ALP, 25-OHD and PTH levels found in obese children and those of the children in the control group. Similarly IR (+) and IR (-) obese pubertal children did not demonstrate any difference for these parameters (data not shown).

OPG and RANKL levels did not have statistically significantly different between obese children and control group (*P*>.05). The mean OPG concentration was 4.33 pmol/L (SD 2.95) in the obese group and in the control group it was 3.96 pmol/L (SD 1.51). The OPG levels showed no statistically significant difference between males and females in the obese and control group as well as IR (+) and IR (-) cases in the obese group (*P*>.05) (Table 3). No correlation was found between OPG levels and the following measures: weight SDS, BMI, BMI SDS, calcium, phosphate, ALP, PTH, 25-OHD, or RANKL.

The mean RANKL concentrations determined in each groups did not show significant differences between males and females. We did not find any difference between the groups. No correlation was found between RANKL and weight SDS, BMI, BMI SDS, calcium, phosphate, ALP, PTH, or 25-OHD (*P*>.05).

No correlation of OPG and RANKL was observed with HOMA-IR and Quick index (*P*>.05) as well as the

Table 2. Results of laboratory tests of obese and non-obese children.

	Obese		Control	p value
	IR (+)	IR (-)		
	(n:33)	(n:33)	(n:22)	
Insulin (mIU/ml)	21.7 (16.5-33.0) *,▲	10.2 (3.8-13.5) *	9.8 (4.9-12.5) ▲	<0.001
FG (mg/dl)	93.7 ± 5.4 *	87.3 ± 5.4 *	90.5 ± 4.0	<0.001
FG / Insulin	4.3 (2.9-5.6) *,▲	8.6 (6.0-24.2) *	9.3 (7.1-17.2) ▲	<0.001
HOMA-IR	5.7 ± 1.2 *,p	2.1 ± 0.5 *	2.0 ± 0.5 ▲	<0.001
QUICKI	0.30 ± 0.01 *, ▲	0.34 ± 0.01 *	0.34 ± 0.01 ▲	<0.001
Total Cholesterol (mg/dL)	164 ± 31	170 ± 31	151 ± 24	>0.05
HDL-Cholesterol (mg/dL)	46 ± 15	47 ± 11	51 ± 7	>0.05
LDL-Cholesterol (mg/dL)	106 ± 26	103 ± 25	89 ± 29	>0.05
Atherogenic indeks	2.8 (0.7-4.7)	2.7 (0.4-4.9)	2.2 (1.6-3.1)	>0.05

* Data are presented with the mean ± SD or median (min-max)
*: p<0.05 : Between Group IR (+) and IR (-)
▲: p<0.05 : Between Group IR (+) and control
Abbreviation:
FG   : Fasting glucose
HOMA-IR   : Homeostasis model assessment for insulin-resistance
QUICKI   : Quantitative insulin-sensitivity check index

Table 3. BMD, OPG and RANKL values of obese and non-obese children.

	Obese		Control	p value
	IR (+)	IR (-)		
	(n:33)	(n:33)	(n:22)	
BMD Z-score	0.4 (-1.9-2.0)	0.1 (-2.4-1.7)	-0.1 (-2-1.1)	>0.05
OPG (pg/ml)	3.86 (1.35-24.17)	4.21 (1.9-7.86)	3.91 (0.56-8.06)	>0.05
RANKL (pg/ml)	0.02 (0-0.98)	0.03 (0-1.5)	0.01 (0-0.35)	>0.05

* Data are presented with the mean ± SD or median (min-max)
Abbreviations:
BMD Z-score   : Bone mineral density Z-score
OPG   : Osteoprotegerin
RANKL   : Receptor activator of nuclear factor kappa B-ligand

atherogenic index (*P*>.05). In addition, there were no differences in OPG and RANKL levels and no correlation to BMD (*P*>.05).

4. Discussion

This study investigated the OPG and RANKL levels among obese children in the pubertal period and the association of these parameters with BMD, insulin resistance, and atherogenic index; but the results obtained did not demonstrate a correlation between these parameters.

OPG is a soluble molecule that is considered within the TNF receptor superfamily, and capable of inhibiting RANKL-mediated osteoblastic bone resorption [5,6]. It has been recently discovered that OPG is produced by various tissues [7,8]-OPG and RANKL levels throughout normal infancy and childhood have been examined in a number studies. Normally, OPG levels show an inverse correlation with age. The course of OPG levels throughout life has been reported as follows: (i) high levels in infancy; (ii) decrease to stable level in childhood and adulthood until the age of 45 and (iii) progressive increase after 45 years until senescence [16].

Analysis of the association of OPG levels with nutritional status revealed that girls with anorexia nervosa had higher OPG levels compared to controls [17], who reported lower serum OPG levels than obese adult females [18]. Another study from Oh ES et al demonstrated that serum OPG levels were higher than other metabolic components in older, obese, and hypercholesterolemic female subjects [19]. However one study failed to show any difference between the OPG levels of obese group and the control group [20]. Our study did not show a statistically significant difference between OPG and RANKL levels of the obese children and the control group.

In accordance with other studies [21], we could not find any correlation between OPG levels or between serum calcium, phosphate or ALP concentrations, nor could we find growth variables such as height-SDS,

weight-SDS, or BMI-SDS. Even puberty does not seem to affect OPG concentrations, however the relatively small number of children that participated in our study make it difficult to draw a conclusion in this regard [16].

Controversial data exist in the literature regarding the association between OPG/RANKL system and BMD [6,7,9-12]. Bucay N et al demonstrated a lower BMD and a higher incidence of fracture among OPG-deficient mice [8]. Rogers et al have also detected a significant association between OPG and BMDs in total body, total hip, and femoral neck, which in fact lost statistical significance after correcting for age and BMI [9]. Review of the literature suggests a correlation between OPG and BMD, particularly in chronic disorders [10,11,17,22]. The cause of this finding is not completely understood, but it might be a compensatory mechanism for a negative balance of bone remodeling in renal bone disease among these children. Browner has reported the association between serum OPG levels with diabetes and cardiovascular mortality, however OPG levels were not correlated with either baseline BMD or the risk of stroke or fracture [6]. In their study on beta-thalassemia patients, Angelopoulos NG et al demonstrated the clinical effectiveness of the OPG/RANKL system as a marker of bone turnover [23]. In our study, we failed to demonstrate a significant correlation between OPG or BMD levels of obese children and control group. However we were not able to make a solid conclusion, as other parameters of osteoporosis were not examined.

Even though serum insulin levels have a reported association with BMD levels, and hyperinsulinemia, it is considered a potential explanation for the association between obesity and BMD today; present data is insufficient to confirm this proposal. Similarly, a correlation of OPG levels to insulin resistance has not been clearly delineated. Insulin resistance in healthy obese adults is associated with lower serum OPG levels and serum OPG level has been proven to be inversely related with fasting blood glucose, fasting insulin and HOMA-IR [24]. On the contrary, Gannage-Yared MH et al. [25] have detected a positive correlation between OPG and the HOMA index. In another study, OPG levels of patients with metabolic syndrome were found to be higher [26]. Kim SM et al. [27] have also determined an association between serum OPG levels and HOMA-IR in both normal and diabetic patients. In our study, we could not establish such a correlation between OPG levels and indexes of insulin sensitivity or insulin resistance.

Animal models have confirmed that OPG is an important inhibitor of osteoclastogenesis and arterial calcification. OPG-deficient mice have been shown to develop arterial calcification in experimental animal models. Min H et al have demonstrated that subcutaneous administration of OPG to OPG-deficient mice prevented arterial calcification, thus they have proposed that OPG/RANKL signaling pathway might have an important role in the regulation of both pathological and physiological calcification [12]. A positive correlation between OPG and atherosclerosis in postmenaposal women has also been reported [28]. Dovio A et al have shown higher levels of OPG among females with Cushing Syndrome, a group that is at increased risk for cardiovascular diseases; this suggests that OPG levels may be associated with a higher risk for coronary disease [29]. Vik A et al have demonstrated a negative correlation between OPG levels and carotid artery calcification [13]. But Rhee EJ et al have investigated the relationship between OPG gene polymorphisms and aortic calcification or coronary artery disease in healthy Korean women and did not observe a correlation between OPG gene polymorphisms and serum OPG levels, or with cardiovascular risk factors [30]. We have not performed ultrasonography examination of carotid artery to determine arterial calcifications. However, we did not detect a correlation between OPG and RANKL levels and the lipid profile or atherogenic indexes.

As for our conclusion, a correlation of OPG levels with BMD, insulin resistance, atherosclerosis that has been determined in studies on adults could not be reproduced in our study in children; this might imply that time is needed before OPG can effect these parameters. OPG changes and their clinical effects among children that were exposed to obesity and insulin resistance for longer are still controversial. Studies in future would elucidate the changes throughout the childhood and detect whether these changes of OPG reported in the literature are the cause or the outcome.

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