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Osteoprotegerin RANKL and Carotid Intima Media Thickness in Diabetics and Prediabetics

Diyabetik ve Prediyabetik Hastalarda Osteoprotegerin RANKL ve Karotis İntima Media Kalınlığı

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Abstract

Purpose: Osteoprotegerin and RANKL (receptor activator of nuclear factor κB) are well known for their roles in osteoporosis, however their effect on atherosclerosis are recently under investigation. This study looked into whether there is an association between osteoprotegerin and RANKL levels and atherosclerosis in prediabetic and diabetic patients without any complications.

Methods: Subjects were grouped as: type 2 diabetics (n: 20), impaired glucose tolerance (IGT) (n:16), impaired fasting glucose (IFG) (n:19) and control group (n:23). Osteoprotegerin and sRANKL were measured by ELISA and carotid intima media thickness (CIMT) on right and left sides was measured and mean value was calculated.

Results: CIMT in diabetics was higher – not significant statistically- when compared to other groups. Groups were not different with regard to osteoprotegerin and RANKL values. Correlation analysis showed a positive correlation between osteoprotegerin and CIMT only in IFG group (r 0,47) and a negative correlation between RANKL and CIMT in the control group (r -0,45). A positive correlation between osteoprotegerin and insulin in diabetics (p<0,05 r 0,50) was found.

Conclusion: We think the possible earlier role of osteoprotegerin and RANKL in atherosclerosis should be evaluated using a more sensitive method to detect atherosclerosis in larger populations.

Key Words: osteoprotegerin, RANKL, atherosclerosis, diabetes, insulin

Özet

Amaç: Osteoprotegerin ve RANKL'ın (*receptor activator of nuclear factor κB*) osteoporozdaki rolleri iyi bilinmektedir fakat yakın zamanda ateroskleroz üzerine etkileri araştırılmaya başlanmıştır. Bu çalışma komplikasyonsuz diyabetik ve prediyabetik hastalarda ateroskleroz ile osteoprotegerin RANKL düzeyleri arasında bir ilişki olup olmadığını araştırmaya yöneliktir.

Yöntem: Olgular tip 2 diyabet (n:20), bozulmuş glukoz toleransı (BGT) (n:16), bozulmuş açlık glukozu (BAG) (n: 19) ve kontrol grubu (n:23) olarak gruplandı. Osteoprotegerin ve çözünebilir RANKL, ELISA yöntemiyle ölçüldü ve sağ ve sol tarafta karotis intima media kalınlığı (KİMK) ölçülerek ortalama hesaplandı.

Bulgular: Diğer gruplarla karşılaştırıldığında diyabetiklerde KİMK daha fazlaydı fakat bu fark istatistiksel olarak anlamlı değildi. Gruplar arasında osteprotegerin ve RANKL düzeyleri açısından fark yoktu. Korelasyon analizi sadece BAG grubunda osteoprotegerin ve KİMK arasında bir pozitif korelasyon (r 0,47) ve kontrol grubunda RANKL ve KİMK arasında bir negatif korelasyon (r -0,45) olduğunu gösterdi. Diyabetik hastalarda osteoprotegerin ve insulin düzeyleri arasında bir pozitif korelasyon (p<0,05 r 0,50) bulundu.

Sonuç: Osteprotogerin ve RANKL'ın aterosklerozdaki muhtemel erken rolünün daha büyük popülasyonlarda, aterosklerozu saptamada daha duyarlı metotlarla değerlendirilmesi gerektiğini düşünmekteyiz.

Anahtar Kelimeler: osteoprotegerin, RANKL, ateroskleroz, diyabet, insülin

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Introduction

RANKL (receptor activator of nuclear factor κB ligand) is a transmembrane protein found in osteoblasts. Osteoprotegerin, blocks the interaction between RANKL and RANK (located on osteoclast precursor) to prevent maturation of osteoclasts and osteoporosis (1, 2).

Similarity between atherosclerotic calcifications and embryonic osteogenesis is interesting. Steps in osteogenesis; like amorphous mineral remodelling, invasion of preosteoblasts followed by trabecular bone formation are also seen in atherosclerosis (3, 4).

Apart from their well assesed role in bone formation, osteoprotegerin and RANKL are recently being investigated in atherosclerosis. According to several studies in the literature, there is a linear relationship between osteoprotegerin levels and cardiovascular mortality (5-8). In an analysis of Dallas Heart Study population, osteoprotegerin is found to be an independent risk factor for coronary atherosclerosis and aortic plaque calcification (9).

Diabetes is a primary risk factor for atherosclerosis (10-14). Whether osteoprotegerin and RANKL levels are high in prediabetics and diabetics without any macrovascular complications is not known. Our aim was to investigate the relationship between carotid intima media thickness and osteoprotegerin and RANKL levels in uncomplicated diabetics and prediabetics.

Materials and methods

Diabetic and prediabetic patients who consulted to the outpatient clinics of Hacettepe University Hospital Department of Endocrinology and Metabolism and who fulfilled the following criteria were accepted to the study; age: 40-65 years, no personal history of malignant disease, cardiovascular, cerebrovascular or peripheral vascular disease. Patients suffering from morbid obesity, patients with a serum creatinine level >1,5 mg/dl or hepatic transaminase level ≥ 3 times normal were not accepted to the study. Control subjects were patients with nontoxic diffuse or nodular goiter who had normal oral glucose tolerance test (OGTT) and had no other known disease. All subjects signed a written informed consent. This cross-sectionally designed study was approved by the local ethics comitee.

Subjects were grouped as diabetics (n: 20), impaired glucose tolerance (IGT) (n: 16), and impaired fasting glucose (IFG) (n: 19) after a 75 g OGTT according to the American Diabetes Association criteria (15) and controls (n: 23). Diabetic patients with a HbA1c level above 9%, with microalbuminuria, or who showed signs of neuropathy or retinopathy on physical examination were not taken to the study. All patients underwent a detailed physical examination and anthropometric measurements of the subjects were recorded. Blood samples for HbA1c, lipids, liver enzymes and creatinine, homocysteine, insulin, high sensitive C reactive protein (hsCRP) as well as osteoprotegerin and sRANKL were taken from the subjects following a ten hour overnight fast. After centrifugation, serum samples for osteoprotegerin and sRANKL were stored in -80°C refrigator. Insulin was measured with an immunoradiometric assay (Immunotech IRMA, Czech Republic). Osteoprotegerin and sRANKL levels were measured via ELISA (enzyme linked immunosorbent assay) method (Biomedica Medizinprodukte GmbH and Co KG, A-1210 Wien, Divischgasse 4). Right and left carotid intima media thicknesses were measured ultrasonographically by the same radiologist - mean of measurements from both sides was taken for each individual. Collected data was analyzed by SPSS 10,0. Kruskall Wallis test was used for comparison of the groups. Statistical differences with a P value lower than 0,05 were accepted as statistically significant. Spearman correlation analysis was used for correlation between continuous variables.

Results

Seventy eight subjects (17 male) were involved in the study. The demographic and anthropometric measurements of the subjects are summarized in Table 1. The body mass index (BMI) was lower in the control group (median: 26,08kg/m²) and median age was younger (median age: 45) when compared to other groups. Sex distribution, menapouse status of women subjects and smoking status of the subjects were not different between groups. When control group was excluded, median BMI and prevalance of hyperlipidemia and hypertension were not different.

Table 1. Demographic characteristics and anthropometric measurements of the participants

Variable	*Type 2 diabetes n=20 Median (minimum-maximum)*	*IGT n=16 Median (minimum-maximum)*	*IFG n=19 Median (minimum-maximum)*	*Controls n=23 Median (minimum-maximum)*	*P value*
Age	49 (45-59)	47 (40-60)	48 (40-63)	45 (40-53)	0,013
BMI (kg/m²)	32,5 (23,8-38,9)	34,9 (23,3-38,8)	33,3 (21,30-39,5)	26,1 (22,6-36,4)	<0,0001
waist circumference (cm)	102 (90-119)	102 (82-124)	102 (82-127)	92 (74-110)	0,002
Skinfold thickness Biceps (cm)	12,5 (4-32)	13,5 (5-25)	14 (2-32)	12 (3-30)	ns
Skinfold thickness Triceps (cm)	20,5 (8-46)	21,5 (7-40)	20,0 (2-35)	20,0 (3-35)	ns
Skinfold thickness Subscapularis (cm)	25,5 (13-37)	27,5 (15-41)	30,0 (12-44)	23,0 (10-33)	ns

Table 2. Metabolic parameters of the participants

Variable	*Type 2 diabetes n=20 Median (minimum-maximum)*	*IGT n=16 Median (minimum-maximum)*	*IFG n=19 Median (minimum-maximum)*	*Controls n=23 Median (minimum-maximum)*	*P value*
Glucose (mg/dl)	114 (82-257)	98 (75-139)	108 (99-124)	84 (72-99)	<0,0001
LDL (mg/dl)	104 (55-169)	127 (80-179)	112 (61-166)	114 (61-159)	ns
Triglyceride (mg/dl)	146 (62-278)	196 (55-385)	123 (57-230)	115 (20-184)	0,008
HDL (mg/dl)	46 (33-68,2)	46 (33,7-70)	47 (33-63,8)	55 (34-87)	ns
sCRP (mg/dl)	0,38 (0,0435-1,37)	0,38 (0,067-1,95)	0,32 (0,05 1,02)	0,19 (0,1-2,15)	ns
Lipo a (mg/dl)	11,8 (2-96,8)	18,7 (2-61,9)	10,7 (0,2-214)	16,9 (3,8-101)	ns
Homocysteine (µmol/L)	11,6 (7,5-19,5)	11,7 (6,5-22,0)	12,5 (6,9-25,6)	11,4 (6,0-17,5)	ns
İnsulin (µIU/ml)	16,0 (7,30-43,0)	13,0 (9,60-41,0)	12,0 (4,40-24,0)	8,1 (2,90-20,0)	ns

Median osteoprotegerin levels were; 0,964 pmol/L in diabetics, 0,965 pmol/L in IGT group, 1,074 pmol/L in IFG group and 0,99 pmol/L in controls. Median sRANKL levels were 0,146 pmol/L, 0,177 pmol/L, 0,149 pmol/L and 0,144 pmol/L in type 2 diabetics, IGT group, IFG group and in controls respectively. There wasn't any difference in osteoprotegerin and sRANKL levels between groups. Other metabolic parameters are summarized in Table 2. Carotid intima media thickness (CIMT) was higher in diabetics but the difference was not statistically significant (p=0,08) (Figure 1).

There was a significant positive correlation between osteoprotegerin and CIMT in IFG group (p<0,05; r 0,47) (Figure 2). In controls there was a correlation between sRANKL and CIMT in a negative direction (p<0,05; r -0,45) (not shown in figure).

Regarding the relation between osteoprotegerin, sRANKL and other parameters; there was a positive correlation between serum insulin levels and osteoprotegerin in type 2 diabetics (p<0,05; r 0,50) (Figure 3), a negative correlation between sRANKL and lipoprotein A in IFG group (p<0,05; r 0,55) and a positive correlation between sRANKL and triglyceride levels in controls (p<0,05; r 0,42) (not shown in figure). There wasn't any significant correlation between osteoprotegerin and RANKL levels and LDL, fasting plasma glucose, homocystein and hsCRP.

In a univariate model, osteoprotegerin and sRANKL were factors affecting the CIMT, however this effect is lost in multivariate analysis (p=0,73 and p=0,38 respectively). BMI, waist circumference, smoking habit, serum insulin, triglyceride, homocysteine or lipoprotein A levels were not determinant on CIMT.

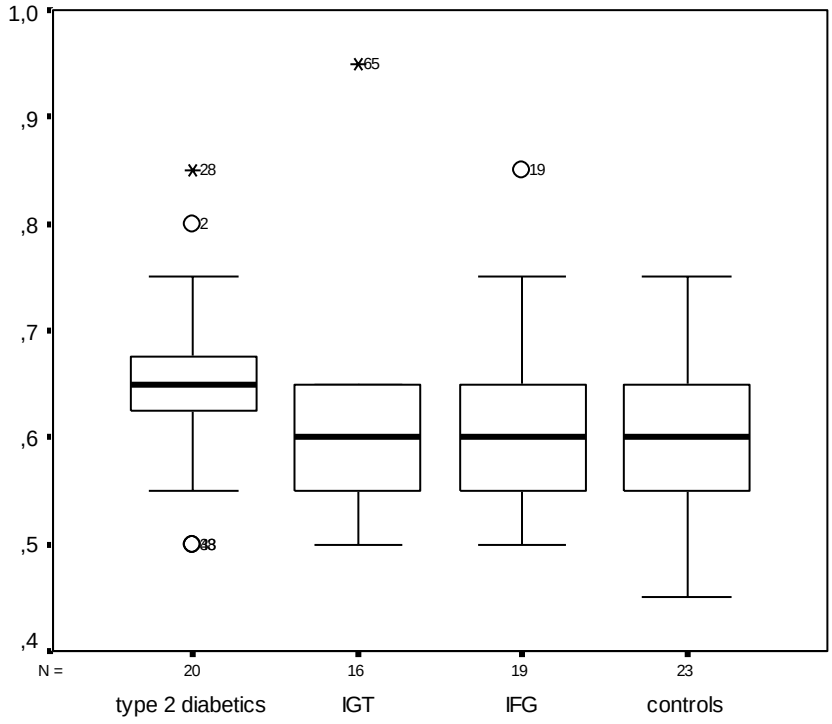

Figure 1. Boxplot graphics for carotid intima media thickness of the participants in IFG group

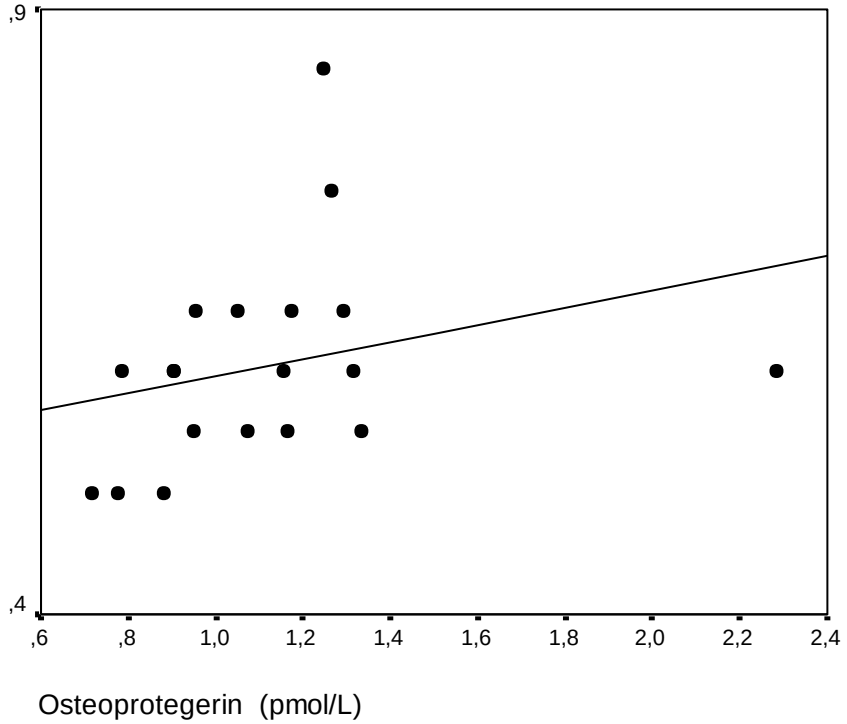

Figure 2. Correlation analysis between carotid intima media thickness and osteoprotegerin

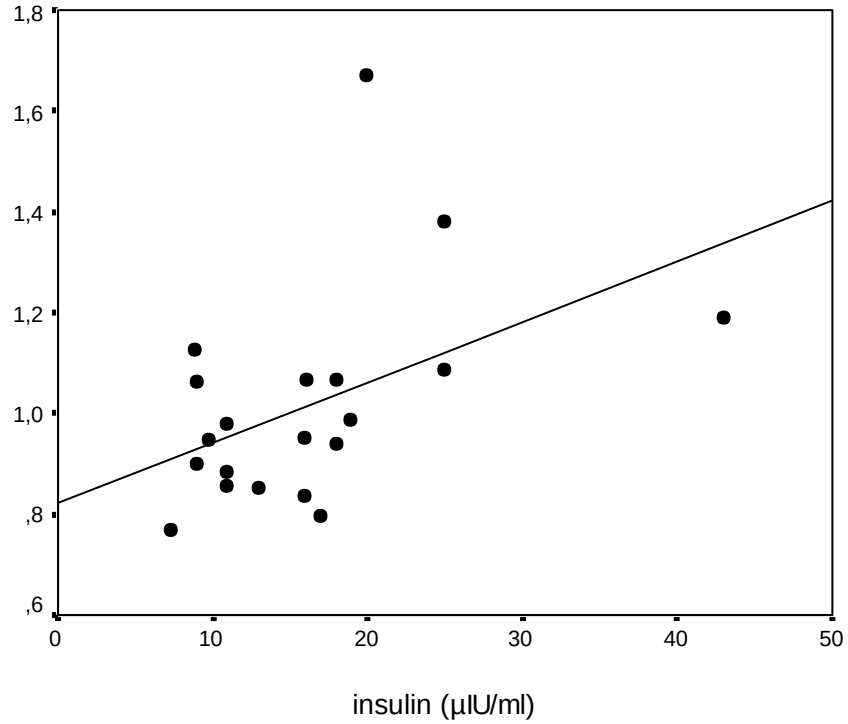

Figure 3. Correlation between serum insulin levels and osteoprotegerin in type 2 diabetics

Discussion

This cross-sectional study investigates the possible correlation between osteoprotegerin and RANKL levels and CIMT in prediabetics and diabetics without any vascular complications. To our knowledge, it is the first study to search for the relationship between osteoprotegerin and RANKL in a prediabetic and uncomplicated diabetic population.

In a prospective study in which 510 type 2 diabetics without any cardiovascular symptoms were involved, coronary artery calcium scores (CCS) were calculated for each subject using computerized tomography. Osteoprotegerin level was significantly higher in the subjects with high CCS. During follow up, 16 cardiovascular events took place and osteoprotegerin was significantly higher in these patients when compared to the subjects without any event. Further analysis showed that osteoprotegerin predicts coronary events more accurately in short term when compared to Framingham score and UKPDS risk score (16). In our study none of the subjects had a known cardiovascular disease or any other vascular complication; besides, carotid atherosclerotic calcification was not detected in any of the subjects. This may explain why we failed to find a correlation between osteoprotegerin and CIMT in the whole study population. But the positive correlation between osteoprotegerin and CIMT in IFG group may imply a role for osteoprotegerin in the very early stage of athersclerosis even in the absence of calcification.

An analysis of Dallas Heart Study population revealed the relationship between osteoprotegerin and coronary artery and aortic plaque calcifications evaluated by computerized tomography or magnetic resonance imaging. 3386 subjects aged between 30-65, were involved in this study (9). We carried out our study in a small population and this may have blunted the possible correlation between osteoprotegerin and atherosclerosis. Another pitfall of our study is that the CIMT is measured with a low resolution ultrasonographic technique which again may have masked minor differences in CIMT.

We couldn't find a correlation between osteoprotegerin and other probable markers of atherosclerosis such as high sensitive CRP and lipoprotein A. In a study in which 104 type 2 diabetics are involved, the investigators evaluated endothelial dysfunction using flow mediated arterial dilation. Osteoprotegerin was significantly higher in patients with endothelial dysfunction whereas hs CRP was not correlated with endothelial dysfunction (17). In another study measuring endothelial dysfunction before and after insulin treatment in type 2 diabetics, fall in osteoprotegerin levels after insulin treatment parallels correction of endothelial

dysfunction however no change occurred in lipoprotein A levels (18).

In this study, we found a negative correlation between CIMT and sRANKL in the control group and lipoprotein A and sRANKL in IFG group. To our knowledge no clinical study yet showed a relation between RANKL and aterosclerosis. Studies yielded controversial results about the relationship between osteoprotegerin and RANKL levels (1, 2). sRANKL is supposed to contribute to the angiogenic process when evaluated at tissue level (19-21). Since we measured the serum levels of RANKL, we are not able to make any further speculations about its contribution to atherosclerosis.

In summary, our study didn't show any correlation between osteoprotegerin and RANKL levels in uncomplicated diabetics but a positive correlation exists in IFG patients. Our study population was small and our patients carried low risk for complications. These factors may have masked the possible correlation in other groups. Further analysis of a larger population of uncomplicated diabetic and prediabetic subjects with a detailed ultrasonographic technique may uncover the subtle differences in CIMT and provide data abut the possible earlier involvement of osteoprotegerin in the atherosclerotic process.

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