Osteopontin and Some Atherosclerotic Risk Factors in Obese and Nonobese, Hypertensive and Normotensive Females

Obez, Obez Olmayan, Hipertansif ve Normotansif Kadınlarda Osteopontin ve Bazı Aterosklerotik Risk Faktörleri

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ABSTRACT Objective: Inflammation is claimed to take part in all stages and complications of atherosclerosis. Osteopontin having matricellular protein properties, is demonstrated to be synthesized in atherosclerotic plaques and is found to have positive relationship with the severity of atherosclerosis. Our aim was to evaluate the relationship of osteopontin with atherosclerotic risk factors in female patients who have hypertension and obesity. **Material and Methods:** Twenty three obese hypertensive, 22 obese normotensive, 20 nonobese hypertensive patients and 22 nonobese normotensive female subjects were evaluated. Body mass index, waist and hip circumference, body fat ratio, systolic and diastolic blood pressure, fasting plasma glucose, post-prandial plasma glucose, total and high density lipoprotein cholesterol, triglyceride, lipoprotein (a), uric acid, c-reactive protein, homeostasis model assessment of insulin resistance (HOMA-IR), fasting and postprandial insulin, osteopontin levels were obtained and low and very low density lipoprotein cholesterol levels were calculated in all. **Results:** Obese hypertensive group had significantly higher plasma osteopontin levels than the rest. Plasma osteopontin levels of obese normotensive group was significantly higher than nonobese hypertensive and nonobese normotensive groups. When the parameters were investigated all together, positive correlations were obtained between osteopontin and body mass index, waist hip ratio, systolic blood pressure, diastolic blood pressure, c-reactive protein. **Conclusion:** Osteopontin was found to be high in obese or hypertensive cases and even higher when obesity and hypertension both existed. The atherosclerotic markers of the latter cases were also higher. Depending on our data we propose that osteopontin may have a specific role in obesity and hypertension, as well as atherosclerosis. We think that studies regarding the blockage of osteopontin biosynthesis in prevention of atherosclerosis and using osteopontin as a marker in asymptomatic atherosclerosis will be noteworthy.

Key Words: Osteopontin; hypertension; obesity; atherosclerosis

ÖZET Amaç: İnflamasyonun aterosklerozun her basamağında ve komplikasyonlarında rolü olduğu kabul edilmektedir. Osteopontinin matrisellüler protein olduğu, aterosklerotik plakta sentezlendiği ve aterosklerozun ciddiyeti ile pozitif ilişkili olduğu gösterilmiştir. Amacımız hipertansiyon ve obezitesi olan kadın hastalarda osteopontin ile bazı aterosklerosklerotik risk faktörlerini değerlendirmek idi. **Gereç ve Yöntemler:** Yirmi üç obez hipertansif, 22 obez normotansif, 20 nonobez hipertansif ve 22 nonobez normotansif kadın çalışmaya alındı. Hepsinde beden kitle indeksi, bel ve kalça çevresi, vücut yağ oranı, sistolik ve diyastolik kan basıncı, açlık kan şekeri, tokluk kan şekeri, total kolesterol ve yüksek dansiteli lipoprotein kolesterol, trigliserid, lipoprotein (a), ürik asit, c-reaktif protein, insülin direncinin homeostazis model değerlendirmesi (HOMA-IR), açlık ve tokluk insülin ve osteopontin seviyelerine bakıldı. Düşük ile çok düşük dansiteli lipoprotein kolesterol seviyeleri hesaplandı. **Bulgular:** Obez hipertansif grubun plazma osteopontin seviyeleri diğer üç gruptan istatistiksel olarak anlamlı yüksek bulundu ($p<0,05$). Obez normotansif grubun plazma osteopontin düzeyi nonobez hipertansif ve nonobez normotansif gruplardan yüksekti. Korelasyon analizinde osteopontin ile beden kitle indeksi, bel kalça oranı, sistolik kan basıncı, diyastolik kan basıncı ve c-reaktif protein arasında anlamlı pozitif ilişki bulundu. **Sonuç:** Hastalarımızda plazma osteopontin düzeyleri obezite veya hipertansiyon varlığında, en fazla da obezite ve hipertansiyon birlikteliğinde yüksek bulundu. Osteopontin düzeyleri yüksek olgularda bazı ateroskleroz belirteçlerinin de yüksek saptanması, osteopontinin aterosklerozda rolü olabileceği gibi hipertansiyon ve obezite etiyopatogenezinde de rol alabileceğini düşündürmüştür. Bu nedenle osteopontinin aterosklerozda bir belirleyici olarak kullanılabileceği ve osteopontin biyosentezini engelleyecek tedavilerin aterosklerozu da önleyebileceği görüşündeyiz.

Anahtar Kelimeler: Osteopontin; hipertansiyon; şişmanlık; aterosklerozis

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Osteopontin (OPN) is a 60 kDa phosphorylated glycoprotein originally found in bone,[1] and is demonstrated to be involved in the formation and calcification of bone.[2] As development continues, it is expressed during gastrulation in the notocord and the embryonic/maternal interface and later in the regions of cartilage condensation, bone formation and in a number of epithelial tissues. In adult, OPN is expressed in activated macrophages and T cells, osteclasts, hepatocytes, smooth muscle, endothelial and epithelial cells.[3]

During development it is secreted in large amounts in embryonic life like many other matricellular proteins. After birth its level lessens fastly and in adults it is synthetized in small amounts physiologically, in order to regulate extracellular matrix. In patological events OPN starts to be synthetized as embryonically, and takes part in inflammation and is synthetized in all the cells found in inflamatory processes.[1,3,4]

Osteopontin is classified as T helper type 1 cytokine that is involved in mineralization of bone and kidney, cell survival, inflammation, cell migration, cell motility and tumor biology.[4-6] OPN induces the expression of a variety of other inflammatory cytokines.[4] Notably it is suggested that OPN plays a role in many diseases characterized by chronic inflammation, including Crohn disease, several types of cancer, autoimmune diseases, obesity, atherosclerosis and cardiac fibrosis.[3,5] Furthermore, OPN is implicated in diabetic macro and microvascular diseases.[7] Recent studies have shown the presence of a complex relationship between inflammation and atherosclerosis, hypertension and obesity.

Atherosclerosis is an inflammatory disease which begins in early childhood in the vascular intima.[4,8,9] Recently OPN has been demonstrated to play a role in human atherosclerosis, [2,10-12] left ventricular hypertrophy[13] and cardiac fibrosis.[14] Hypertension is a major contributing factor in the development of vascular complications associated with diabetes mellitus, atherosclerosis, obesity and chronic renal failure.[15] Hypertensive patients without any other medical conditions were reported to have high circulating levels of inflammatory cytokines.[16,17] Following the demonstration of higher OPN levels in patients with primary aldosteronism than the ones with essential hypertension,[17] plasma OPN levels were found to correlate with carotid morphological and haemodynamic changes in patients with asymptomatic essential hypertension.[15,18]

Obesity is associated with alterations in myocardial and vascular structure as well as physiology, which are accompanied by an adverse risk factor profile leading to the development of cardiovascular disease.[19] The chronic low-grade inflammation associated with obesity is characterized by a number of inflammatory cytokines such as OPN.[19-22] It was shown that OPN is extensively upregulated in the adipose tissue of obese humans, as well as of diet-induced and genetically (db/db) obese mice.[20,21]

Considering about the complex interaction among atherosclerosis, hypertension and obesity, we investigated the relationship of OPN and metabolic or anthropometric parameters, in women who have the burden of atherosclerotic risk factors: hypertension and obesity.

MATERIAL AND METHODS

PATIENTS

A total of 65 females, 23 obese hypertensive, 22 obese normotensive and 20 nonobese hypertensive patients, aged from 40-50 years, were recruited from the outpatient Clinic of Internal Medicine, Ankara Education and Research Hospital from September 2008 to December 2008. 22 age matched nonobese normotensive female subjects constituted the control group. Patients who were taking antihypertensive drugs or patients had mean blood pressure levels over 140/90 mmHg were put into hypertensive group according to The Joint National Commitee on the Detection, Evaluation and Treatment of High Blood Pressure VII. In spite of the idea of putting patients with mean blood pressure 120-139/80-89 mmHg in the prehypertensive

group and patients with mean blood pressure below 120/180 mmHg in normal group, for beter understanding the subjects with mean blood pressure levels below 140/90 mmHg were put into normotensive group.

The study was performed according to the Helsinki Declaration 2008**.** The local ethics comitee approved this study and all the subjects gave written informed consent.

Patients with male gender, with conditions that disorders which may affect metabolic parameters, such as; polycystic ovary syndrome or past/present story of thyroid dysfunction, pregnant cases, the ones with chronic diseases (diabetes mellitus, chronic renal disease, chronic hepatic failure and malignancies), infections, coronary artery disease (stable or unstable angina pectoris or history of myocardial infarction) were excluded. Participants had not been on any medications for at least 6 months before the start of the study including oral contraceptives, glucocorticoids, ovulation induction agents, anti-diabetic and anti-obesity drugs, estrogenic, anti-androgenic anti-hyperlipidemic medications.

Following detailed physical examination, body weights, heights, waist and hip circumferences, body fats were measured, and waist-hip ratios (WHR) were calculated for each participant. Blood was withdrawn after 12h of overnight fasting, at 08.30 a.m. for fasting plasma glucose (FPG), fasting insulin (FI), serum total and high density lipoprotein (HDL) cholesterol, triglyceride (TG), c-reactive protein (CRP), lipoprotein (a) [(Lp (a)], uric acid, and osteopontin levels. Another blood sample was taken for postprandial plasma glucose (PPBG) and postprandial insulin (PPI) 2 h after breakfast. Patients having FBG levels over 126 mg/dL and PPBG levels over 200 mg/dl were diagnosed as diabetes mellitus and excluded.

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Subjects were classified according to BMI such as obese ≥ 30 kg/m^2, and nonobese < 30 kg/m^2. Systolic and diastolic blood pressures (SBP and DBP) were measured after a 5 min rest in the semi-sitting position with a sphygmomanometer. Blood pressure was measured at least three times from the right upper arm, and the mean was used for the analysis.

Waist and hip circumferences of the subjects were measured during overnight fasting by a non elastic measurement, in upright position. Body fats were estimated by Tanita body composition analayser TBF -300 after the subjects rested 30 minutes.

LABORATORY METHODS

Plasma glucose, total cholesterol, TG and HDL concentrations were determined by enzymocalorimetric spectrophotometric method in a Roche/Hitachi molecular PP autoanalyser. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol were calculated by the Friedewald Formula (LDL: Total cholesterol HDL-TG/5). Insulin was measured by means of DRG Diagnostics (DRG Instruments GmbH, Germany) ELISA kits.

An indirect measure of insulin resistance was calculated from the formula: fasting plasma insulin (μunite/mL) x fasting plasma glucose (mmol /L)/ 22.5 named as Homeostasis Model Assessment of Insulin Resistance (HOMA-IR).

High sensitivity CRP was measured by immunnefolometric tests by Beckman-Cutler device. Uric acid was measured by calorimetric, Lp (a) was measured by nephelometric methods.

For the measurements of OPN, 5 mL blood samples were collected in EDTA containing tubes. These blood samples were centrifuged 4000 cycle/min in 30 minutes. Plasma was then stored at -80˚C. Plasma OPN levels were measured by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Human OPN assay kit, Biotec USA). This ELISA kit was recently developed based on the method reported by Kon et al.,[23] and it measures total concentration of non-phosphorylated and phosphorylated forms of OPN in plasma.

STATISTICAL ANALYSIS

Calculations were performed using SPSS version 11.5 (Customer ID 30000105 930). Data are presented as mean ± SD. Student t-test was used to compare the groups in a parametric way. One way variation analysis (ANOVA) was used to compare all study groups with each other. Tukey's multiple comparison test was used for post hoc analysis. A p value of < 0.05 was considered as statistically significant. Pearson correlation co-efficient was used for the correlation analysis.

RESULTS

This study was performed on 23 obese hypertensive, 22 obese normotensive, 20 nonobese hypertensive patients and 22 nonobese normotensive female subjects. All the demographic and laboratory findings of the groups were demonstrated in Table 1.

TABLE 1: Characteristics of the groups.

	Obese hypertensive (n= 23) Group I	Obese normotensive (n= 22) Group II	Nonobese hypertensive (n= 20) Group III	Nonobese normotensive (n= 22) Group IV
Age (yr)	42.78 ± 7.26	39.36 ± 6.64	43.65 ± 9.08	40.86 ± 6.25
Height (cm)	154.60 ± 4.99	155.00 ± 3.59	157.80 ± 5.91	157.72 ± 6.80
Weight (kg)	79.41 ± 7.99 [a,b,c]	87.02 ± 14.25 [d,e]	59.77 ± 6.68 [f]	55.26 ± 6.41
BMI (kg/m^2)	33.31 ± 3.19 [b,c]	36.31 ± 6.34 [d,e]	23.91 ± 1.77 [f]	22.28 ± 2.62
W. Cir. (cm)	95.26 ± 5.61 [b,c]	97.36 ±8.94 [d,e]	82.10 ± 8.74 [f]	72.86 ± 6.32
H. Cir. (cm)	114.00 ± 5.55 [b,c]	118.8 ± 10.25 [d,e]	103.35 ± 7.45 [f]	97.68 ± 5.52
WHR	0.83 ± 0.03 [b,c]	0.82 ± 0.03 [d,e]	0.79 ± 0.04 [f]	0.74 ± 0.04
Body fat (%)	31.51 ± 6.84 [b,c]	36.21 ± 9.59 [d,e]	18.09 ± 5.16 [f]	13.87 ± 5.31
SBP (mmHg)	147.82 ± 21.09 [a,c]	119.54 ± 13.61 [d,e]	144.50 ± 23.05 [f]	109.54 ± 11.74
DBP (mmHg)	92.17 ± 16.22 [a,c]	78.86 ± 9.75 [d,e]	92.75 ± 11.63 [f]	73.18 ± 8.38
FBG (mg/dL)	99.04 ± 11.49 [c]	96.90 ± 11.94 [e]	97.75 ± 10.84 [f]	88.27 ± 10.08
PPBG (mg/dL)	117.04 ± 25.07 [c]	118.18 ± 22.10 [e]	112.30 ± 23.32 [f]	97.31 ± 17.58
Cholesterol (mg/dL)	223.00 ± 41.05 [a,c]	191.50 ± 35.54	198.46 ± 38.79 [f]	174.59 ± 27.03
TG (mg/dL)	148.08 ± 60.79 [c]	152.27 ± 73.86 [e]	154.75 ± 86.87 [f]	103.86 ± 62.06
LDL (mg/dL)	136.43 ± 39.92 [a,c]	112.95 ± 31.71	117.65 ± 38.35	98.77 ± 21.32
VLDL (mg/dL)	29.61 ± 12.15 [c]	30.45 ± 14.77 [e]	30.95 ± 17.37 [f]	20.77 ± 12.41
HDL (mg/dL)	56.78 ± 13.10 [a]	48.13 ± 9.28 [e]	49.80 ± 10.82	55.18 ± 12.33
Uric acid	4.89 ± 1.21 [b,c]	4.43 ± 1.03 [e]	4.08 ± 0.72 [f]	3.55 ± 0.65
CRP (mg/dL)	4.54 ± 3.68 [c]	4.37 ± 3.60 [d,e]	3.05 ± 2.61 [f]	1.46 ± 1.35
Lp a (mg/dL)	269.14 ± 249.22	334.04 ± 276.76 [e]	381.43 ± 250.22 [f]	147.30 ± 111.59
FI (µu/mL)	13.46 ± 5.81 [c]	13.20 ± 5.50 [e]	12.38 ± 6.57	9.02 ± 4.72
PPI (µu/mL)	44.58 ± 20.90 [c]	49.58 ± 24.29 [e]	34.57 ± 25.58	29.94 ± 15.28
HOMA-IR	3.35 ± 1.66 [c]	3.21 ± 1.54 [e]	3.03 ± 1.87 [f]	1.99 ± 1.08
OPN (ng/mL)	308.76 ± 113.56 [a,b,c]	237.31 ± 71.22 [d,e]	188.22 ± 59.37 [f]	143.40 ± 30.44

BMI: Body mass index, W. Cir: Waist circumference, H. Cir: Hip circumference, WHR: Waist- hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, PPBG: Postprandial blood glucose, TG: Triglyceride, LDL: Low density lipoprotein cholesterol, VLDL: Very low density lipoprotein cholesterol, HDL: High density lipoprotein cholesterol, CRP: C- reactive protein, Lp a: Lipoprotein a, FI: Fasting insulin, PPI: Postprandial insulin HOMA-IR: Homeostasis model assessment insulin resistance index, OPN: Osteopontin. Data are presented as mean ± SD.

a : Difference between Group I and II is statistically significant (p< 0.05)

b : Difference between Group I and III is statistically significant (p< 0.05)

c : Difference between Group I and IV is statistically significant (p< 0.05)

d : Difference between Group II and III is statistically significant (p< 0.05)

e : Difference between Group II and IV is statistically significant (p< 0.05)

f : Difference between Group III and IV is statistically significant (p< 0.05)

Comparing obese hypertensive and obese normotensive groups we found that OPN, total, LDL and HDL cholesterol levels of obese hypertensive patients were statistically higher than their normotensive peers. Obese normotensive patients had higher weight than hypertensive ones. No statistically significant difference were found in uric acid, CRP, Lp (a), FI, PPI and HOMA-IR levels of these two obese groups.

When obese hypertensive group and nonobese hypertensive groups were compared, uric acid and OPN levels were found to be statistically higher in obese hypertensives. Their Lp (a), FI, PPI, HOMA-IR levels exhibited insignificant difference.

Significantly higher FI, PPI, HOMA-IR, uric acid, CRP, total, LDL, VLDL cholesterol and TG and OPN levels were observed in obese hypertensive patients than nonobese normotensive patients. However no difference was observed regarding their Lp (a).

OPN and CRP levels of obese normotensive group were statistically higher than nonobese hypertensive group. Their FI, PPI, HOMA-IR, uric acid, Lp (a) levels did not differ.

TG, VLDL, FI, PPI, HOMA-IR, OPN, uric acid, CRP and Lp (a) levels were statistically higher in obese normotensive group than nonobese normotensive group. HDL leves were higher in nonobese group.

When nonobese hypertensive and nonobese normotensive groups were compared, we found that nonobese hypertensive group had statistically higher OPN, weight, BMI, waist circumference, hip circumference, WHR, body fat, total and VLDL cholesterol, TG, FBG, PPBG, HOMA-IR, uric acid, CRP, Lp (a) levels, but FI, PPI levels were similar.

Correlation analyses were performed using the parameters. Positive correlations of OPN may be listed as:

OPN and SBP, DBP, BMI, WHR, CRP (r= 0.402, p= 0.001; r= 0.245, p= 0.022; r= 0.476, p= 0.001; r= 0.447, p= 0.001; r= 0.275, p= 0.01 respectively).

DISCUSSION

Recently osteopontin has been investigated in a number of vascular pathologies and its expression is shown to be augmented in atherosclerosis, arterial restenosis, myocardial infarction, stroke, stenosis of native and bioprotetic valves.[24] It is reported to be a novel component of human atherosclerotic plaques and its expression on atherosclerotic plaques is shown to be closely associated with the severity of atherosclerosis and calcification.[2,11,12,25,26]

As OPN is reported to be primarily expressed in adipose tissue and women have abundance of adipose tissue, in order to obtain a homogenous group we included only women in our study. Our data showed that OPN levels of obese hypertensive patients were significantly higher than other three groups. Moreover plasma OPN levels of obese normotensive patients were also found to be significantly higher than nonobese (hypertensive or normotensive) subjects. Nonobese hypertensive patients had significantly higher plasma OPN levels than nonobese normotensive subjects. We also found positive correlation between OPN and SBP, DBP, BMI, WHR, CRP. Though obesity acts as a cardiovascular risk factor by mechanisms that are not fully understood, it is found to be associated with alterations in myocardial and vascular structure and physiology.[19,27] In literature it was shown that obese and overweight patients exhibited significantly increased circulating OPN concentrations as compared with lean subjects, and a significant positive correlation was found between OPN levels and body fat.[4,19,28] Accordingly we also found that OPN levels were higher in obese patients than the nonobese ones.

Osteopontin gene expression in omental and subcutaneous adipose tissue was shown to be highly positively correlated with waist circumference, BMI, body fat percentage, serum leptin, and markers of glucose homeostasis.[4,14] Positive correlation was found between OPN and BMI and WHR in our patients. While our obese pateints (either

hypertensive or normontensive) compared with nonobese ones had higher OPN levels, in nonobese groups also hypertensive ones who had higher OPN levels also exhibited higher weight, BMI, waist, hip circumference, WHR and body fat percentage. This result may strenghten the idea of the positive relationship of OPN between adipose tissue. We hope that more satisfying results will be obtained, if we continue to work with larger group of patients.

As OPN is a multifunctional protein involved in various inflammatory processes, it may be speculated that OPN can be a critical regulator in obesity induced adipose tissue inflammation and insulin resistance. In the literature relationship between OPN and hyperglycemia-insulin resistance was demonstrated.[4,19] Nevertheless we could not find correlation between OPN and insulin and HOMA-IR. We think that this result may be explained by the relatively small size of our study population.

In many prospective studies, it was demonstrated that in healthy subjects even small CRP elevations in normal limits, may augment future risks of stroke, myocardial infarction and peripheric arterial disease.[29] High CRP levels in obesity was also claimed to be a marker of inflammation of adipose tissue.[30] In some studies, like ours, positive relationship between OPN and CRP was determined.[29,31,32] In our hypertensive and obese patients CRP and OPN levels were high, we wonder if we can use OPN as an atherosclerotic risk marker as CRP.

The association among hyperuricemia, metabolic syndrome and atherosclerotic vascular disease has been reported in adults and children[33,34]. Keeping in accordance with this information serum uric acid levels were high in obesity and hypertension in the present study and even higher when obesity and hypertension occured together.

Hypertension is an important risk factor in vascular complications related to diabetes, atherosclerosis, obesity. Inflammatory cytokines like OPN may trigger the vascular damage in cardiovascular pathologies and plasma levels may correlate with atherosclerosis in patients with hypertension.[16,18,20] In our hypertensive patients the OPN levels were higher and positive correlation was found between OPN and systolic and diastolic pressures. We speculate that OPN levels may serve as a useful marker for atherosclerosis in patients with hypertension and blocking OPN biosynthesis may have therapeutic value.

As all the groups were evaluated, the OPN levels of obese patients were higher than hypertensive patients, but the highest OPN levels were determined in obese as well as hypertensive patients. This finding makes us think that obesity may be a more important factor than hypertension.

In conclusion, our data showed that in obesity, hypertension, OPN plays an important role. It is also related to atherosclerotic inflammatory markers such as; CRP and uric acid levels. Further studies are needed to clarify whether inhibiting OPN biosynthesis may be useful in the prevention of atherosclerosis or not. The value of determining plasma OPN levels in evaluating the results of preventative measures of cardiovascular diseases also worths inquiring. We suggest that OPN may be a marker in in asymptomatic phase of cardiovascular diseases.

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