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Full Length Research Paper

Osteopontin a new probable marker for atherosclerosis in obese women?

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Obesity is associated with a state of chronic, low-grade inflammation characterized by abnormal cytokine production and macrophage infiltration into adipose tissue, which may contribute to the development of insulin resistance and type 2 diabetes. It was emphasized that inflamation takes part in all stages and complications of atherosclerosis. Osteopontin (OPN) is a multifunctional protein involved in various inflammatory processes, cell migration, and tissue remodeling. This study evaluated the role of OPN in female patients who have at least one risk factor for atherosclerosis such as obesity. The study included 45 morbidly obese normotensive female patients and 22 age and sex-matched control subjects. As well as making physical and antropometric examinations, fasting plasma glucose and insulin, post prandial plasma glucose and insulin, lipid profile, C-reactive protein, lipoprotein a, OPN levels were obtained in all female person. In this study we investigated atherosclerosis indirectly. Our obese group had significantly higher levels in glucose and lipid parameters as well as antropometric measures. Obese group also had significantly higher plasma OPN and C -reactive protein levels than the control group ($p < 0.05$). We also performed the correlation analysis of the groups and found positive correlations in OPN levels and body mass index, C-reactive protein, fasting insulin. In conclusion, our data may point toward a role of OPN in atherosclerosis and obesity.

Key words: Osteopontin, atherosclerosis, obesity.

INTRODUCTION

Osteopontin (OPN) is a chemokine-like, extracellular matrix associated protein involved in monocyte motility and inflammatory immune response, mineralization in bone and kidney, cell survival, inflammation, and tumour biology (Irita et al., 2006; Kurata et al., 2006; Ohmori et al., 2003; Scetana et al., 2007; Kiefer et al., 2008; Luomala et al., 2007).

It was originally found in bone (Heinegard et al., 1989) and determined that it was involved in the formation and calcification of bone (Ohmori et al., 2003/) . OPN is expressed in activated macrophages and T cells, osteoclasts, hepatocytes, smooth muscle, endothelial and epithelial cells (Irita et al., 2006; Kurata et al., 2006; Ohmori et al., 2003; Scetana et al., 2007; Kiefer et al., 2008; Luomala et al., 2007). Since its clonning in 1986, OPN has been found to be associated with a great range of pathological responses.

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OPN plasma levels are found elevated in various diaeases including atherosclerosis, inflammatory bowel disease, granulomatous inflammatory disease, rhematoid arthritis, multiple sclerosis, several types of cancer, obesity, and cardiac fibrosis (Kiefer et al., 2008; Luomala et al., 2007;Bertola et al., 2009).

The atherosclerotic lesion is proved to be highly inflammatory (Scetana et al., 2007). Inflammatory cytokines are accepted to predict cardiovascular events such as coronary heart disease, stroke and congestive heart failure (Kurata et al., 2006; Ohmori et al., 2003; Scetana et al., 2007; Luomala et al., 2007). OPN is also agreed to be an inflammatory mediator, that can enhance pro-inflammatory process in vascular diseases (Luomala et al., 2007).

Since OPN levels were found to be asso-ciated with the presence and extent of coronary artery disease, it may be speculated that increased expression of OPN may promote the development of atherosclerosis (Ohmori et al., 2003; Golledge et al., 2004). Obesity and hypertension are important risk factors for atherosclerosis. The chronic

low-grade inflammation associated with obesity is determined by increased systemic concen-trations of inflammatory markers and cytokines in patients and animal models of obesity (Kiefer et al., 2008). The main origin of this systemic inflammatory response is shown to be located in adipose tissue (Weiseberg et al., 2003). Adipose tissue produces a number of inflam -matory cytokines, and some of them are found elevated in the serum of obese patients (Bautista et al., 2005).

Keeping in mind the complex relations among athero-sclerosis and obesity, we investigated the relationship of OPN and metabolic and antropometric parameters, in women who have a burden of an atherosclerotic risk factor such as obesity.

PATIENTS AND METHODS

This study carried out between September 2008 to December 2008 in the Outpatient Clinic Ankara Education and Research Hospital after the approval of local ethics comitee. This study is a prospective trial.

The study included 45 morbidly obese normotensive female patients and 22 age and sex-matched female control subjects. As OPN is reported to be primarily expressed in adipose tissue and women have abundance of adipose tissues, in order to obtain a homogenous group we included only women in our study. Our control subjects were normotensive and nonobese. Patients with male gender, diabetes mellitus, glucose intolerance, conditions which may effect metabolic parameters (such as thyroid dysfunc-tions in history or nowadays), pregnancy, chronic diseases such as hypertension, infection, coronary artery disease and who were taking medicine were excluded. All the subjects gave written informed consent.

After detailed physical examination, body weight, height, waist, hip circumference, waist -hip ratio (WHR) and body fat of all the subjects were measured. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Women were classified according to BMI such as obese 30 kg/ m^2. All the female subjects' waist and hip circumference were measured when fasting by a non-elastic masurement, as upright position. Body fat were estimated by Tanita body composition analayser TBF -300 after the subjects rested 30 min.

Sistolic and diastolic blood pressure (SBP and DBP) were measured after a 5 min rest in the semi-sitting position with a sphygmomanometer. Blood pressure was determined at least three times at the right upper arm, and the mean was used in the analysis. The patients who were taking antihypertensive drugs or patients whose determined mean blood pressure levels 140/90 mmHg were accepted as hypertensive.

Blood was withdrawn after 12 h of overnight fasting, at 08.30 a.m. for fasting plasma glucose (FPG), insulin (FI), serum total and high density lipoprotein (HDL) cholesterol, triglyceride, C-reactive protein (CRP), lipoprotein a (Lp a), and osteopontin levels. Another blood sample was taken for postprandail plasma glucose (PPPG) 2 h after breakfast.

Laboratory methods

Plasma glucose, total cholesterol, triglyceride (TG) and HDL cholesterol concentrations were determined by enzimochalorimetric spectrophotometric methods in a Roche/Hitachi molecular PP autoanalyser. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol were calculated by the Friedewald Formula (VLDL = TG/5; 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 fasting plasma insulin (unite / ml) x fasting plasma glucose (mmol /l) / 22. 5 formula as homeostasis model assessment (HOMA) . 2.7 HOMA levels were accepted as present insulin resistance, < 2.7 HOMA levels were accepted as absent insulin resistance. High sensitivity CRP was measured by immunnefolometric tests by Beckman- Cutler device. 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 santrifuged 4000 cycle / min in 30 min. 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. Gomez-Ambrosi et al. (2007) measures total concentration of non phosphorylated and phosphorylated forms of OPN in plasma.

After calculating the statistical analysis of the obese and nonobese normotensive control groups we made the correlation analysis of obese group. By using SPSS version 11.5 (Customer ID 30000105 930) we performed the calculations. We presented our data 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. We used Tukey's multiple comparison test for post hoc analysis. A p value of < 0.05 was considered as statistically significant. Pearson correlation coefficient was used for the correlation analysis.

RESULTS

We performed the study with 45 obese patients and 22 control subjects. All the demographic and laboratory findings of obese patients and control groups were compared and demonstrated in Table 1.

In Table 1 obese and nonobese groups were compared. As expected BMI, waist and hip circumference, WHR, body fat measurements were found to be significantly higher in obese group than nonobese group (respectively $p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.008$, $p < 0.001$) (Table 1). Signifcantly higher FBG (fasting blood glucose), PPBG (post prandial blood glucose), total and LDL, VLDL cholesterol, TG, CRP, Lp a, FI, HOMA (homeostasis model assessment) and OPN levels were observed in obese patients than nonobese subjects (respectively $p < 0..001$, $p < 0.001$, $p = 0.001$, $p = 0.004$, $p = 0.008$, $p = 0.008$, $p < 0.001$, $p < 0.001$, $p = 0.015$, $p = 0.003$, $p < 0.001$, $p = 0.026$) (Table 1). However no differences were observed in obese patients and control women as age, HDL cholesterol levels were concerned.

We then made the correlation analysis in obese group and found weak positive correlation between OPN and BMI, CRP, FI levels, between BMI and HOMA, FI. We also obtained weak positive correlation between CRP and HOMA, FI. We found weak negative correlation between HDL cholesterol and FI, and strong positive correlation between HOMA and FI (Table 2). We could not find any positive or negative correlations among any other paramters, so we did not include them in the table.

Table 1. Characteristics of patient and control groups.

	Patients (n:45)	control (n:22)	P
Age (yr)	43. 65 ± 9.08	40.60 ± 7.96	NS
BMI (kg/m^2)	34. 78 ± 5.15	22.28 ± 2.62	<0.001
Waist circumference (cm)	97.60 ± 11.35	72.86 ± 6.32	<0.001
Hip circumference (cm)	115.22 ± 10.36	97.68 ± 5.52	<0.001
WHR	0.85 ± 0.18	0.74 ± 0.04	=0.008
Body fat (%)	33.81 ± 8.54	13.87 ± 5.31	<0.001
FBG (mg/dl)	98.00 ± 11.63	88.27 ± 10.08	<0.001
PPBG (mg/dl)	117.60 ± 23.40	97.31 ± 17.58	<0.001
Cholesterol(mg/dl)	207.60 ± 41.22	174.59 ± 27.03	<0.001
TG (mg/dl)	150.13 ± 66.75	103.86 ± 62.06	=0.008
LDL (mg/dl)	124.95 ± 37.65	98.77 ± 21.32	=0.004
VLDL (mg/dl)	30.02 ± 13.35	20.77 ± 12.41	=0.008
HDL (mg/dl)	52.55 ± 12.08	55.18 ± 12.33	NS
CRP (mg/dl)	4.46 ± 3.60	1.46 ± 1.35	<0.001
Lp a (mg/dl)	300.87± 262.09	147.30±171.59	=0.015
FI (u/ml)	13.33 ± 5.59	9.02 ± 4.72	=0.003
HOMA	2.95 ± 1.43	1.79 ± 0.97	<0.001
OPN (ng/ml)	289.99 ± 82.03	245.87± 55.24	=0.026

Data are presented as mean ± SD. NS: nonsignificant. BMI: Body mass index, WHR: waist- hip ratio, FBG: fasting blood glucose, PPBG: post prandial blood glucose, TG: triglyceride, LDL: low density lipoprotein cholesterol, VLDL: very low density lipoprotein, HDL: high density lipoprotein cholesterol, CRP: C- reactive protein, Lp a: lipoprotein a, FI: fasting insulin, HOMA: homeostasis model assessment, OPN: osteopontin.

Table 2. Summary of correlation analysis of obese patients.

N=45	OPN	BMI	C R P	HOMA	FI	HDL
OPN	1	$p < 0.05$ $r = 0.302$	$p < 0.05$ $r = 0.320$		$p < 0.05$ $r = 0.329$	
BMI	$p < 0.05$ $r = 0.302$	1		$p < 0.05$ $r = 0.355$	$p < 0.05$ $r = 0.335$	
CRP	$p < 0.05$ $r = 0.320$		1	$P < 0.05$ $r = 0.361$	$P < 0.05$ $r = 0.359$	
HOMA		$p < 0.05$ $r = 0.355$	$p < 0.05$ $r = 0.361$	1	$p < 0.001$ $r = 0.953$	
FI	$p < 0.05$ $r = 0.329$	$p < 0.05$ $r = 0.335$	$p < 0.05$ $r = 0.359$	$p < 0.001$ $r = 0.953$	1	$p < 0.05$ $r = -0.307$
HDL					$p < 0.05$ $r = -0.307$	1

BMI: Body mass index, CRP: C- reactive protein, HOMA: homeostasis model assessment, FI: fasting insulin, HDL: high density lipoprotein cholesterol, OPN: osteopontin.

DISCUSSION

Our data showed that OPN levels of obese patients were significantly higher than those levels of nonobese controls. In obese group OPN levels were found to be correlated with CRP, BMI and FI levels. Obesity as well

as being a cardiovascular risk factor is also an important risk factor for insulin resistance (Grundy, 2007; Lawlor et al., 2006; Despres and Lemieux, 2006). Incresed systemic concentrations inflammatory markers were determined in obese animals and humans, such as adiponectin, leptin, resistin, visfatin, omentin, interleukin-6, and tumor necrosis factor- .

These systemic inflammatory responses are mainly derived from adipose tissue (Hotami lıgil et al., 1993). OPN is evaluated to be one of those inflammatory markers. OPN also induces the expression of other inflammatory cytokines and chemokines in peripheral blood mononuclear cells (Xu et al., 2005).

After elevated OPN gene expression in murine obesity was demonstrated (Todoric et al., 2006), Keifer and co workers showed up-regulated OPN gene and protein expression in diet induced and genetic murine obesity (Kiefer et al., 2008). In human, OPN primarily, reported to be expressed in adipose derived stem cells (Hicok et al., 2004), then Gomez-Ambrosi and co-workers demonstrated for the first time the presence of OPN in human omental adipose tissue (Gomez-Ambrosi et al., 2007). Moreover these authors observed that plasma OPN levels were increased in overweight and obese patients, and further elevated in obesity associated diabetes. Keifer and co workers suggested a local impact of OPN within obese adipose tissue, which was not reflected by systemic OPN concentrations(Kiefer et al., 2008). According to their study, analysis of adipose tissue cellular fractions revealed that OPN was highly expressed in adipose tissue macrophages in humans and mice, and this adipose macrophage expression was found to be strongly up-regulated by obesity. In a recent interesting study Xue et al. declared that simple obesity was associated with reduced breast arterial calcification and increased circulating osteopentin concentrations (Xue et al., 2008). All these data proves the specific role of OPN in obesity.

OPN gene expression in omental and subcutaneous adipose tissue was shown to be highly positively correlated with waist, hip circumference, BMI, percent body fat (Kiefer et al., 2008). We only found positive correlation between OPN and BMI. Waist, hip circumference, WHR and body fat of our obese patients were significantly elevated as well as BMI of them, but we were not able to show positive correlation between OPN and these paremeters, we think that it was about comparably smaller sample size of our study. We hope that more satisfying results will be obtained, if we continue our studies with larger patient groups.

Recently Nomiyama and co- workers have identified OPN as a link between adipose tissue inflammation and insulin resistance in a murine model of diet induced obesity by examining the role of OPN in the accumulation of adipose tissue macrophages (Nakamachi et al., 2007; Nomiyama et al., 2007). After Weiseberg et al. identified macrophage accumulation possibly derived from the circulation in obese adipose tissue (Weiseberg et al., 2003) Xu et al. characterized these macrophages as an important mediator of insulin resistance (Xu et al., 2003). The findings about macrophages that infiltrate adipose tissue are from the circulation have focused attention on possible mechanisms by which these cells are recruited into obese adipose tissue. Accumulating evidence has demonstrated that these cells recruitement during inflammation is dependent on the expression of OPN (Ashkar, 2000; Bruemmer, 2003).

In our study we could not find positive correlation with OPN and HOMA, but found positive correlation with OPN and FI levels. When we compared our obese and nonobese groups, HOMA was significantly high in our obese patients. As we determined positive correlation between HOMA and FI, as well as HOMA and CRP levels and OPN and CRP levels of our obese patients, it may be speculated that if our study population will be expanded more satisfying results would have been obtained as correlation between OPN and HOMA is concerned. Besides, our obese patients and control subjects were normoglycemic.

In cardiovascular events CRP has been shown to be a marker of the degree of inflammatory activity and a valuable tool for the risk assessment (Fichtschere et al., 2000). In many prospective studies, it was demonstrated that even in healthy subjects small CRP elevations in normal limits may augment future risks of stroke, myocardial infarction and peripheric arterial disease, (Fichtschere et al., 2000; Rifai and Ridker, 2001). The proinflammatory association between CRP and OPN was questioned recently (Suezawa et al., 2005; Tanaka et al., 2006). Gomez-Ambrosi et al. stated that excess adiposity may contribute to the obesity associated low grade chronic inflammation, with OPN emerging as an additional element of this condition. It was also speculated that as some agents lower levels of CRP, elevated CRP and osteopontin levels might benefit from these treatments (Ridker et al., 2008, 2009; Kurata et al., 2007). In JUPITER study, in healthy persons without hyperlipidemia but with elevated CRP levels, rosuvastatin significantly reduced the incidence of major cardiovascular events (Ridker et al., 2008, 2009). A number of authors have shown that weight loss resulted with a strong reduction in OPN expression in adipose tissue, as well as decreased plasma CRP and an improvement of insulin sensitivity (Cancello et al., 2005; Clement and Langin, 2007). In a recent study, Keifer et al. Demonstrated that antibody mediated neutralization of OPN action significantly reduced insulin resistance in obesity (Keifer et al., 2010).

In our study the CRP levels of obese patients were significantly higher than our control subjects as well as OPN levels. We also found positive correlation in OPN and CRP levels of our obese patients. These results reminds us that OPN plays a part in obesity associated inflammatory state, which takes place in the obesity associated cardiovascular derangements.

In conclusion, data obtained in our study showed that in

obesity which is a risk factor of atherosclerosis, plasma OPN level is high. Although, our sample size is small, our study, besides reminding us about the measures obesity, makes us think about using OPN as a probable marker of atherosclerosis and the value of determining OPN plasma levels in evaluating the results of preventative measures of cardiovascular diseases. We also speculate that targeting OPN could provide an useful approach for the treatment of obesity and related metabolic and cardiovascular disorders.

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