INVERSE RELATIONSHIP BETWEEN ADIPONECTIN AND PLASMINOGEN ACTIVATOR INHIBITOR-1 IN METABOLIC SYNDROME PATIENTS

BILGILI SIBEL, CELEBILER AYDAN C., DOGAN AYLIN, KARACA BAYSAL

The Ministry of Health, Izmir Education and Research Hospital, Clinical Biochemistry Department, Turkiye, e-mail: bilgili.sibel@yahoo.com.tr

Objectivs. To determine the serum levels of adiponectin and plasminogen activator inhibitor-1 in metabolic syndrome versus healthy controls and to see the relation of them with each other and with the metabolic syndrome components.

Methods. Adiponectin and plasminogen activator inhibitor-1 levels were measured in 53 subjects with metabolic syndrome and 30 healthy controls by ELISA. All subjects of metabolic syndrome had the criterias of metabolic syndrome (obesity, insulin resistance, hypertension, dyslipidemia, glucose metabolism disorders). Adiponectin and plasminogen activator inhibitor-1 levels in serum were compared between the groups and relations of them with each other, with metabolic syndrome components, also HbA1c and C peptide were examined. For statistical analysis student-t test and pearson's correlations were used.

Results. Metabolic syndrome group had significantly lower adiponectin and higher plasminogen activator inhibitor-1 levels than healthy controls ($p<0.001$). There was no difference between the average age of both groups. There was an inverse relationship between plasma adiponectin and plasminogen activator inhibitor-1 ($r = -0.653$, $p<0.001$). Also adiponectin levels were inversely correlated with body mass index (BMI), waist and hip circumferences, systolic and diastolic pressures, fasting plasma glucose, 2 hour postprandial serum glucose, HbA1c, C peptide, triglycerides, total cholesterol, insulin and insulin resistance (HOMA). Plasminogen activator inhibitor-1 levels were correlated positively with these parameters.

Conclusion. Hypoadiponectinemia and elevated plasminogen activator inhibitor-1 levels were closely associated with the metabolic syndrome and its components, inverse relationship was present between adiponectin and plasminogen activator inhibitor-1 levels in metabolic syndrome patients. It is suggested that measuring and regulating the plasma concentration of adiponectin and plasminogen activator inhibitor-1 may be useful for management of the metabolic syndrome so this may prevent the development of obesity, cardiovascular diseases and diabetes.

Keywords: Metabolic syndrome – Adiponectin - Plasminogen activator inhibitor-1.- Obesity

Visceral fat accumulation has been shown to play crucial roles in the development of cardiovascular disease as well as the development of obesity-related disorders such as diabetes mellitus, hyperlipidemia, hypertension and the so-called metabolic syndrome (MS) (MATSUZAWA 2006).

MS occurs in about 25% of the adult population in industrialised countries. The pathogenesis of this syndrome is associated with insulin resistance. Insulin resistance has negative effects on arterial and arteriolar function throughout the body (MICZKE et al.2005). MS is associated with an increased risk for cardiovascular

Corresponding author: Sibel Bilgili, MD, The Ministry of Health, Izmir Education and Research Hospital, Clinical Biochemistry Department – Bozyaka, Izmir, Turkiye, e-mail: bilgili.sibel@yahoo.com.tr

diseases and diabetes mellitus type 2 (Wilson et al. 2005). There are growing evidence that proteins produced in adipose tissue can play a role in development of insulin resistance. One of them is adiponectin, that circulates in human plasma at high level (5-30 μg/ml) (Miczke et al. 2005). Adiponectin is a newly found adipose tissue-specific collagen-like protein, that is down-regulated in obese individuals. Hypoadiponectinemia has been identified as an independent risk factor for type 2 diabetes, coronary artery disease and hypertension. Experimental studies show that adiponectin plays a protective role in the development of insulin resistance, atherosclerosis, and inflammation (Ouchi et al. 2006). Most importantly, the role of adiponectin in the body and the results of experimental evidences suggest the possible future use of adiponectin in the treatment of diabetes, atherosclerosis and obesity. Impairment of the fibrinolytic system participates into the cardiovascular complications of obesity. This defect has been linked to high concentrations of plasminogen activator inhibitor-1 (PAI-1), a factor which is the primary physiological inhibitor of fibrinolysis (Guerre-Milla 2004). Also PAI-1 overexpression may participate in this process. Interestingly, recent in vitro and in vivo studies showed that besides its role in atherothrombosis, PAI-1 is also implicated in adipose tissue development and in the control of insulin signaling in adipocytes. These findings suggest PAI-1 inhibitors serve in the control of atherothrombosis and insulin resistance (Alessi-Juhan 2006). Adiponectin has antiatherogenic properties while PAI-1 is closely involved in the development of atherosclerosis (Maruyoshi et al. 2004). The aim of this study was to search the relationship between adiponectin and PAI-1 in patients with MS and with it's components.

Materials and Methods

This study was planned as a prospective, case control study in the Ministry of Health Izmir Education and Research Hospital Clinic Biochemistry Department in 2006.

The local ethics committee approved the study. Informed consent was taken from all the subjects. 53 patients with MS (35 women, 18 men) (group 1), 30 healthy subjects without any known disease (17 women, 13 men) (group 2) were included in this study. All subjects were found positive for at least 3 criteria for MS according to NCEP-ATP III (2001).

Non of the patients were treated with drugs for hypertension, dyslipidemia and diabetes. Blood for lipid analysis was collected after a 12-hour overnight fast. 75 g oral glucose tolerance test (OGTT) was applied to all subjects. Glucose tolerance status was determined according to the WHO criteria (WHO/NCD/NCS 1999.2.). Glycemia, total cholesterol, triglyceride and HDL-c were measured by routine biochemical methods. The value of LDL-c was calculated using Friedwald's formula (Friedwald et al. 1972). HbA1c was measured by using HPLC in the hospital clinical laboratory. Serum insulin and C peptide assays were performed by chemiluminassay, and the insulin resistance of the subjects is calculated with the homeostasis model using the formula HOMA: insulin (μIU/ml) x fasting glucose (mmol/L) / 22.5 (Matthews et al. 1985)

The body mass index (BMI), waist circumference, hip circumference, systolic and diastolic blood pressures were measured.

For the estimation of plasma adiponectin concentration, enzyme immunoassay was done using a commercial kit (B-Bridge International,Inc. human Adiponectin ELISA Kit) and for plasma PAI-1 concentration, enzyme immunoassay was done using a commercial kit (Imubind Plasma PAI-1 Elisa Kit, Germany). Venous samples taken for plasma adiponectin and PAI-1 concentration measurements were immediately stored at –70 °C.

All obtained variables BMI, waist and hip circumferences, systolic and diastolic blood pressures, serum lipids (cholesterol, triglyceride, HDL and LDL), glycemia and serum insulin, insulin resistance indexes, HbA1c, C peptide, adiponectin also PAI-1 were compared between the groups; MS versus control. The interrelations between serum adiponectin and PAI-1 also other variables were assessed.

For statistical analysis; comparisons between groups were performed by student-t test and for correlation analysis pearson's correlations were used. Data were analyzed using SPSS 13 software for Windows. All group data were reported as mean ± SD. Significant differences were assumed when $p< 0.05$.

Results

Subjects with MS (n:53; 18 males and 35 females, average age 51.1 +/-11.8) and healthy controls (n:30; 13 males and 17 females, average age 46.2 +/- 12.4) were about the same age. All subjects of MS group had BMI >25 and also other criteria of MS (e.g. obesity, insulin resistance, possible disorders of glucose metabolism, dyslipidemia and hypertension) were man-

Table 1
Clinical and biochemical characteristics of the study groups

Measures	MS group N = 53	Control group N = 30	p
Age (years)	51.1 ± 11.8	46.20 ± 12.4	NS
Systolic blood pressure (mmHg)	132 ± 23	112 ± 11	<0.001
Diastolic blood pressure (mmHg)	86 ± 15	72 ± 6	<0.001
BMI (kg/m^2)	30.5 ± 4.5	19.1 ± 3.0	<0.001
Waist circumference (cm)	102.5 ± 9.0	90.1 ± 9.4	< 0.001
Hip circumference (cm)	114.3 ± 8.7	104.8 ± 9.7	< 0.001
Fasting serum glucose (mg/dL)	108 ± 11	94 ± 9	<0.001
2 h Postprandial glucose (mg/dl)	127 ± 41	100 ± 21	0.002
Insulin (μIU/ml)	13.4 ± 5.8	8.4 ± 4.4	<0.001
Insulin resistance(homa)	3.5 ± 1.6	2.0 ± 1.09	<0.001
C peptide (ng/ml)	3.4 ± 1.1	2.5 ± 0.8	<0.001
HbA1c (%)	6.3 ± 1.2	5.6 ± 0.5	0.003
Total cholesterol mg/dl)	206 ± 42	157 ± 23	<0.001
Triglycerides (mg/dl)	195 ± 113	82 ± 18	<0.001
HDL (mg/dL)	41 ±11	47 ± 13	0.032
LDL (mg/dL)	125 ± 40	108 ± 50	NS
Adiponectin (μg/ml)	15.6 ± 5.8	29.1 ± 10.9	<0.001
PAI-1 (ng/ml)	165.1± 4.5	82.4 ± 32.7	<0.001

NS = not significant

ifested in all of them. Among the subjects who underwent OGTT, 25 of MS patients were positive for impaired glucose tolerance. There was no diabetic patient among the MS and the control group.

MS patients had significantly higher PAI-1, BMI, waist and hip circumferences, systolic and diastolic pressures, fasting glycemia, 2 hour postprandial serum glucose, total cholesterole, triglyceride, HDL cholesterole, C peptide, HbA1c, insulin level and insulin resistance (HOMA) (table1). In contrast plasma adiponectin levels were lower in subjects with MS when compared with controls (p<0.001). Adiponectin levels were found to be higher in women than men (23.75 +/- 9.8 and 18.6 +/- 10.2 respectively, p=0.026). Lower adiponectin levels were associated with different components of MS. Correlations of adiponectin with other parameters are shown in table 2. Adiponectin levels were correlated negatively with PAI-1, also with BMI, waist and hip ratios, systolic and diastolic blood pressures, fasting plasma glucose, 2 hour postprandial glucose, HbA1c, C peptide, triglycerides, cholesterole, insulin and insulin resistance, in contrast PAI-1 levels correlated positively with these parameters

Table 2
Correlation coefficients between serum adiponectin and other clinical parameters

Measures	Correlation coefficients	p
Age (years)	0.036	NS
Systolic blood pressure (mmHg)	-0.323	0.003
Diastolic blood pressure (mmHg)	-0.446	<0.001
BMI (kg/m^2)	-0.666	<0.001
Waist circumference (cm)	-0.310	0.007
Hip circumference (cm)	-0.340	0.003
Fasting serum glucose (mg/dl)	-0.313	0.004
2 h Postprandial glucose (mg/dl)	-0.393	<0.001
Insulin (μIU/ml)	-0.365	0.001
Insulin resistance (HOMA)	-0.364	0.001
C peptide (ng/ml)	-0.382	<0.001
HbA1c (%)	-0.344	0.001
Total cholesterol (mg/dl)	-0.343	0.002
Triglyceride (mg/dl)	-0.356	0.001
HDL (mg/dl)	0.067	NS
LDL (mg/dl)	0.065	NS
PAI-1 (ng/ml)	-0.653	<0.001

NS = not significant

Table 3
Correlation coefficients between serum PAI-1 and other clinical parameters

Measures	Correlation coefficients	p
Age (years)	0.011	NS
Systolic blood pressure (mmHg)	0.350	<0.001
Diastolic blood pressure (mmHg)	0.487	<0.001
BMI (kg/m^2)	0.745	<0.001
Waist circumference (cm)	0.285	0.005
Hip circumference (cm)	0.315	0.002
Fasting serum glucose (mg/dl)	0.315	<0.001
2 h Postprandial glucose (mg/dl)	0.350	<0.001
Insulin (μIU/ml)	0.353	<0.001
Insulin resistance (HOMA)	0.351	<0.001
C peptide (ng/ml)	0.368	<0.001
HbA1c (%)	0.384	<0.001
Total cholesterol (mg/dl)	0.363	<0.001
Triglyceride (mg/dl)	0.341	<0.001
HDL (mg/dl)	0.041	NS
LDL (mg/dl)	0.067	NS
Adiponectin (μg/ml)	-0.653	<0.001

NS = not significant

as shown in table 3. There were no correlation between adiponectin and HDL, LDL cholesteroles, similarly PAI-1 and HDL, LDL cholesterole correlations were not significant.

Significant finding of this study was the negative correlation between adiponectin and PAI-1 levels in MS patients ($r = -0.653$, $p<0.001$).

Discussion

There is increasing evidence that new cases of cardiovascular diseases are closely associated with increased prevalence of insulin resistance and type 2 diabetes due to excessive weight and sedentary lifestyle. Much of the recent work on obesity has highlighted the key role of adipose tissue as an endocrine organ secreting a large number of substances that mediate vascular and metabolic complications of obesity (Bahia et al.2006). Most of the coagulation and fibrinolytic proteins may contribute to the development of cardiovascular diseases or associated with them (Heinrich et al. 1994). Increased plasma levels of PAI-1, a main inhibitor of fibrinolysis, reflect increased coagulability and are correlated with the augmentation of coronary vascular heart diseases (Juhan-Vague et al. 1991). The biological role of PAI-1 extends beyond regulation of fibrinolysis, since PAI-1 has been shown to influence cell migration and angiogenesis by competing with integrin binding on extracellular matrix vitronectin. In adipose tissue PAI-1 could impair pre-adipocyte migration and attachment to vitronectin, as suggested by its potent inhibitory effect on these processes in vitro (Crandall et al. 2000).

Adiponectin is a peptide inhibiting the food intake, affects the transfer of insulin signal, increases insulin sensitivity, glucose utilization, free fatty acid transport and oxidation in muscles, inhibits gluconeogenesis and decreases glycemia. It is an endogenous inhibitor of angiogenesis as well as of the proliferation and migration of endothelial cells, also shows anti-inflammatory effects (Tajtakova et al. 2006). Although the association of adiponectin with several diseases remains contraversial, many clinical studies have demonstrated that low plasma adiponectin levels associate closely with obesity related diseases, including atherosclerotic cardiovascular diseases, type II diabetes, hypertension and dyslipidemia (Okamato et al. 2006). Low level of adiponectin in obese individuals may be considered as a marker predicting a possibility of the development of MS. It is suggested that early regulation of serum adiponectin levels in obese subjects by treatment of obesity, especially in young ones, could result in a lowering the risk of mainly cardiovascular diseases associated with MS. High adiponectin plasma concentrations are associated with favorable lipid profiles. Adiponectin might have a protective role in vascular function (Tajtakova et al. 2006).

In this study we also found that adiponectin was indeed decreased and PAI-1 levels were higher in MS patients compared with control group and there was a significant negative correlation between them. The mechanisms linking PAI-1 activity to adiponectin levels are not clear at the moment. PAI-1 could have a direct down-regulating effect on adiponectin expression and secretion in adipose tissue (Ma et al. 2004). On the other hand relationship between PAI and adiponectin could be a consequence of the another factor such as insulin resistance, inflammation, dyslipidemia (Ilse Mertens et al. 2005). The second finding of the present study was; adiponectin levels were negatively correlated with PAI-1, BMI, systolic and diastolic blood pressures, fasting serum glucose, 2 hour postprandial serum glucose, HbA1c, insulin, insulin resistance (HOMA), C peptide, triglyceride, total cholesterol. Our results were in accor-

dance with many other studies (Bahia et al.2006, Tajtakova et al.2006, Kim et al.2006, Mojiminiyi et al.2006, Mohan et al.2005, Ryo et al.2004, Santaniemi et al.2006). Also concordant with other studies PAI-1 levels were significantly higher in the MS group than the control group (Pannacciulli et al.2002, Bastard et al.2000). These findings suggest PAI-1 inhibitors may serve in the control of atherothrombosis and insulin resistance. Given the low levels of adiponectin in subjects with the MS, and the beneficial effects of the adiponectin in animal studies, there is exciting potential for adiponectin replacement therapy in insulin resistance and related disorders (Whitehead et al. 2006). We found no significant correlation with HDL and LDL cholesterols with adiponectin and PAI-1 although total cholesterol and triglycerides negatively correlated. This may be due to characteristic of our study group and if we could study in a large group of MS patients, as reported by others adiponectin and PAI-1 might show a correlation with HDL and LDL cholesterols.

Beside these findings, further clinical and experimental investigations will be needed to illuminate the significance of adiponectin and PAI-1 in MS. This work was important to add concordant data into this topic. So we conclude, hypoadiponectinemia, also elevated PAI-1 are closely associated with the MS and its components, measuring the plasma concentration of adiponectin and PAI-1 may be useful for management of the MS, so this may prevent the development of obesity, cardiovascular diseases and diabetes.

References

Alessi MC, Juhan-Vague: *PAI-1 and the metabolic syndrome: links, causes, and consequences.* Arterioscler Thromb Vasc Biol. **26,** 2200-2207, 2006

Bahia L, Aguiar LG, Villela N, Bottino D, Godoy-Matos AF, Geloneze B, Tambascia M, Bouskela E. Relationship between adipokines, inflammation, and vascular reactivity in lean controls and obese subjects with metabolic syndrome. Clinics **61**, 433-440, 2006

Bastard JP, Pieroni L, Hainque B: Relationship between plasma plasminogen activator inhibitor 1 and insulin resistance. Diabetes Metab Res Rev **16**, 192-201, 2000

Crandall DL, Busler DE, McHendry-Rinde B, Groeling TM, Kral JG. Autocrine regulation of human preadipocyte migration by plasminogen activator inhibitor-1. J Clin Endocrinol Metab **85**, 2609-2614, 2000

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry **18**, 499-502, 1972

Guerre-Milla M: Adipose tissue and Adipokines: for beter or worse. Diabetes Metab **30**, 13-19, 2004

Heinrich J, Balleisen L, Schulte H, Assmann G, van de Loo J: Fibrinogen and factor VII in the prediction of coronary risk. Results from the PROCAM study in healthy men. Arterioscler Thromb **14**, :54–59, 1994

Juhan-Vague I, Alessi M, Vague P: Increased plasma PAI-1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia **34**, 457–462, 1991

Kim SM, Cho KH, Park HS: Relationship between plasma adiponectin levels and the metabolic syndrome among Korean people. Endocrine J **53,** 247-254, 2006

Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, Brown NJ, Swift LL, McGuinness OP, Wasserman DH, Vaughan DE, Fogo AB: Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. Diabetes **53,**:336-346, 2004

Maruyoshi H, Kojima S, Funahashi T, Miyamoto S, Hokamaki J, Soejima H, Sakamoto T, KawanoH, Yoshimura M, Kitagawa A, Matsuzawa Y, Ogava H: Adiponectin is inversely related to plasminogen activator inhibitor type 1 in patients with stable exertional angina. Thromb Haemost **91,** 1026-1030, 2004

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC:. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. **28**, 412-419, 1985

Matsuzawa Y: The metabolic syndrome and adipocytokines. FEBS Lett. **580**, 2917-2921, 2006

Mertens I, Bellaux D, Funahashi T, Matsuzawa Y, Van der Planken M, Verrijken A, Ruige JB, Van Gaal LF: Inverse relationship between plasminogen activator inhibitor-I activity and adiponectin in overweight and obese women. Interrelationship with visceral adipose tissue, insulin resistance, HDL-chol and inflammation. Thromb Haemost **94**, 1190-1195, 2005

MICZKE A, BRYL W, SZULINSKA M, PUPEK-MUSIALIK D: The role of adiponectin in pathogenesis of insulin resistance and metabolic syndrome. Pol Merkur Lekarski **19**, 723-726, 2005

MOHAN V, DEEPA R, PRADEEPA R, VIMALESWARAN KS, MOHAN A, VELMURUGAN K, RADHA V Association of low adiponectin levels with the metabolic syndrome-the Chennai Urban Rural Epidemiology Study. Metabolism **54**, 476-481, 2005

MOJIMINIYI OA, ABDELLA NA, AI AROUJ M, BEN NAKHI A. Adiponectin, insulin resistance and clinical expression of the metabolic syndrome in patients with Type 2 diabetes. Int J Obes **31**, 213-220, 2007

OKAMATO Y, KIHARA S, FUNAHASHI T, MATSUZAWA Y, LIBBY P: Adiponectin: a key adipocytokine in metabolic syndrome. Clin Sci (Lond) **110**, 267-278, 2006

OUCHI N, SHIBATA R, WALSH K: Cardioprotection by adiponectin. Trends Cardiovasc Med **16**,141-146, 2006

PANNACCIULLI N, MITRIO VD, MARINO R, GIORGINO R, PERGOLA GD: Effect of Glucose Tolerance Status on PAI-1 Plasma Levels in Overweight and Obese Subjects. Obesity Research. **10**, 717-725, 2002

RYO M, NAKAMURA T, KIHARA S, KUMADA M, SHIBAZAKI S, TAKAHASHI M, NAGAI M, MATSUZAWA Y, FUNAHASHI T: Adiponectin as a biomarker of the metabolic syndrome. Circ J **68,**:975-981, 2004

SANTANIEMI M, KESANIEMI YA, UKKOLA O: Low plasma adiponectin concentration is an indicator of the metabolic syndrome. Eur J Endocrinol **155**, 745-750, 2006

TAJTAKOVA M, PETRASOVA D, PETROVICOVA J, PYTLIAK M, SEMANOVA Z. Adiponectin as a biomarker of clinical manifestation of metabolic syndrome. Endocr Regul **40**, 15-19, 2006

WHITEHEAD JP, RICHARDS AA, HICKMAN IJ, MACDONALD GA, PRINS JB: Adiponectin-a key adipokine in the metabolic syndrome. Diabetes Obes Metab **8**, 264-280, 2006

WORLD HEALTH ORGANIZATION. Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO Consultation. WHO/NCD/NCS 99.2. Geneva,1999.

WILSON PW, D'AGOSTINO RB, PARISE H, SULLIVAN L, MEIGS JB: Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation **15,** 3066-3072, 2005