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The Relationship Between Proinflammatory Cytokine Levels and Fibrinolytic System in Obese Patients[*]

Obez Hastalarda Proinflamatuvar Sitokinler ile Fibrinolitik Sistem Arasındaki İlişki

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Objectives: The aim of this study was to investigate the relationship between proinflammatory cytokines (TNF-α and IL-6), and fibrinolytic system parameters (t-PA, and PAI-1) and insulin resistance in obese individuals.

Patients and Methods: The study included 54 obese subjects (BMI ≥30 kg/m^2; 41 females, 13 males; mean age 33.5 years) and 30 non-obese healthy individuals (BMI <25 kg/m^2; 19 females, 11 males; mean age 22.3 years). Fibrinogen levels were measured by the coagulometric method and the measurements of TNF-α, IL-6, t-PA and PAI-1 were carried out by the ELISA method.

Results: Compared with non-obese subjects, obese individuals had significantly higher fibrinogen (p<0.01), PAI-1 (p<0.001), TNF-α (p<0.01), and IL-6 (p<0.001) levels, and significantly lower t-PA level (p<0.001) and t-PA/PAI-1 ratio (p<0.001). We also found an inverse relationship between TNF-α and t-PA levels (p=0.007) and t-PA/PAI-1 ratio (p=0.016) in obese individuals. The presence or absence of insulin resistance did not affect proinflammatory cytokines and fibrinolytic system parameters in obese individuals.

Conclusion: Our findings indicate increased inflammatory cytokine levels especially in TNF-α level, and decreased fibrinolysis in obese individuals. These changes may contribute to atherosclerotic process independent from insulin resistance in obesity.

Key Words: Cytokines; fibrinolysis; insulin resistance; obesity/complications.

Amaç: Obez kişilerde proinflamatuar sitokinlerden TNF-α ve IL-6, fibrinolitik sistem parametrelerinden t-PA ve PAI-1 ve insülin direnci arasındaki ilişki araştırıldı.

Hastalar ve Yöntemler: Çalışmaya obez (VKİ ≥30 kg/m^2) olarak değerlendirilen 54 kişi (41 kadın, 13 erkek; ort. yaş 33.5) ve obezite sorunu olmayan (VKİ <25 kg/m^2) 30 kişi (19 kadın, 11 erkek; ort. yaş 22.3) alındı. Fibrinojen düzeyleri koagülometrik olarak ve TNF-α, IL-6, t-PA, PAI-1 düzeyleri ELISA yöntemiyle ölçüldü.

Bulgular: Kontrol grubuyla karşılaştırıldığında, obez kişilerde fibrinojen (p<0.01), PAI-1 (p<0.001), TNF-α (p<0.01) ve IL-6 düzeyleri (p<0.001) anlamlı derecede yüksek, t-PA düzeyi (p<0.001) ve t-PA/PAI-1 oranı (p<0.001) anlamlı derecede düşük bulundu. Obezlerde TNF-α ile t-PA (p=0.007) ve t-PA/PAI-1 oranı (p=0.016) arasında ters ilişki saptandı. İnsülin direnci olan ve olmayan obez kişilerde parametreler arasında fark yoktu.

Sonuç: Obezitede adipoz dokudan salgılanan özellikle TNF-α gibi inflamatuar sitokinlerin artması fibrinolizde azalmaya yol açar. Obez kişilerde görülen bu değişiklikler, insülin direncinden bağımsız olarak ateroskleroza neden olabilir.

Anahtar Sözcükler: Sitokinler; fibrinoliz; insülin direnci; obezite/komplikasyon.

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Obesity is an important problem for public health whose prevalence has been increasing rapidly all over the world. Along with obesity, hypertension, insulin resistance, diabetes mellitus, and dyslipidemia prevalence have also been increasing and the development of atherosclerosis has been accelerated.

Obesity has been reported to be the major risk factor for cardiovascular diseases in both females and males.[1] Obesity and insulin resistance that is closely related to obesity cause endothelial dysfunctions which lead to atherosclerosis and thrombosis.[2] It has been indicated that there is a close and independent correlation between the levels of body mass index (BMI) which is one of the diagnostic criteria for obesity and fibrinogen, von Willebrand Factor (vWF), tissue plasminogen activator (t-PA), and plasminogen activator inhibitor-1 (PAI-1) levels.[3-5] It has also been reported that the levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) which are pro-inflammatory cytokines secreted from adipose tissue increase in obesity.[6,7] Moreover, the relationship between the inflammatory cytokines and endothelial dysfunction and the effects of this relationship on the development of atherosclerotic lesions have been shown.[2,8] In this study, it was aimed to examine the relationship between the inflammatory cytokines and endothelial dysfunction, in obese patients.

PATIENTS AND METHODS

Fifty four obese patients (BMI≥30 kg/m^2; 41 females, 13 males; mean age 33.5 years; range 31.6 to 37.5 years) who applied to the Obesity Polyclinic of Endocrinology Department, and 30 non-obese healthy control subjects (BMI<25 kg/m^2; 19 females, 11 males; mean age 22.3 years; range 20.9 to 24.7 years) were included in the study. All the participants gave their informed consent and the study was approved by the ethical committee of the university.

Patient group consisted of subjects who were accepted as obese according to the criteria of World Health Organization (WHO, BMI≥30 kg/m^2), and the control group was selected from the non-obese people with BMI<25 kg/m^2.[9] The subjects who were smoking or had cardiovascular diseases, hypertension, dyslipidemia, type 2 diabetes mellitus, failure of liver or kidneys, systemic diseases, malignancies, hypo or hyperthyroidism, anemia, leukocytosis, thrombocytosis, high urea and creatinine levels were excluded. The ones whose laboratory findings indicated metabolic syndrome according to WHO criteria,[10] the subjects who were taking anti-obesity drugs or those that had abnormal liver function test results, the ones who were suspected to have cortisol-metabolism dysfunction according to the results of 1 mg dexamethasone test or the women in menopause were not accepted in the study. Anthropometric measurements of all participants (height, weight, waist circumference and hip circumference) were recorded by a standardized protocol. Weight was measured to the nearest 0.1 kg; height was measured to the nearest 0.5 cm. Waist circumference was measured on widest circumference over the great trochanters. Body mass index and waist-hip ratio (WHR) were calculated.

Fasting insulin levels were measured by chemical immunoassay method with BIODPC-Immulite 2000 Automatic Analyzer. Insulin resistance was calculated by Homeostasis Model Assessment test {HOMA= fasting plasma glucose (mg/dL) x fasting plasma insulin (uIU/ml) x 0.05551/22.5}.[11] The upper level of HOMA was accepted as 2.24 as it was asserted in the only wide research that had been done in Turkey.[12]

Plasma samples were stored in a deep freezer at the temperature of -80 °C.

vWF (Asserachrom, 00240 Diagnostica STAGO9, 92600 Asnieres, France), PAI-1 (Asserachrom, 002962 Diagnostica STAGO9, 92600 Asnieres, France), t-PA (Tint Elize M12612, Biopool Trinity Biotech, Ireland), IL-6 (Diaclone 1006-30, France) and TNF-α (Diaclone 1100-41, France) levels of these samples were measured by ELISA (Enzyme-Linked ImmunoSorbent Assay) method.

Statistical analysis

All statistical analyses were performed on SPSS 9.0 software for Windows. Numeric vari-

ables were tested for normal distribution by the Shapiro Wilks test. Normally distributed variables were tested by independent samples t-test but the other variables were tested by Mann-Whitney U-test because the distributions of these variables were non-normal. Data were expressed as mean±standard deviation (SD) for normally distributed variables while as median (25%-75% percentiles) for non-normally distributed variables. Chi-square test was used to compare dichotomize variables between groups. Pearson correlation was calculated. P<0.05 was considered statistically significant.

RESULTS

All the clinical and laboratory parameters of both obese and healthy (non-obese) control groups are shown on Table 1. There was no significant difference in terms of age and gender between two groups. BMI values (p<0.001), the waist circumferences (p<0.001), WHR (p<0.001), systolic blood pressures (p<0.001), and diastolic pressures (p<0.004) of the obese group were found significantly higher than those of the control group (Table 1).

There was no statistical difference between the lipid profiles of the groups. HOMA, which is an indicator for insulin resistance, was significantly higher in obese patients when compared to control group (p<0.001). Fibrinogen value was higher in obese patients than in the control group (p<0.01). There was not a significant difference in vWF values between two groups.

PAI-1 value was higher in obese group than in the control group (p<0.001). The other indicators such as t-PA and t-PA/PAI-1 ratio were found in lower levels in obese patients than in the control group (p<0.001, p<0.001 respectively). TNF-α and IL-6 levels were higher in obese group than in the control group (p<0.001, p=0.001 respectively).

In obese group; PAI-1 levels had a positive correlation with waist circumferences (r=0.378, p=0.005), WHR (r=0.359, p=0.008) and t-PA lev-

Table 1. The clinical and laboratory features in obese and healthy control groups

	Obese group (n=54)			Control group (n=30)			
	n	range	Ort±SD	n	range	Ort±SD	*p*
Age (year)	30	25-40		29	26-34		0.677
Gender (female/male)	41/13			19/11			0.331
Body mass index (kg/m^2)	33.5	31.6-37.5		22.3	20.9-24.7		<0.001
Waist circumference (cm)			103.4±10.6			75.5±8.8	<0.001
Waist/hip ratio	0.87	0.83-0.91		0.76	0.73-0.86		<0.001
Systolic blood pressure (mmHg)	120	110-121.3		110	110-120		0.001
Diastolic blood pressure (mmHg)	80	70-80		70	60-80		0.004
Triglylceride (mg/dl)	72.5	50-111		47.5	36.8-95.5		0.049
Total cholesterol (mg/dl)			173.5±25.3			167.4±22.7	0.270
LDL-cholesterol (mg/dl)	108.5	92.6-126		105	84.6-115.9		0.251
HDL-cholesterol (mg/dl)	51	43.8-56.3		54	47.3-58.39		0.455
Homeostasis model assessment	2.29	1.59-3.55		1.34	0.97-1.96		<0.001
Fibrinogen (ng/ml)			292.7±55.2			251.3±52.1	0.001
von Willebrant factor (ng/ml)			102.1±23.3			92.1±28.1	0.085
PAI-1 (ng/ml)	68	34.8-113.3		35.5	21-60.8		<0.001
t-PA (ng/ml)			10.2±4.3			15.1±6.0	<0.001
t-PA/PAI-1 ratio	0.18	0.08-0.25		0.42	0.24-0.70		<0.001
TNF-α (mg/dl)	39.6	16.6-101.2		15.7	1.6-44.6		<0.001
IL-6 (ng/ml)	1.6	0.6-3.6		0.3	0.1-0.06		<0.001

LDL-cholesterol: Low density lipoprotein cholesterol; HDL-cholesterol: High density lipoprotein cholesterol; PAI-1: Plasminogen activator inhibitor; t-PA: Tissue plasminogen activator; TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin-6; NS: Nonsignificant.

Table 2. The correlations between the parameters of fibrinolytic system, cytokines and anthropometric parameters in obese group

Parameters	Releated parameters	r	*p*
Plasminogen activator inhibitor	Waist circumference	0.378	0.005
	Waist/hip ratio	0.359	0.008
	Tissue plasminogen activator	0.413	0.002
Tissue plasminogen activator	Waist circumference	0.336	0.013
	Waist/hip ratio	0.390	0.004
t-PA/ PAI-1 ratio	Waist circumference	–0.249	0.069
	Waist/hip ratio	–0.261	0.057
Tumor necrosis factor alpha	Tissue plasminogen activator	–0.369	0.007
	t-PA/ PAI-1 ratio	–0.333	0.016

PAI-1: Plasminogen activator inhibitor; t-PA: Tissue plasminogen activator.

els (r=0.413, p=0.002), and t-PA levels had also positive correlation with waist circumferences (r=0.336, p=0.013) and WHR (r=0.390, p=0.004); but TNF-α, had a negative relationship with t-PA levels (r=–0.369, p=0.007) and t-PA/PAI-1 ratio (r=–0.333, p=0.016), t-PA/PAI-1 ratio had also a negative relationship with waist circumferences (r=–0.249, p=0.069), and WHR (r=–0.261, p=0.057). There was no relationship between any of those parameters and IL-6 (Table 2).

Table 3. The correlation among fibrinolytic, cytokine and obesity parameters in all subjects

Parameters	Releated parameters	r	*p*
Fibrinogen	Body mass index	0.393	<0.001
	Waist circumference	0.249	0.025
von Willebrant factor	Body mass index	0.241	0.028
	Waist circumference	0.251	0.022
Plasminogen activator inhibitor	Body mass index	0.377	<0.001
	Waist circumference	0.427	<0.001
	Waist/hip ratio	0.367	0.001
Tissue plasminogen activator	Body mass index	–0.388	<0.001
	Waist circumference	–0.272	0.012
	Tumor necrosis factor-α	–0.399	<0.001
t-PA/PAI-1 ratio	Body mass index	–0.536	<0.001
	Waist circumference	–0.540	<0.001
	Waist/hip ratio	–0.466	<0.001
	Homeostasis model assessment	–0.266	0.014
	Tumor necrosis factor-α	–0.320	0.004
Tumor necrosis factor alpha	Body mass index	0.360	0.001
	Waist circumference	0.249	0.025
Homeostasis model assessment	Body mass index	0.470	<0.001
	Waist circumference	0.465	<0.001
	Waist/hip ratio	0.293	0.007
	Fibrinogen	0.275	0.011
	Triglylcerid	0.236	0.031

PAI-1: Plasminogen activator inhibitor, t-PA: Tissue plasminogen activator.

When all subjects were regarded, fibrinogen, vWF, PAI, and TNF-α levels had positive correlations with BMI, and waist circumferences, whereas t-PA levels and t-PA/PAI-1 ratios had negative correlations with BMI, waist circumferences, WHR and TNF-α (Table 3).

Obese subjects were divided into two groups according to whether they had insulin resistance or not. In the group with insulin resistance, there was higher levels of BMI (p=0.023) when compared to the group of no insulin resistance. Among those two groups, there was not a significant difference in the other parameters (Table 4).

DISCUSSION

Obesity prevalence has been increasing rapidly throughout the world. It is accepted that obesity is a major risk factor for cardiovascular diseases for both women and men.[13] The malfunctions of haemostatic and fibrinolytic systems which appear in obesity are also important risk factors for the development of cardiovascular diseases.[1] These malfunctions include thrombocyte hyperactivity, hypercoagulability, and hypofibrinolysis, all of which are the elements of the atherosclerotic process. Thrombocyte hyperactivity is related to increased vWF levels. Hypercoagulability is caused by increased fibrinogen, factor VII, and factor VIII levels, and hypofibrinolysis is the result of increased PAI-1.[14]

In this research, we examined haemostatic, fibrinolytic, and proinflammatory factors which are the potential risks for cardiovascular diseases in obese subjects who do not have dyslipidemia, hypertension and diabetes mellitus and compared with those values of non-obese individuals of the control group who have similar features in terms of age and gender. Although systolic and diastolic blood pressure levels of two groups were in normal range, they were higher in obese patients. This situation is consistent with the argument that obesity can elevate blood pressure.[15]

Table 4. Parameters of the groups with insulin resistance and non insulin resistance

	The group with insulin resistane (n=29)			The group with non-insulin resistane (n=25)			
	n	range	Ort±SD	n	range	Ort±SD	*p*
Age (year)	30	27-39		27	23-43		0.439
Gender (female/male)	22/7			20/5			0.516
Body mass index (kg/m^2)	35.5	32.9-38.1		32.4	31.0-35.1		0.023
Waist circumference (cm)			105.6±10.0			100.8±10.9	0.096
Waist/hip ratio	0.87	0.85-0.91		0.85	0.82-0.90		0.245
Systolic blood pressure (mmHg)	120	110-122.5		120	110-122.5		0.392
Diastolic blood pressure (mmHg)	80	70-80		80	70-80		0.687
Triglylcerid (mg/dl)	77	51.5-110.5		67	46-119.5		0.314
Total cholesterol (mg/dl)			172.1±24.4			175.2±26.7	0.666
LDL-cholesterol (mg/dl)	109	90.9-126.5		108	99.5-126		0.609
HDL-cholesterol (mg/dl)			51.78±8.1			51±9.2	0.736
Fibrinogen (ng/ml)	289	252.5-329.5		284	258-329.5		0.986
von Willebrant factor (ng/ml)			103.1±25.3			101.0±21.2	0.741
PAI-1 (ng/ml)	65	36.5-139		73	33-107		0.386
t-PA (ng/ml)	10.2	8.5-13.9		8.9	6.2-12.1		0.157
t-PA/ PAI-1 ratio	0.15	0.08-0.22		0.18	0.07-0.32		0.952
TNF-α (mg/dl)	36.8	18.1-101.2		45.9	13.8-103.5		0.905
IL-6 (ng/ml)	1.6	0.7-4.4		1.7	0.5-3.5		0.673

LDL-cholesterol: Low density lipoprotein cholesterol; HDL-cholesterol: High density lipoprotein cholesterol; PAI-1: Plasminogen activator inhibitor; t-PA: Tissue plasminogen activator; TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin-6.

vWF has a role in thrombocyte adhesion in the process of cardiovascular disease development. In our study, although there was not a significant difference, the levels of vWF were higher in obese patients than in the individuals of the control group. When all subjects were taken into consideration, the level of vWF had a positive correlation with BMI and waist circumference, but we didn't determine this correlation in the obese group. Thus, we think that vWF is not a primary determinant of hypercoagulability in obesity. In ARIC research[16] there was a positive correlation between BMI and WHR and vWF in males and females. Also, vWF antigen, independent from other metabolic variances, had a positive correlation with waist circumference in the research done by De Pergola et al.[17] Folsom et al.[18] had reported that vWF was closely related to the risk of cardiovascular disease in healthy women and men.

In this research, fibrinogen was found significantly higher in obese subjects when compared to control group. There was also a positive relationship between the fibrinogen levels and BMI and the waist circumference when all subjects were evaluated, but there was no correlation among these same parameters in obese group. In the previous researches, significantly higher levels of fibrinogen were found in obese patients which showed a positive correlation with BMI and waist circumference.[3,14] Increased fibrinogen level is an independent risk factor for cardiovascular diseases and can partly explain the high mortality prevalence of cardiovascular diseases in obese people.[5]

In various researches, PAI-1 that is the inhibitor of fibrinolytic system was found to be an important cardiovascular risk factor.[14,19] PAI-1 is produced from hepatocytes, adipocytes, mesangial cells, fibroblasts and vascular smooth muscle cells. In plasma PAI-1 promotes clot formation which plays a key role in the pathogenesis of cardiovascular events.[20] The presence of a defect concerning fibrinolysis in morbid obese patients had been supported by the increased antigen PAI-1 and by the decreased levels of t-PA.[5,21,22] It had been shown that, there was a correlation between the increase in waist circumference and WHR which were accepted as the indicators of body fat distribution and elevated levels of PAI-1.[17,19] The correlation between PAI-1 level and abdominal fat seem to be independent from total fat mass, triglyceride levels and age.[18,23] In our research, we determined that PAI-1 level was significantly higher in obese group than in control group. We saw that as BMI, waist circumference, and WHR increased, the levels of PAI-1 also increased, which resulted in the inhibition of fibrinolytic system in obese patients.

In our research, t-PA levels and t-PA/PAI-1 ratios of obese patients were significantly lower than that of the subjects of control group. This finding is consistent with the opinion which states that t-PA which is a plasminogen activator decreases in obese patients.[5] In our study when all subjects were evaluated, we found that as BMI, waist circumference and WHR increased, t-PA levels and t-PA/PAI-1 ratio decreased. However, there was a positive correlation between the increase of PAI-1 and the increase of t-PA as BMI and WHR increased in obese group. As a result, we think that rather than using increased levels of PAI-1 and t-PA separately, using t-PA/PAI-1 ratio provides much more information in the obese. Thögersen et al.[24] showed a positive correlation between the t-PA antigen and PAI-1 antigen and activity in the population in which the prevalence of cardiovascular diseases was high. In the Prospective Caerphilly Study,[25] high levels of t-PA antigen had a correlation with the development of major cardiovascular diseases. The results of our research support the idea that obesity inhibits the fibrinolytic activity. As a result, this situation increases the risk of atherosclerosis.

It has been shown that a chronic and low grade of inflammatory process develops in obesity.[26,27] IL-6 which is secreted from the subcutaneous tissue is one of the major proinflammatory cytokines.[28,29] In subjects with obesity, IL-6 levels increase.[8,30] There is also a positive correlation between this increase and BMI.[29,30] After weight loss, IL-6 levels decrease.[30,31] In our research, we found that IL-6 levels were significantly higher in obese group than in

the control group. It is a known fact that it is abdominal obesity which creates a serious risk for metabolic dysfunctions and it is related with waist circumference and WHR.[9] We did not find a relationship between the levels of waist circumference, WHR and IL-6. We think that this result is appropriate because IL-6 is secreted from subcutaneous adipose tissue.

TNF-α is another proinflammatory cytokine which is secreted from visceral adipose tissue.[29,32] Visceral obesity is characterized by the increased levels of insulin, triglycerides, free fatty acids and TNF-α. They can activate endothelial cells, hepatocytes and/or adipose tissue for secreting PAI-1. This situation explains the relationship between hypofibrinolysis and obesity.[14] It is found that there is a strong relationship between abdominal obesity and TNF-α level, and positive correlation between TNF-α and WHR ratio.[8,22,31] In our study, the levels of TNF-α were significantly higher in obese group than in the control group. Unlike IL-6, the increase in the levels of TNF-α had a positive correlation with waist circumference (Table 3). This situation makes us think that, for the obese patients, the increase in the levels of TNF-α is closely related with visceral fat accumulation because visceral adipose tissue plays the major role in the TNF-α secretion. On the other hand, the negative correlation between the increased TNF-α and decreased t-PA/PAI-1 ratio support the decreased process of fibrinolysis in the obese group.

Insulin resistance is the key factor in the relation between the obesity and type 2 diabetes mellitus.[33] In our research, the values of PAI-1 were higher in insulin resistant group than in the non-insulin resistant group, but the results were not statistically significant. There was not a relationship between insulin resistance and the values of fibrinogen, vWF, t-PA, and t-PA/PAI-1 ratios. Also the levels of IL-6 and TNF-α did not differ between the insulin resistant and the non-insulin resistant groups. These results may be interpreted that obesity contributes to endothelial dysfunction and the process of proinflammatory atherosclerosis in great amounts and independent from insulin resistance.

In conclusion; our findings indicate increased inflammatory cytokine concentrations, especially TNF-α, and decreased fibrinolysis in obese, otherwise healthy subjects. These changes may contribute to atherosclerotic process independent from insulin resistance in obesity.

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