

PREDİYABETTE ERKEN ATEROSKLEROZ BELİRTECİ E-SELECTİN

E-SELECTIN AS AN EARLY ATHEROSCLEROSIS MARKER IN PREDIABETES

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ABSTRACT

INTRODUCTION: Activation of endothelial dysfunction cascade and procoagulant pathways are one of the main pathophysiological mechanisms that leads to beta cell dysfunction, insulin resistance and vascular complications in prediabetes and diabetes. The present study aimed to investigate the relation between E-selectin and carotid intima media thickness (CIMT) in prediabetic patients.

METHODS: Seven patients, who were diagnosed with prediabetes on 75gr oral glucose tolerance test (OGTT) based on ADA criteria, were included in the present study. Sixty-six healthy volunteers were included as the control group. The patient and the control groups were examined in terms of E-selectin and fasting insulin levels. CIMT was measured by B-mode ultrasonography and HOMA-IR was calculated.

RESULTS: The mean E-selectin level was 35.19 (9.56-118.46) ng/mL in the prediabetic patient group and 16.60 (1.55-116.62) ng/mL in the control group. The difference between the groups was statistically significant ($p=0.001$). CIMT was statistically significantly higher in prediabetic patients group than control group ($p<0.001$, 0.62 ± 0.10 mm and 0.54 ± 0.09 mm respectively). In the prediabetic patients group a significant positive correlation was found between E-selectin and HbA1c ($r=0.306$, $p=0.01$), HOMA-IR ($r=0.243$, $p=0.043$), LDL-C ($r=0.329$, $p=0.005$), and TG ($r=0.252$, $p=0.035$) but there was a negative correlation with waist/hip ratio.

CONCLUSION: We found that E-selectin level, which is one of the markers of endothelial dysfunction, was increased in prediabetes. This result supports the idea that endothelial dysfunction and early stage atherosclerosis start in the prediabetic period.

Keywords: Prediabetes, atherosclerosis, e-selectin

ÖZET

AMAÇ: Endotel disfonksiyonu kaskadı ve prokoagülan yollarının aktivasyonu, prediyabet ve diyabette beta hücre fonksiyon bozukluğuna, insülin direncine ve vasküler komplikasyonlara yol açan ana patofizyolojik mekanizmalardan biridir. Bu çalışmada prediyabetli hastalarda E-selektin ve karotis intima media kalınlığı (CIMT) arasındaki ilişkiyi araştırmak amaçlanmıştır.

YÖNTEM: ADA kriterlerine göre 75gr oral glukoz tolerans testi (OGTT) ile prediyabet tanısı konan 70 hasta çalışmaya dahil edildi. Altmış altı sağlıklı gönüllü de kontrol grubu olarak değerlendirildi. Hasta ve kontrol grupları E-selektin ve açlık insülin düzeyleri açısından incelendi. CIMT, B-mod ultrasonografi ile ölçüldü ve HOMA-IR hesaplandı.

BULGULAR: Prediyabetik hasta grubunda ortalama E-selektin seviyesi 35.19 (9.56-118.46) ng/mL ve kontrol grubunda 16.60 (1.55-116.62) ng/mL idi. Gruplar arasındaki fark istatistiksel olarak anlamlıydı ($p=0.001$). CIMT, prediyabetik hasta grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksek bulundu (sırasıyla $p<0.001$, 0.62 ± 0.10 mm ve 0.54 ± 0.09 mm). Prediyabetik hasta grubunda E-selektin ile HbA1c ($r=0.306$, $p=0.01$), HOMA-IR ($r=0.243$, $p=0.043$), LDL-C ($r=0.329$, $p=0.005$) ve TG ($r=0.252$, $p=0.035$) arasında anlamlı bir pozitif ilişki bulundu, fakat bel/kalça oranı ile negatif bir korelasyon vardı.

SONUÇ: Prediyabette endotel disfonksiyonunun belirteçlerinden biri olan E-selektin düzeyinin arttığını bulduk. Bu sonuç endotel disfonksiyonunun ve erken evre aterosklerozun prediyabetik dönemde başladığı düşüncesini desteklemektedir.

Anahtar kelimeler: Prediyabet, ateroskleroz, e-selectin

INTRODUCTION

Diabetes mellitus (DM) is an important cause of morbidity and mortality that reduces quality of life and survival. Diabetes causes microvascular (nephropathy, retinopathy, and neuropathy) and life-threatening macrovascular complications such as atherosclerotic

cardiovascular diseases due to chronic hyperglycemia. In 2017, there were 425 million diabetics and 352 million people (7.3% of adults) with impaired glucose tolerance (IGT) worldwide. The prevalence of type 2 diabetes mellitus is increasing around the world. Accordingly, it was assumed that in 2045 the number

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of diabetics will be 629 million and the number of people with impaired glucose tolerance will be 531 million (8.3%) (1). Prediabetes is the main risk factor for type 2 diabetes. The risk of diabetes is increased by 4 to 7 fold for impaired fasting glucose (IFG) and 6 fold for IGT as compared with people with normal glucose tolerance (2). Elevated glucose levels are a direct cause of atherosclerosis or clinical cardiovascular disease (CVD) (3-6). Chronic inflammation plays a key role in the progression of atherosclerosis and is closely related with endothelial dysfunction (7). Leukocytes passing into the vascular endothelium and migrating in the subendothelial area is the major process for the development of atherosclerosis. This is mediated by adhesion molecules expressed on the vascular endothelial cell surface. During inflammation, E-selectin plays an important part in recruiting leukocytes to the site of injury. VCAM-1 (vascular cellular adhesion molecule-1) and ICAM-1 (interstitial cellular adhesion molecule-1) play a role in transendothelial migration and the firm attachment of leukocytes (8). E-selectin is a surface glycoprotein molecule that is activated by cytokines and expressed in endothelial cells (9). E- and P-selectin function in both early and advanced stages of atherosclerotic lesion development (10). Pathologic studies demonstrated that expression of cellular adhesion molecules is enhanced in both endothelial cells and other types of tissue. E-selectin, VCAM-1 and ICAM-1 are increased in the circulation over the course of inflammatory situation and are detected in the plasma (11-13). Previous studies have examined the relationship of E-selectin concentrations with carotid atherosclerosis and the risk of coronary heart disease, but the results were variable (14-17). CIMT is an important marker for atherosclerosis staging. It can be measured relatively simply and non-invasively, it is well suited for use in large-scale population studies. Measurement of the CIMT via B-mode ultrasonography provides easy evaluation of atherosclerosis. CIMT is an established measure of early atherosclerotic changes and is used as a surrogate endpoint of vascular outcomes in clinical trials (18,19). This parameter is included in European guidelines in the prevention of cardiovascular disorders. Similar to classic risk factors such as diabetes, hyperlipidemia and obesity, this is an independent risk factor for CVD. The present study aimed to investigate the relation of E-selectin with insulin resistance, CIMT, and cardiovascular risk factors in prediabetes.

MATERIALS AND METHODS

Study population

Seventy patients with prediabetes were included in the study. Sixty-six volunteers were included as the control group. Participants who had overt diabetes mellitus, CVD or hypertension history, and younger than 18 years old were not included in this study. None of the prediabetic patients had not received lipid-lowering medications. The study was performed at the Endocrinology Department of Diskapi Yildirim Beyazit

Training and Research Hospital between January 2012 and July 2012. The protocol was approved by the local Ethics Committee and all participants provided written informed consent before the study procedures. Categories of glucose tolerance were defined according to American Diabetes Association (ADA) criteria (20). Prediabetes is defined by an elevated fasting plasma glucose (FPG) concentration between 100-125 mg/dL or elevated 2-hour plasma glucose concentration between 140-199 mg/dL after a 75-g glucose load in the oral glucose tolerance test (OGTT) in the presence of an FPG concentration <126 mg/dL. None of the patients with prediabetes had received metformin.

Clinical and biochemical evaluation

All participants (prediabetics and controls) underwent physical examinations, anthropometric measurements, biochemical screening, and their history of medications or cardiovascular disease was investigated. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). The waist circumference was measured at the narrowest level between the costal margin and iliac crest, and the hip circumference was measured at the widest level over the buttocks while the subjects were standing and breathing normally. Blood samples were taken in the morning between 8.00 AM and 11.00 AM from each patient after a 12-h overnight fasting to determine insulin and glucose levels. Fasting serum insulin levels were measured using a chemiluminescent immunoassay (Advia Centaur XP, Siemens Healthcare Diagnostics Inc., Tarrytown USA). Insulin resistance was calculated using the homeostasis model assessment insulin resistance index (HOMA-IR) (21) with the following formula: $\text{fasting plasma glucose (mmol/L)} \times \text{fasting serum insulin (mU/mL)} / 22.5$. The cut-off value was taken as 2.7 for HOMA-IR. HbA1c was measured by high performance liquid chromatography (HPLC). The total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were determined with enzymatic colorimetric assays by spectrophotometry (BioSystems S.A., Barcelona, Spain). The Friedewald formula was used to calculate the low-density lipoprotein cholesterol (LDL-C).

Measurement of Carotid Atherosclerosis

Measurement of CIMT provides easy evaluation of atherosclerosis. CIMT was measured via high-resolution B-mode ultrasonography using a 13-MHz linear probe (Hitachi EUB 7000 HV). Three arterial wall segments of the common carotid artery were measured bilaterally after imaging from a fixed lateral transducer angle and designated as mean CIMT. The IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia. To avoid subjective error, CIMT measurements were taken by the same person.

Measurement of E-selectin

Fasting blood samples were taken from the participants and centrifuged. Serum samples were stored frozen

at -80°C until the day of analysis. Serum E-selectin levels were measured on all samples using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, USA). The microplate in the kit was precoated with anti-E-selectin antibody. E-selectin present in the sample or standard binds to antibodies adsorbed in the microwells. Horseradish peroxidase (HRP) was added, which binds to E-selectin captured by the first antibody. Following incubation and a wash step, substrate solution was added to the wells. The reaction was terminated by the addition of acid, and absorbance was measured at 450 nm. The analytical range was 0.33-50 ng/mL. The calculated overall coefficient of variation was 5.4%. The calculated overall coefficient of variation was 6.0%.

Statistical Analyses

Descriptive values of data are presented as mean±SD, median (minimum-maximum), number and % frequencies. The data were evaluated using the Kolmogorov-Smirnov test and those with a homogenous distribution were analyzed using parametric tests (Student t-test) whereas non-parametric tests (Mann Whitney U test) were used for those with a non-

homogenous distribution. The difference between groups for qualitative variables was evaluated using the Chi-square test. The correlation analyses were performed using Pearson's and Spearman's correlation tests. A p value lower than 0.05 was considered as the level of statistical significance, and calculations were made using Predictive Analytics Software (PASW) (SPSS, version 18).

Results

The demographic characteristics and biochemical parameters of the patients with prediabetes and the control subjects are shown in Table 1. Serum E-selectin levels were significantly elevated in patients with prediabetes compared with the control subjects (p=0.001, **Table 1**). The mean HbA1c was 5.69±0.48% in the prediabetic group. Similarly, CIMT was statistically significant higher in prediabetic patients groups (0.62±0.10 mm and 0.54±0.09 mm respectively, p<0.001). The total cholesterol, LDL-C, and TG levels were statistically significant elevated in prediabetic patients group compared with the control group (Table 1). Correlation analysis revealed a relation between E-selectin and weight (r=0.239, p=0.005), fasting blood glucose (r=0.236, p=0.006), total cholesterol

Table 1 Demographic characteristics and biochemical data of prediabetic patients and control subjects

	Prediabetic patients (n=70)	Control (n=66)	p
Age (year)	46.27±9.50	43.87±10.20	0.160
Gender (%)			
Female	29 (%41,4)	38(%57,6)	0.086
Male	41(%58,6)	28(%42,4)	
Height (cm)	165.58±9.9	163.28±8.0	0.138
Weight (kg)	82.81±16.08	75.04±11.09	0.001
BMI (kg/m²)	30.19±5.40	28.68±3.42	0.055
Waist circumference (cm)	106.11±11.45	93.68±11.56	<0.001
Hip circumference (cm)	106.50±11.52	107.37±9.06	0.623
Waist/Hip Ratio	1.00±0.09	0.87±0.06	<0.001
E-selectin (ng/mL)*	35.19 (9.56-118.46)	16.60 (1.55-116.62)	0.001
CIMT (mm)	0.62±0.10	0.54±0.09	<0.001
Fasting blood glucose (mg/dl)	107.88±13.67	88.93±8.71	<0.001
Insulin (µIU/ml)*	10.31(2.05-42.84)	10.00(5.00-23.70)	0.846
HOMA-IR*	2.50(0.51-9.94)	2.11 (1.00-5.20)	0.254
Total cholesterol (mg/dL)	207.80±26.04	198.57±28.17	0.049
LDL-C (mg/dL)*	136.0(59.0-158.0)	123.50(47.0-157.0)	0.001
HDL-C (mg/dL)	42.07±7.50	47.36±10.11	0.001
TG (mg/dL)	175.94±70.98	147.57±66.15	0.017

Data with parametric distribution are presented as mean±SD.

*Data with non-parametric distribution are presented as median (minimum-maximum).

CIMT, carotid intima media thickness; HOMA-IR, homeostasis model assessment insulin resistance index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride

($r=0.409$, $p<0.001$), LDL-C ($r=0.445$, $p<0.001$), and TG ($r=0.362$, $p<0.001$) and in all participants, but negative correlation was found between E-selectin HDL-C ($r=-0.298$, $p<0.001$) in all participants. In the prediabetic group, e-selectin was correlated positively with HbA1c ($r=0.306$, $p=0.01$), HOMA-IR ($r=0.243$, $p=0.043$), LDL-C ($r=0.329$, $p=0.005$), TG ($r=0.252$, $p=0.035$) and negatively correlated with waist/hip ratio ($r=-0.393$, $p=0.001$). Moreover, a statistically significant relation was determined between CIMT and age ($r=0.572$, $p<0.001$), weight ($r=0.352$, $p=0.003$), BMI ($r=0.394$, $p=0.001$), waist circumference ($r=0.413$, $p<0.001$), fasting blood glucose ($r=0.411$, $p<0.001$), HOMA-IR ($r=0.242$, $p=0.044$), total cholesterol ($r=0.242$, $p=0.044$), or TG ($r=0.268$, $p=0.025$), in patients with prediabetes. CIMT was found as an independent variable by multiple regression analysis ($p=0.001$) (Table 2).

Table 2. Regression analysis of lipid profile outcome in the prediabetic patients and control group

	B	S.E.	Sig.	Exp(B)	95% C.I.forEXP(B)	
					Lower	Upper
CIMT	6,688	2,090	,001	802,758	13,342	48300,384
E-Selectin	,002	,008	,762	1,002	,987	1,018
LDL-C	,014	,010	,177	1,014	,994	1,034
HDL-C	-,050	,026	,053	,951	,904	1,001
TG	-,001	,003	,876	,999	,993	1,006

CIMT, carotid intima media thickness; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride

DISCUSSION

The endothelium is the largest organ in the body and secretes numerous important physiologic and pathophysiologic inflammatory mediators. Acute and chronic inflammation is also associated with endothelial dysfunction (22,23). Endothelial dysfunction occurs due to chronic exposure to various stressors, which result in chronic inflammation such as oxidative stress and impaired nitric oxide production. In addition, hyperglycemia-related production of advanced glycation end products (AGE) leads to endothelial dysfunction. A series of biochemical and mechanical events over the course of the development of diabetes results in endothelial dysfunction and vascular inflammation. In patients with type 2 diabetes mellitus, the major cause of mortality and morbidity is CVD. Patients with type 2 diabetes mellitus have an increased risk for premature atherosclerosis. Atherosclerosis and coronary artery disease may be inflammatory conditions and E-selectin plays a key role in the initial process of inflammation. Observational studies have shown that elevated levels of E-selectin have been observed in acute myocardial infarction, coronary heart disease, restenosis following peripheral arterial angioplasty, and stable or unstable angina (24-27). Increased concentrations of plasma

adhesion molecules such as ICAM-1, VCAM-1, and E-selectin have been detected in patients with developing diabetes and atherosclerosis. An increased plasma concentration of adhesion molecules has been suggested as a marker of early atherosclerosis (28). Expression of E-selectin, ICAM-1, von Willebrand factor (vWF) and plasminogen activator inhibitor (PAI-1), which appears due to endothelial cell activation, is enhanced in both patients who are prediabetic and diabetic (29-31). The present study confirms that serum levels of E-selectin were increased in patients with prediabetes, and demonstrates that elevated circulating serum levels of E-selectin may provide important prognostic information for patients with prediabetes. Endothelial dysfunction is a well-recognized consequence of diabetes that leads to both micro- and macrovascular disease complications (32). However, endothelial dysfunction may occur before the onset of diabetes. This may result in diabetes by facilitating glucose dysregulation and insulin resistance (33-35). Prediabetes was initially recognized as a condition associated with increased risk of progression to type 2 diabetes mellitus. Prediabetes, as with type 2 diabetes, is characterized particularly by impaired insulin secretion, and insulin resistance. Processes that lead to endothelial dysfunction and vascular inflammation are potentialized in the presence of insulin resistance, and form the basis for micro- and macrovascular complications of diabetes (36). There is no certain plasma glucose threshold for the development of microangiopathy. As compared with individuals with normal glucose tolerance, the prevalence of microangiopathic complications such as nephropathy (e.g. microalbuminuria, increased GFR), retinopathy, and neuropathy is increased in the prediabetic period (37-42).

Reynolds et al. showed that E-selectin and adiponectin were found to serve as independent predictors of carotid plaque (43). The threshold value for IMT is considered to be 0.9 mm. An increase in IMT over the threshold value indicates progression of atherosclerosis. A positive correlation has been reported between IMT and degree of atherosclerotic changes (19). Sakurai et al identified a positive association of serum levels of E-selectin and hsCRP with carotid atherosclerosis, and E-selectin levels were significantly associated with carotid wall thickening and were strongly associated with the presence of heterogeneous plaque. Zemlin AE et al. showed that E-selectin concentrations were raised in hyperglycemic subjects, no relationship was observed between these E-selectin concentrations and CIMT (44). Similarly Leinonen et al. found that E-selectin levels were higher in diabetics, but not associated with CIMT (45). Babar et al. showed that adolescents with type 1 DM who exhibit significantly elevated HbA1c experience a greater degree of vascular oxidative stress and endothelial dysfunction as seen with elevated E-selectin. This study also shows that E-selectin can be used as a marker of oxidative stress to identify patients with suboptimal glycemic control. Furthermore, CIMT, arterial stiffness and fibrinogen increased with rising HbA1c, indicating that suboptimal glycemic control in

adolescence is associated with endothelial dysfunction and early atherosclerosis. This indicates that as the glycemic control worsens, the degree of oxidative stress, endothelial dysfunction, CIMT and arterial stiffness would also increase (46). In our study, a surrogate marker of early atherosclerosis, which CIMT was significantly higher in the prediabetic groups than in the control group. However, we determined no significant relation between CIMT and serum E-selectin concentration. The probable cause of this is that our patients are prediabetic and have not poor glycemic regulation.

Obesity is an important risk factor for developing diabetes mellitus. The relative risk of type 2 diabetes for the 90th percentile of body mass index (BMI) (BMI=29.9 kg/m²) versus the 10th percentile (BMI=20.1 kg/m²) was 11.2 (46). Studies have demonstrated that obesity is a state of chronic inflammation significantly associated with increased plasma concentrations of CRP, IL-6, TNF- α , ICAM-1, and VCAM-1 and E-selectin. Obesity causes insulin resistance and the release of proinflammatory and procoagulant substances from adipose tissue by activating the inflammation cascade (47). Our study showed that E-selectin levels were significantly associated with HOMA-IR and negatively correlated with waist/hip ratio in patients with prediabetes. Excess adipose tissue releases excess fatty acids and a variety of adipokines that seemingly elicit metabolic risk factors that predispose to both diabetes and CVD. Although further work is needed, it seems clear that these biomarkers are predictors of increased morbidity in patients who are prediabetic or diabetic.

In conclusion, E-selectin is one of the markers of endothelial dysfunction in circulation. Therefore, measurement of such biomarkers in the circulation enables early diagnosis and treatment and also gives physicians a chance to determine new therapeutic goals. This might reduce the risk for development and appearance of disease-related potential complications.

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