RJDNMD, **18**(4):293-302 *http://www.jrdiabet.ro*

THE RELATIONSHIP BETWEEN *sE-Selectin* CONCENTRATIONS, MICROALBUMINURIA AND INSULIN-SENSITIVITY IN TYPE 2 DIABETES PATIENTS

Elena Violeta Băcanu [1, ✉]***, Virginia Borza*** [2]***, C. Ionescu-Tîrgoviste*** [1]

[1] „N Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania
[2] Institute of Atomic Physics, Magurele, Bucharest, Romania

received: August 08, 2011 *accepted*: November 11, 2011

available online: December 15, 2011

Abstract

The aim of the study was to examine the effects of poor metabolic control on vascular endothelium, expressed by increased soluble E-selectin concentration, microalbuminuria and their relationship with insulin sensitivity in type 2 diabetes. We studied 42 patients with type 2 diabetes and 36 subjects without diabetes. The patients with type 2 diabetes were divided in two study subgroup: group A (HbA1c<8%) and group B (HbA1c≥ 8%). Patients with type 2 diabetes had both significantly high levels of sE-selectin and AER compared with control subjects (p<0.001). A poor metabolic control, expressed by a difference in HbA1c of 1.4% (mean value) between group A and group B, resulted in a significant increase of sE-selectin (p<0.001), suggesting endothelial damage. In patients with type 2 diabetes, poor metabolic control influences sE-selectin and microalbuminuria. We found a positive correlation between these two markers of endothelial dysfunction.

Key words*: sE-selectin, microalbuminuria, insulin sensitivity, endothelial dysfunction.*

Introduction

E-selectin is an adhesion molecule that helps leukocytes roll on vascular endothelium and attach to the endothelium, serving as a marker of inflammation and atherosclerosis (processes promoted by the traditional risk factors and by dysglycemia/hyperglycemia, as well) [1].

Endothelial dysfunction is associated with cardiovascular risk factors such as: hypercholesterolemia, hypertension, diabetes, smoking, inflammation and decreased insulin sensitivity.

Hyperglycemia promotes leukocyte adhesion to endothelial cells through up regulation of cell-surface expression of E-selectin, intercellular adhesion molecule (ICAM-1), and vascular cell adhesion molecule (VCAM-1) [2]. Stimulation of endothelial cells with glycated albumin (advanced glycation end product/ bovine

✉ 5-7 Ion Movila Street, Bucharest, District 2, Postal Code 11420
corresponding author e-mail: drelena_bac@yahoo.com

serum albumin) also increases expression of these adhesion molecules [2, 3], which can be detected in soluble [4] forms (sE-selectin, sICAM-1, and sVCAM-1) in the circulation [5, 6]. In patients with type 2 diabetes, concentrations of serum sE-selectin, which is expressed exclusively by endothelial cells, have been increased in most [4, 7, 8] but not all [9, 10] comparisons. A positive correlation with glycemic control was reported in two studies [9, 11]. These cross-sectional data suggest that sE-selectin may be a more sensitive indicator of hyperglycemia induced endothelial activation in vivo than sVCAM-1 or sICAM-1, although the expression of all three is regulated by glucose in vitro [12]. Leena Ryysy et al. demonstrated on 81 patients with type 2 diabetes that improvement of glycemic control by 1 year of insulin therapy leads to a sustained decrease in sE-Selectin concentrations [13]. Other studies show that the decrease in sE-selectin level is independent of the pharmacological agent used to lower glucose concentrations. Moreover, the results of the same study suggest that the concentration of sE-selectin, but not of sVCAM-1, is sensitive to changes in glycemic control [13, 14].

Decreased insulin sensitivity leads to endothelial dysfunction through several mechanisms: indirectly, in association with major cardiovascular risk factors (hyperglycemia, dyslipidemia, and hypertension) and directly, through its action on the vascular wall [15]. Insulin, in addition to metabolic actions, has a direct vasodilator effect by stimulating endothelial NO synthesis [16, 17]. When insulin sensitivity is decreased, the ability of insulin to stimulate NO synthesis is diminished [16].

Assessment of endothelial function plays an important role in identifying the actions of both cardiovascular risk factors and various therapeutic interventions on vascular wall. In clinical practice, endothelial function is assessed by determination of endothelium-dependent vasodilatation in both coronary circulation and peripheral arteries and measurement of urine albumin excretion.

Increased urine albumin excretion is associated with most cardiovascular risk factors, hyperglycemia, hypertension, renal dysfunction, dyslipidemia, hyperhomocysteinemia, increased protein intake, smoking, and presence of acute phase markers. Otherwise, diabetes and hypertension are the major risk factors for development of microalbuminuria [18].

Epidemiological data show that the prevalence of microalbuminuria is 30% in diabetic adults and 10-15% in non-diabetic adults [19]. Microalbuminuria, a result of increased renal endothelium permeability, is a marker of systemic endothelial dysfunction as well [19].

ADA defines microalbuminuria as the presence of albumin excretion rate in the range of 30-299 mg in a 24 h urine collection or as an albumin-to-creatinine ratio in the range of 30-299 mg/g measured in a random spot urine collection [20]. According to the 2011 ADA recommendations this has been shown to be the earliest stage of diabetic nephropathy in type 1 diabetes and a marker for development of nephropathy in type 2 diabetes [20]. Microalbuminuria is also a well-established marker of increased cardiovascular risk [19].

The aim of the study was to examine the effects of poor metabolic control on vascular endothelium, expressed by increased soluble

E-selectin concentration, albumin excretion rate and their relationship with insulin sensitivity in type 2 diabetes.

Patients and methods

Our study included 76 subjects: 42 patients with type 2 diabetes (20 men and 22 women) and 36 subjects without diabetes (20 men and 16 women). Diabetic patients were recruited from the outpatient department of 'N.C.Paulescu' National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania and subjects from control group were healthy volunteers. The clinical characteristics of the subjects of these two groups are given in Table 1.

To establish to what extent poor metabolic control affects the serum level of E-selectin and for a better assessment of its correlation with insulin sensitivity (HOMA IR) and microalbuminuria, patients with type 2 diabetes were divided in two study subgroups: group A: 21 patients, 10 men and 11 women, with type 2 diabetes and HbA1c< 8% (6.5% to 7,9%, mean HbA1c 7.23% ±0.12); group B: 21 patients, 10 men and 11 women, with type 2 diabetes and HbA1c≥8% (8% to 9.6%, mean HbA1c 8.63%±0.11).

The inclusion criteria in the group with type 2 diabetes were: positive diagnosis of type 2 diabetes, age 40–70 years, BMI ≤35kg/m2, fasting blood glucose level >7mmol/l (>126mg/dl), HbA1c ≥ 6,5%, duration of diabetes >3 years, previous oral anti diabetic therapy or nutrition therapy. Type 2 diabetes was defined according to the criteria of American Diabetes Association [20].

Exclusion criteria included the following: urinary infection, congestive heart failure, myocardial infarction, or stroke in the past 6 months; epilepsy or other severe disease; liver disease, serum creatinine concentration >120μmol/l (1.36mg/dl), or macroalbuminuria; proliferative retinopathy or severe maculopathy; previous insulin therapy for >2 weeks; excessive alcohol consumption (>20 g/day); and night work as previously described.

Other condition who is associated with high level of E-selectin such as discoid lupus erythematosus [21], localized sclerodermia [22] palmar and plantar pustulosis [23] and malignancies such as those in metastatic breast carcinoma [24], was exclusion criteria for this study.

All patients gave written informed consent to participate in the study, which was approved by the local ethical committee from NIDNMD "NC Paulescu Institute".

Percent body fat (%) was measured using a bioelectrical impedance analyzer (Omron BF 500). We performed anthropometric measurements: weight, height, BMI (Body Mass Index). The (BMI) was calculated as weight in kilograms divided by height in square meter [25].

Microalbuminuria was determined by measuring the albumin excretion rate from three non consecutive first morning urine samples. Normal albumin excretion rate was <30mg/g creatinine and microalbuminuria was defined as AER in the range of 30 and 299 mg/g creatinine [20]. The ACR (albumin to creatinine ratio) was calculated as follows: urinary albumin concentration (mg/liter)/ urinary creatinine concentration (mg/dl). The mean value of each patient's three ACRs was used to indicate the level of albumin excretion [20]. Results were transformed logarithmically because the AER followed a log-normal distribution.

Laboratory assay: The blood samples were obtained after 12 hours of overnight fasting. Morning urine sample was collected in a container for analysis of creatinine and microalbuminuria. Fasting blood glucose, glicosylated hemoglobin (reference range 4.0-6.0%), total cholesterol, high density lipoprotein, triglyceridemia, serum creatinine, urea and uric acid were measured using standard techniques. To estimate low-density lipoprotein (LDL) cholesterol we used Fridewald formula as follows: LDL-cholesterol=Total cholesterol-HDL cholesterol-triglycerides/5. Serum levels of specific insulin and proinsulin were measured by Elisa (DRG international, Inc) on a TECAN analyzer. Serum sE-selectin was measured by ELISA kit Human E-selectin-Milipore-MN. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the product of fasting plasma insulin value (micro units per ml) and fasting plasma glucose value (mg per deciliter) divided by 405. Statistical analysis:

Data were analyzed using the SPSS package for Windows version 16 and Excels 2003. As a first step analysis, comparisons between patients and controls were performed using the unpaired

Student's t test. Patient's characteristics are given as mean values ±SD and data skewed distributed were expressed as median (interquartile range). Pearson's correlation was calculated to measure the relationship between variables. All p-values < 0.05 were considered statistically significant.

Results

Clinical and biochemical variables of diabetic patients and control group were given in table 1.

In table 2 is given the relationship between diabetic patients (with HbA1c <8% and respectively ≥8%) vs. control for E-selectin, albumin excretion rate, HOMA-IR, plasma specific insulin and plasma proinsulin.

Table 1. Clinical and biochemical characteristics of the diabetic patients and controls.

	Diabetic patients	Controls	p value
	N=42	N=36	ns
sex	M/F=20/22	M/F=20/16	ns
smokers	30.95%	2%	p<0.001
Duration of diabetes (years)	6.33±0.47	–	–
BMI (Kg/m2)	28.18±0.60	23.12±5.	p<0.005
SBP (mmHg)	126±3.61	118.4±2.3	p<0.05
DBP (mmHg)	74±0.9	70.17±1.	p<0.001
Blood glucose (mg/dl)	160±4.01	84.53±0.08	p<0.001
HbA1c (%)	7.93±0.13	4.94±1.4	p<0.001
Cholesterol (mg/dl)	201.98±5.78	178.8±0.09	p<0.05
Triglyceride (mg/dl)	117.7±12.68	119.33±1.1	ns
Uric acid (mg/dl)	6.10±0.24	4.21±1.5	p<0.05
Creatinine (mg/dl)	0.77±0.02	0.60±0.02	p<0.001
Urea(mg/dl)	34±1.3	30.9±1.3	Ns
Albumin-to-creatinine ratio (mg/g)	21.9±2.48	5.36±0.41	p<0.001
Plasma insulin (μu/ml)	13.21±1.10	7.19±6.	p<0.05
HOMA-IR (arbitrary units)	5.27±0.49	1.26±0.08	p<0.001

Plasma proinsulin (pmol/ml)	39.56±3.09	11.87±0.69	p<0.001
E-selectin (ng/ml)	11.6±0.53	7.45±0.6	p<0.001

Data in table are presented as mean±SD; N=number; BMI=body mass index, HbA1c=Glycated hemoglobin, SBP and DBP=systolic and diastolic blood pressure; HOMA-IR=homeostasis model assessment of insulin resistance

Table 2. Markers of endothelial dysfunction, insulin sensitivity and pancreatic secretion.

	Patients with T2DM		Patients without T2DM	Statistical significance for		
	HbA1c<8	HbA1c≥8		Diff group A/B	Diff group A/C	Diff group B/C
	GROUP A	GROUP B	CONTROL GROUP	p=value	p=value	p=value
E-selectin-Both groups (ng/ml)	11.61±/0.53		7.48±0.59			
E-selectin (ng/ml)	9.67±0.61*	13.57±0.66**	7.48±0.59	<0.001	0.014	<0.001
Albumin-to-creatinin ratio(mg/g)	15.53±2.65**	28.34±3.77**	5.37±0.42	0.008	0.001	<0.001
HOMA-IR (arbitrary units)	4.54±0.64**	6.01±0.74**	1.27±0.08	0.139	<0.001	0.001
Plasma insulin(μU/ml)	12.33±1.7*	14.1±1.62**	7.19±0.7	0.454	0.012	0.001
Plasma Proinsulin(pmol/ml	34.41±4.46**	44.72±4.09**	11.87±1.7	0.096	<0.001	<0.001
Proinsulin/Insulin ratio	3.28±0.45	3.89±0.53*	2.22±0.59	0.381	0.152	0.041

* - p value< 0.05 -statistically significant

** -p value< 0,001 – highly statistically significant

Serum levels of E-selectin were 56% higher in patients with type 2 diabetes (11.61±0.53ng/ml) than in control subjects (7.48±0.59ng/ml), p<0.001 (Table 2). There was no gender difference in sE-selectin concentration between women with type 2 diabetes (11.77± 0.63ng/ml) and men (11.43±0.89). We also found, in keeping with data by Bannan et al [12], that normal women had lower levels than normal men and that this difference was abolished by type 2 diabetes. The gender difference in normal subjects may be explained by estrogen, because estradiol decreases sE-selectin level. In group B of patients we found a mean level of E-selectin significantly increased compared with the control group (13.57±0.66ng/ml vs. 7.48±0.59ng/ml, p value <0.001). In group A of patients we found a mean level of sE-selectin increased compared with control group (9.67±0.61ng/ml vs. 7.48±0.59ng/ml, p value<0.05) (Figure 1).

A poor metabolic control, namely a difference of 1.4% in terms of HbA1c mean level: 7.23 ±0.12% (group A) and 8.63 ± 0.11% (group B), was associated with a significant increase in E-selectin serum level in group B (13.57±0.56ng/ml) versus group A (9.67±0.61ng/ml), p value <0.001.

A statistical significant difference in albumin-to-creatinine ratio was found both between group B and control group (28.34±3.67mg/g vs. 5.37±0.42mg/g, p value<0.001) and between group A (15.53±2.65 mg/g) and control group (5.37±0.42 mg/g, p value<0.001) (Figure 2).

We also observed a significant difference in the mean level of albumin-to-creatinine ratio between group B (28.34±3.67mg/g) and group A (15.53±2.67mg/g), p value<0.05 (Figure 2).

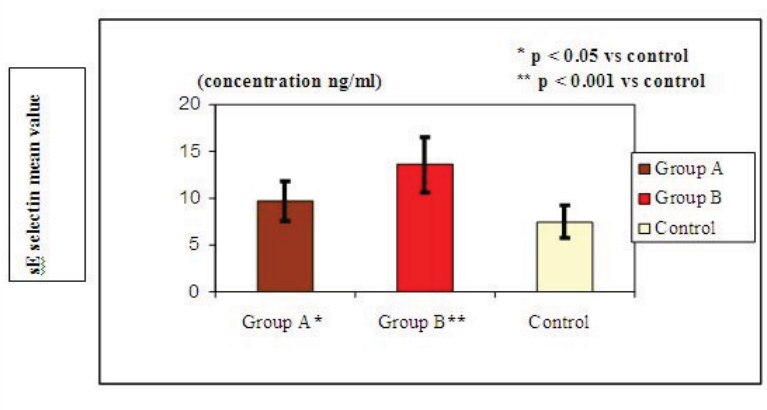

Figure 1. sE-Selectin mean value recorded in the three study groups:
group A – with type 2 diabetes with HbA1c < 8% and group B with HbA1c ≥ 8%

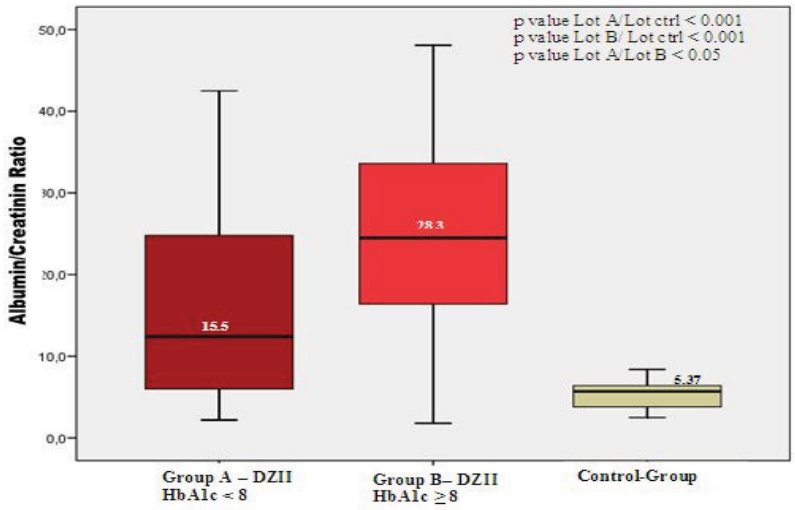

Figure 2. Box plot graph ACR

Regarding the β cell function (serum level of specific insulin and proinsulin), we found the following results: in group B plasma insulin was 14.1±15.2 μU/ml vs. 7.19±0.7μU/ml in control group, p value<0.001, while in group A was 12.33±1.7μU/ml versus 7.19±0.7 μU/ml control group, p value <0.05. The higher value of specific plasma insulin in diabetic patients vs controls is explained by the higher blood glucose value in diabetic patients vs controls (see table 1). Plasma level of proinsulin was also significantly increased in both groups: group B vs. control group (44.72±0.9pmol/ml, 11.87±1.7pmol/ml, respectively, p value<0.001) and in group A vs. control group (34.41±4.46pmol/ml, 11.87±1.7pmol/ml, respectively, p value<0.001). This increase might reflect a decrease in β cell function.

Overall, HOMA IR (group A + group B) was positively correlated with both albumin-to-creatinine ratio (r=0.475, p=0.001) and serum level of E-selectin (r=0.307, p<0.05) (Table 3). Our data show a strong positive

correlation between serum level of E-selectin and urine albumin-to-creatinine ratio (r=0.591; p value<0.001) (Figure 3). We also found that serum level of E-selectin was positively correlated with systolic blood pressure (r=0.394; p=0.01), total cholesterol, LDL-cholesterol and BMI. This study showed a positive correlation between: ACR and systolic blood pressure (r=0.380; p<0.05); ACR and HBA1c (r=0.561; p<0.001); ACR and HOMA IR (r=475; p=0.001); ACR and plasma insulin (r=0.332; p<0.05) (Table 3).

Table 3. Person's correlation between sE selectin concentration, ACR and selected variables

DIABETIC PATIENTS(GROUP A+B)	sEselectin		ACR(Albumin-to-creatnine ratio)	
	r	p	r	p
sE- Selectin	1		0.0591(**)	0.000
Albumin-to-creatinine ratio	0.059(**)	0	1	
age	0.113	0.475	–0.248	0.114
BMI	0.008	0.960	0.273	0.081
Duratio of diabetes(years)	–0.009	0.953	–0.119	0.453
SBP	0.394(**)	0.010	0.380(*)	0.013
DBP	0.024	0.880	0.286	0.066
Cholesterol	0.177	0.261	0.053	0.739
Triglycerides	0.088	0.577	0.101	0.524
HDL	–0.005	0.973	0.126	0.427
LDL	0.143	0.368	0.024	0.879
Blood glucose	0.520(**)	0.000	0.558(**)	0.000
HbA1c	0.601(**)	0.000	0.561(**)	0.000
Plasma insulin	0.211	0.181	0.332(*)	0.032
Plasma proinsulin	0.461(**)	0.002	0.172	0.277
HOMA-IR	0.307(*)	0.048	0.475(**)	0.001

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

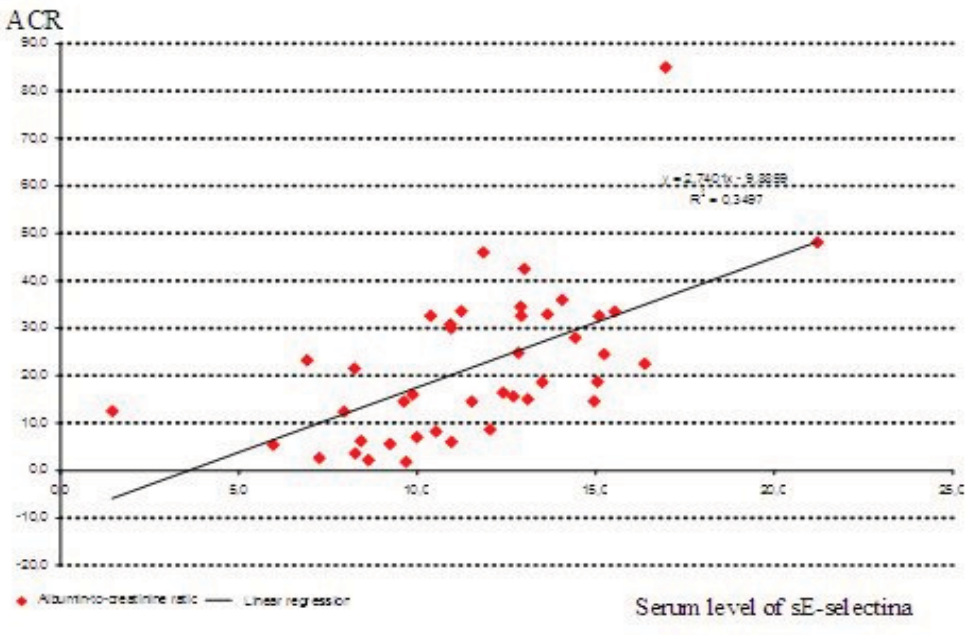

Figure 3. Correlation between sE-selectin and ACR (r=0.591, p<0.001)

Discussions

Several abnormalities contribute to the relationship between endothelial dysfunction and insulin sensitivity, such as synthesis of reactive oxygen species (super oxide), inflammation, cytokine synthesis (e.g., TNF α), and activation of renin angiotensin system and increased concentration of endothelin.

In this study, early markers of endothelial dysfunction (serum level of E-selectin and microalbuminuria, respectively) are increased in patients with type 2 diabetes, even in patients in group A. Moreover, poor metabolic control, expressed by a HbA1c increase of 1.4% (the difference between group A and group B), resulted in a significant increase in E-selectin serum level, suggesting that improve metabolic may offer benefits in early endothelial dysfunction. As other studies have shown [12], sustained improvement in glycemic control was associated with a significant decrease in E-selectin serum level. Other studies showed that this association is independent of the pharmacological agent used (oral antidiabetic drugs or insulin) [14, 26].

Regarding the mechanisms linking hyperglycemia and sE-selectin concentration, it seems possible that glucose-induced increased oxidative stress is involved [2, 27]. Glycosylated hemoglobin concentration are associated with reduced total plasma antioxidant trapping capacity in patients with type 2 diabetes [28], a finding consistent with the concept that clinically relevant hyperglycemia increases oxidative stress. Because treatment of hyperglycemia has reduced sESelectin levels in three studies [8, 13, 29], glycemic control could be considered a better tool than antioxidants to lower sE-selectin concentration in type 2 diabetes.

No data presently exist regarding the predictive value of sE selectin concentration for future vascular events in patients with type 2 diabetes, and no data exist to demonstrate that lowering of se-Selectin reflects a beneficial change in vascular function.

The identification of endothelial dysfunction markers associated with the development of nephropathy and cardio-vascular disease in patients with type 2 diabetes has considerable clinical implica-tions.

Current management strategies are increasingly focusing on preventive measures following early detection of markers of endothelial dysfunctions. Early detection of endothelial dysfunction markers allows the control of beneficial therapeutic intervention on endothelial damage by improving metabolic control.

Determination of serum E-selectin, an early biomarker of endothelial dysfunction, allows assessing the benefits of improved metabolic control on endothelial function.

Conclusion

In patients with type 2 diabetes, poor metabolic control influences serum level of sE-selectin and microalbuminuria. In this study we found a positive correlation between both these two markers of endothelial dysfunction.

The level of sE selectin seems to be an early biomarker of endothelial dysfunction, suggesting the urgent need for improvements in the metabolic control in order to prevent the end stage diabetic complications.

Abreviation: ADA=American Diabetes Association; ACR=albumin-to-creatinine

ratio; AER=albumin excretion rate; BMI=body mass index; HOMA-IR=homeostasis model assessment of insulin resistance; sE-selectin = soluble E-selectin.

REFERENCES

1. **Albelda SM, Smith CW, Ward PA**. Adhesion molecules and inflammatory injury. FASEB J. 8:504–512, 1994.

2. **Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, Remuzzi A, Zoja C, Remuzzi G**: Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. *J Clin Invest* 101: 1905-1915, 1998.

3. **Kunt T, Forst T, Harzer O, Buchert G, Pfutzner A, Lobig M, Zschabitz A, Stofft E, Engelbach M, Beyer J**: The influence of advanced glycation endproducts (AGE) on the expression of human endothelial adhesion molecules. *Exp Clin Endocrinol Diabetes* 106: 183-188, 1998.

4. **Fasching P, Waldhausl W, Wagner OF**: Elevated circulating adhesion molecules in NIDDM-potential mediators in diabetic macroangiopathy (Letter). *Diabetologia* 39: 1242-1244, 1996.

5. **Newman W, Beall LD, Carson CW, Hunder GG, Graben N, Randhawa ZI, Gopal TV, Wiener-Kronish J, Matthay MA**: Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock. *J Immunol* 150: 644-654, 1993.

6. **Pigott R, Dillon LP, Hemingway IH, Gearing AJ**: Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem Biophys Res Commun* 187: 584-589, 1992.

7. **Steiner M, Reinhardt KM, Krammer B, Ernst B, Blann AD**: Increased levels of soluble adhesion molecules in type 2 (non-insulin dependent) diabetes mellitus are independent of glycaemic control. *Thromb Haemost* 72: 979-984, 1994.

8. **Kvasnicka J, Skrha J, Perusicova J, Kvasnicka T, Markova M, Umlaufova A, Pecen L**: Haemostasis, cytoadhesive molecules (sE-selectin and sICAM-1) and inflammatory markers in non-insulin dependent diabetes mellitus (NIDDM). *Sb Lek* 99: 97-101, 1998.

9. **Kado S, Nagata N**: Circulating intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 46: 143-148, 1999

10. **Devaraj S, Jialal I**: Low-density lipoprotein postsecretory modification, monocyte function, and circulating adhesion molecules in type 2 diabetic patients with and without macrovascular complications: the effect of alpha-tocopherol supplementation. *Circulation* 102: 191-196, 2000.

11. **Lim SC, Caballero AE, Smakowski P, LoGerfo FW, Horton ES, Veves A**: Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals without microalbuminuria. *Diabetes Care* 22: 1865-1870, 1999.

12. **Bannan S, Mansfield MW, Grant PJ**: Soluble vascular cell adhesion molecule-1 and E-selectin levels in relation to vascular risk factors and to E-selectin genotype in the first-degree relatives of NIDDM patients and in NIDDM patients. *Diabetologia* 41: 460-466, 1998

13. **Leena Ryysy, Hannele Yki-Järvinen**: Improvement of glycemic control by 1 year of insulin therapy leads to a sustained decrease in sE-selectin concentrations in type 2 diabetes. *Diabetes Care*, volume 24, number 3, march 2001.

14. **Yki-Järvinen H, Ryysy L., Nikkilă K, Tulokas T, Vanamo R, Heikkilă M**: Comparison of bedtime insulin regimens in patients with type 2 diabetes mellitus: a randomized, controlled trial. *Ann Intern Med* 130: 389-396, 1999.

15. **Caballero AE**: Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease, *Obes Res* 11: 1278-1289, 2003.

16. **Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD**: Insulin- mediated skeletal muscle vasodilation is nitric oxide dependent. *J Clin Invest* 94: 1172-1179, 1994.

17. Steiberg HO, Baron AD: Vascular function, insulin resistance and fatty acids. *Diabetologia* 45: 623-34, 2002

18. Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK, He J: The metabolic syndrome and chronic kidney disease in U.S. Adults. *Ann Intern Med* 140 (3): 167-174, 2004.

19. Gerstein HC, Mann JFE, Yi Q, Zinman B, Dinneen SF, Hoogwerh B, Halle JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S, for the Hope Study Investigators: Albuminuria and risk of cardiovascular events, death and heart failure in diabetic and nondiabetic individuals. *JAMA* 286: 421-426. 2001.

20. ADA recommendation 2011, Standards of Medical Care in Diabetes- *Diabetes Care* Volume 34, supplement 1, January S13- 39, 2011

21. Nyberg F, Stephansson E: Elevated soluble E-selectin in cutaneous lupus erythematosus. *Adv Exp Med Biol* 455: 153-159, 1999.

22. Yamane K, Ihn H, Kubo M, Yazawa N, Kikuchi K, Soma Y, Tamaki K: Increased serum levels of soluble vascular cell adhesion molecule 1 and E-selectin in patients with localized scleroderma. *J Am Acad Dermatol* 42: 64-69, 2000.

23. Kitamura T, Tamada Y, Kato M, Yokochi T, Ikeya T: Soluble E-selectin as a marker of disease activity in pustulosis palmaris et plantaris. *Acta Derm Venereol* 79: 462–464, 1999.

24. Zhang GJ, Adachi I: Serum levels of soluble intercellular adhesion molecule-1 and E-selectin in metastatic breast carcinoma: correlations with clinicopathological features and prognosis. *Int J Oncol* 14:71-77, 1999.

25. Available at www.nhlbisupport.com/bmi/bmi-m.htm

26. Cominacini L, Fratta PA, Garbin U,Campagnola M, Davoli A, Rigoni A, Zenti MG, Pastorino AM, Lo CV: E-selectin plasma concentration is influenced by glycaemic control in NIDDM patients: possible role of oxidative stress. *Diabetologia* 40: 584-589, 1997.

27. Marui N, Offermann MK,Swerlick R, Kunsch C, Rosen CA, Ahmad M,Alexander RW,Medford RM: Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant –sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 92: 1866-1874, 1993.

28. Măkimattila S, Liu-M-L, Vakkilainen J, Schlenzka A, Lahdenperă S, Syvănne M, Măntysaari M, Summanen P,Bergholm R,Taskinen M-R, Yki Jărvinen H: Impaired endothelium-dpendent vasodilatation in NIDDM: relation to LDL size, oxidized LDL and antioxidants. *Diabetes Care* 22: 973-981, 1999

29. Albertini JP, Valensi P, Lormeau B, Aurousseau MH, Ferriere F, Attali JR, Gattegno L: Elevated concentrations of soluble E-selectin and vascular cell adhesion molecule-1 in NIDDM: effect of intensive insulin treatment. *Diabetes Care* 21: 1008-1013, 1998.