PREDİYABETTE ERKEN ATEROSKLEROZ BELİRTECİ E-SELECTİN

E-SELECTIN AS AN EARLY ATHEROSCLEROSIS MARKER IN PREDIABETES

İlknur Öztürk ÜNSAL[1], Nujen Colak BOZKURT[2], Evrim CAKIR[3], Mustafa ÖZBEK[1], Erman CAKAL[1]

[1]Sağlık Bilimleri Üniversitesi, Dışkapı Yıldırım Beyazıt Eğitim Araştırma Hastanesi
[2]İstanbul Aydın Üniversitesi VM Medical Park Hastanesi
[3]Alanya Alaaddin Keykubat Üniversitesi Tıp Fakültesi

Ankara Eğt. Arş. Hast. Derg. (Med. J. Ankara Tr. Res. Hosp.) Cilt / Volume: 52 Sayı / Number: 2 Yıl / Year: 2019 ISSN:1304-6187
Sayfa/Page :95-100

Geliş Tarihi / Submitted : Nisan 2019 / April 2019 Kabul Tarihi / Accepted : Mayıs 2019 / May 2019

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-sixhealthy 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 yolaklarının 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 ever 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

Sorumlu Yazar / Corresponding Author:
İlknur Öztürk ÜNSAL
Çağdaş sok No:76/9 Aydınlıkevler 06130 Ankara, Türkiye
Tel: +90 312 596 29 22 **Gsm:** + 90 532 552 97 76 **E-posta:** ilknur_dr@yahoo.com

of diabetics will be 629 million and the number of peoplewith 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-selectinconcentrations 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 ofatherosclerosis. 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 thepatients 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: fasting plasma glucose (mmol/L) × 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. CIMTwas 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-intimainterface to the leading edge of the mediaadventitia. 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 levelswere 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	Control	p
	(n=70)	**(n=66)**	
Age (year)	46.27±9.50	43.87±10.20	0.160
Gender (%) Female Male	 29 (%41,4) 41(%58,6)	 38(%57,6) 28(%42,4)	0.086
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-selectinwere 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/m^2) versus the 10th percentile (BMI=20.1 kg/m^2) 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-1and 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 ratioin 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 andalso gives physicians a chance to determine new therapeutic goals. This might reduce the risk for development and appearance of disease-related potential complications.

Acknowledgements :None
Conflicts of interest:There are no conflicts of interest for all authors.

REFERENCES

1.) Cho NH, Kirigia J, Mbanya JC et al. IDF Diabetes Atlas. Eighth edition, 2017. www.diabetesatlas.org
2.)Gerstein HC, Santaguida P, Raina P, et al. Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic over view and meta analysis of prospective studies. Diabetes Res Clin Pract 2007;78:305–312.
3.)Chait A, Bornfeldt KE. Diabetes andatherosclerosis: is there a role for hyperglycemia? J Lipid Res 2009; 50: 335–339.
4.)Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events. Diabetes Care 1999;22:233– 240.
5.)Huxley R, Barzi F, Woodward M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. BMJ 2006;332:73– 78.
6.)Alexander CM, Landsman PB, Teutsch SM, Haffner SM, fort he NHANES II and NCEP Investigators. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes 2003;52:1210–1214.
7.)Libby P. Inflammation in atherosclerosis. Nature 2002, 19-26;420(6917):868-74.
8.)Albelda SM, Smith CW, Ward PA. Adhesion molecules and inflammatory injury. FASEB J. 1994;8:504–512.
9.)Bevilacqua MP, Stengelin S, Gimbrone MA Jr, Seed B. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 1989;243: 1160–1165.
10.)Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagner DD. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest 1998;102: 145– 152.
11.)Ballantyne CM, Mainolfi EA, Young JB et al. Relationship of increased levels of circulating intercellular adhesion molecule 1 after heart transplantation to rejection: human leukocyte antigen mismatch and survival. J Heart Lung Transplant. 1994;13:597–603.
12.)Nakai K, Itoh C, Kawazoe K et al. Concentration of soluble vascular cell adhesion molecule-1 (VCAM-1) correlated with expression of VCAM-1 mRNA in the human atherosclerotic aorta. Coron Artery Dis. 1996;6:497–502.
13.)Gearing AJH, Newman W. Circulating adhesion molecules in disease. Immunol Today. 1993;14:506–512.
14.)Blann AD, Farrell A, Picton A, McCollum CN. Relationship between endothelial cell markers and arterial stenosis in peripheral and carotid artery disease. Thromb Res. 2000; 97:209-216.
15.)Blann AD, Amiral J, McCollum CN. Circulating endothelial cell/leucocyte adhesion molecules in ischaemic heart disease. Br J Haematol.1996; 2:263-265.
16.)Porta B, Baldassarre D, Camera Met al. ; MIAMI Study Group. E-selectin and TFPI are associated with carotid intima-media thickness in stable IHD patients: the baseline findings of the MIAMI study. Nutr Metab Cardiovasc Dis. 2008;4:320-328.
17.)Hwang SJ, Ballantyne CM, Sharrett AR et al. E Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk InCommunities (ARIC) study. Circulation. 1997; 96:4219-4225.
18.)Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997;96:1432-1437.
19.)Knapp M, Lisowska A, Sobkowicz B, Tycinska A, Sawicki R, Musial WJ. perfusion and intima-media thickness in patients with subclinical hypothyroidism. Advances in MedicalSciences.2013; 58: 44-49.
20.)Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes 2019 American Diabetes Association. Diabetes Care 2019 Jan; 42(Supplement 1): S13-S28.
21.)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 1985;28:412–419.
22.)Hingorani AD, Cross J, Kharbanda RK et al. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. Circulation 2000; 102:994–999.
23.)Bacon PA, Raza K, Banks MJ, Townend J, Kitas GD. The role of endothelial cell dysfunction in the cardiovascular mortality of RA.Int Rev Immunol 2002; 21:1–17.
24.)Wang X, Zhang J, Du X, Song M, Jia C, Liu H. Association of A561C and G98T Polymorphisms in E Selectin Gene with Coronary Artery Disease: A Meta-Analysis. PLoSOne. 2013; 8: 79301.
25.)Squadrito F, Saitta A, AltavillaDet al. Thrombolytic therapy

with urokinase reduces increased circulating endothelial adhesion molecules in acute myocardialinfarction. Inflamm Res 1996;45: 14–19.
26.)Hwang SJ, Ballantyne CM, Sharrett ARet al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incidentcoronary heart diseasecases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1996; 4219–4225.
27.)Belch JJ, Shaw JW, Kirk Get al. The White blood cell adhesion molecule E-selectin predicts restenosis in patients with intermittent claudication undergoing percutaneous transluminal angioplasty. Circulation 1997;95:2027–2031.
28.)Bluher M, Unger R, Rassoul F et al. Relation between glycaemic control, hyperinsulinemia and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or type II diabetes. Diabetologia. 2002;45:210–216.
29.)Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA. 2004;291:1978–1986.
30.)Thorand B, Baumert J, Doring A et al. Association of cardiovascular risk factors with markers of endothelial dysfunction in middle-aged men and women. Results from the MONICA/KORA Augsburg Study. Thromb Haemost. 2006;95:134–141.
31.)Vischer UM. vonWillebrand factor, endothelial dysfunction, and cardiovascular disease. J ThrombHaemost. 2006;4:1186–1193.
32.)Tooke JE. Microvascular function in human diabetes. A physiological perspective. Diabetes. 1995;44:721–726.
33.)Rossi R, Cioni E, Nuzzo A, Origliani G, Modena MG. Endothelial-dependent vasodilation and incidence of type 2 diabetes in a population of healthy post menopausal women. Diabetes Care. 2005;28:702–707.
34.)Meigs JB, O'Donnell CJ, Tofler GH et al. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: the Framingham Offspring Study. Diabetes. 2006;55:530–537.
35.)Lyon CJ, Law RE, Hsueh WA. Mini review: adiposity, inflammation, and atherogenesis. Endocrinology. 2003;144:2195–2200.
36.)Ferrannini E, Gastaldelli A, Iozzo P. Pathophysiology of prediabetes. Med Clin North Am 2011;95:327–339.
37.)Tikellis G, Wang JJ, Tapp R et al. The relationship of retinal vascular calibre to Diabetes and retinopathy: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. Diabetologia 2007;50:2263–2271.
38.)Tapp RJ, Tikellis G, Wong TY, Harper Ca, Zimmet PZ, Shaw JE. Australian Diabetes Obesity and Lifestyle Study Group. Longitudinal association of glucose metabolism with retinopathy: results from the Australian Diabetes Obesity and Lifestyle (AusDiab) study. Diabetes Care 2008;31:1349 –1354.
39.)Sprague RS, Ellsworth ML. Vascular disease in pre-diabetes: new insights derived from systems biology. MoMed 2010;107:265–269.
40.)Singleton JR, Smith AG, Russell JW et al. Microvascular complications of impaired glucose tolerance. Diabetes 2003;52:2867–2873.
41.)Gabir MM, Hanson RL, Dabelea D et al. Plasma glucose and prediction of microvascular disease and mortality: evaluation of 1997 American Diabetes Associationand 1999 World Health Organization criteria for diagnosis of diabetes. Diabetes Care 2000;23:1113– 1118.
42.)Franklin GM, Kahn LB, Baxter J, Marshall JA, Hamman RF. Sensory neuropathy in non-insulin-dependent diabetes mellitus. The San Luis Valley Diabetes Study. Am J Epidemiol 1990;131:633-643.
43.)Reynolds HR, Buyon J, Kim M et al. Association of plasma soluble E-selectin and adiponectin with carotid plaque in patients with systemic lupus erythematosus. Atherosclerosis 2010; 210: 569–574.
44.)Zemlin AE, Matsha TE, Kengne AP, Hon GM, Erasmus RT. Correlation of E-selectin concentrations with carotid intima-media thickness and cardio-metabolic profile of mixed ancestry South Africans: a cross-sectional study. Ann Clin Biochem. 2017 Jan;54(1):92-100.
45.)Leinonen ES, Hiukka A, Hurt-Camejo E, et al. Low-grade inflammation, endothelial activation and carotid intima-media thickness in type 2 diabetes. J Int Med 2004; 256: 119–127.
46.)Babar G, Clements M, Dai H, Raghuveer G. Assessment of biomarkers of inflammation and premature atherosclerosis in adolescents with type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2019 25;32 (2):109-113.
47.)Carey VJ, Walters EE, Colditz GA et al. Body Fat Distribution and Risk of Non-lnsulin-dependent Diabetes Mellitus in Women The Nurses' Health Study. Am J Epidemiol 1997; 145:614-619.
48.)Hartge MM, Unger T,Kintscher U. The endothelium and vascular inflammation in diabetes. Diabetes and Vascular Disease Research 2007;4: 84-88.