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Coronary Microvascular Endothelial Dysfuction Initiates Obvious Coronary Artery Diseases in Patients with Diabetes Mellitus

Nursen Keles[1]*, Necibe Nur Keles[2], Soe Moe Aung[3] and Mustafa Caliskan[1]

[1]Department of Cardiology, Istanbul Medeniyet University, Turkey

[2]Uskudar Government Hospital Department of Neurology, Turkey

[3]Fatih University Department of Cardiology, Turkey

***Corresponding author:** Nursen Keles, Istanbul Medeniyet University, Goztepe Training and Research Hospital, Department of Cardiology, Doktor Erkin Caddesi, Kadıköy /Istanbul, Turkey, Tel: (90)507 987 7745; Fax: (90) 216 344 0093; Email: drnursenkeles@yahoo.com.tr

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ABSTRACT

Cardiovascular diseases are the most common cause of the mortality in patients with Diabetes Mellitus. The risk of cardiovascular mortality in patients with type 2 diabetes is higher than that of the general population. Coronary artery diseases may be related to the loss of the protective properties of the endothelium. An early and reversible event in the pathogenesis of atherosclerosis is endothelial dysfunction. Both *in vivo* and *in vitro* studies of small arteries and arterioles of diabetic subjects show impaired endothelial function without anatomic lesions.

Hyperglycemia, insulin, insulin resistance, inflammation and oxidative stress play roles in the mechanism of coronary microcirculatory endothelial dysfunction in patients with diabetes mellitus. Coronary flow reserve reflects coronary microvascular endothelial dysfunction and many studies demonstrated that coronary flow reserve could be measured with non- invasive methods by using both mutislice computed tomography and transthorasic Doppler echocardiography.

Coronary microcirculatory endothelial dysfunction promotes obvious coronary artery diseases in patients with diabetes mellitus and recent studies demonstrated that coronary microcirculatory endothelial dysfunction can be detected with non- invasive techniques in asymptomatic diabetic patients. Therefore; coronary microcirculatory endothelial dysfunction should be defined by non-invasive methods and should be taken early steps to reduce cardiovascular mortality in diabetic patients.

INTRODUCTION

Cardiovascular Diseases **(CVD)** are liable for up to 80% of the deaths in patients with type 2 diabetes mellitus **(DM)** [1]. The age-adjusted relative cardiovascular mortality risk in patients with type 2 DM is 3 times higher than that of the normal population [1,2]. A recent study revealed that CVD mortality was 7.5 times greater among type 2 patients without a previous myocardial infarction than in those without DM [1]. Moreover, the incidence of CVD mortality was 3 times higher in diabetes patients who had a history of myocardial infarction when compared to nondiabetic individuals.

The obstruction of epicardial coronary arteries was defined as the reason of angina pectoris more than two centuries ago, and sudden thrombotic blokage of an epicardial coronary artery has been determined as the culprit of acute myocardial infarction since over 100 years [3]. Coronary artery diseases **(CAD)** such as stable and unstable angina, acute myocardial infarction, peripheral artery disease, and stroke may be related to the loss of the protective properties of the endothelium, which in normal conditions preserves vascular tone, and inhibit thrombosis and inflammation [4]. An early and reversible event in the pathogenesis of atherosclerosis is endothelial dysfunction which is associated with increased vascular tone, arterial stiffening, and intimamedia thickness. Hirata et al.Recently reported that the severity of endothelial dysfunction was correlated with the risk of primary or recurrent cardiovascular events. Moreover, cardiovascular risk-reducing interventions such as physical exercise and alimentation also improve endothelial functions [5,6]. Thus, it is possible to consider the endothelial function as an indicator of cardiovascular health which can be used to guide patient management. The coronary microvasculature (vessels <300 μm in diameter) cannot be directly imaged *in vivo*, but a number of invasive and noninvasive techniques can be used to assess parameters of coronary microvascular function [7]. The coronary flow reserve **(CFR)** is one of the parameters used to assess endothelial at the level of the coronary circulation [8]. From a pathophysiological point of view, reduced CFR can result from the combination of different alterations such as impaired vasodilation, enhanced vasoconstrictor responsiveness, and/or structural remodeling of the coronary microvasculature.

Not only *in vitro* but also *in vivo* trials of small arteries and arterioles of diabetic subjects demonstrate impaired endothelial function without anatomic lesions. CFR is a surrogate marker of coronary microcirculatory endothelial function in diabetic patients without significant stenosis of the associated epicardial coronary artery.

CORONARY MICROVASCULAR DYSFUNCTIONETIOLOGY IN DIABETES

Hyperglycemia and Coronary Microvascular Dysfunction

Hyperglycemia plays clearly the major role in the pathopysiological mechanisms of complications of DM. It initiates both repeated acute alterations in intracellular metabolism including activation of the diacylglycerol **(DAG)-**protein kinase C **(PKC)** and polyol pathway, elevated oxidative stress, glycocalyx perturbation of endothelial cell [9] and cumulative long-term alterations in the macromolecule texture and function via developed glycation end products. In both diabetic and healthy individuals, endothelial-mediated vasodilation and flow-mediated dilation **(FMD)** are impaired by hyperglycemia [10-13]. Oxidative stress is increased by elevated activity of pro-oxidant enzyme and reduced production of antioxidant glutathione in endothelial cells, under hyperglycemia [14]. Both type 1 and type 2 DM; glycemic control indicating [15] the intervention of other factors is similar. However; undiagnosed high blood glucose with long term duration and lipid profile impairment or increased insulin resistance could be associated with type 2 DM. Moreover, good control of blood glucose in type 1 DM can rehabilitate the early acetylcholine-provoked endothelium-dependent function impairment [16]. On the other hand; that effect of good blood glucose control has never been demonstrated in type 2 DM.

The Effects of Insulin and Insulin Resistance in Endothelium-Mediated Dilation of the Vessels

Insulin receptors are expressed by endothelial cells. Insulin is associated with Nitric Oxide-dependent vasodilation in skeletal muscle [17]; Nitric oxide generation may be induced by insulin directly or indirectly via a second messenger. *In vitro* studies show that the L-arginine-nitric oxide vasodilator pathway is activated by insulin in men [18] and it also induces the release of endothelium-derived relaxing factors including prostaglandin I2 **(PGI2)** and nitric oxide. Increased L-arginine metabolism and decreased L-arginine recycling may reduce the L-arginine level for endothelial nitric oxide synthase **(NOS)**. Hein et al. [19] showed that enhanced arginase activity reduces L- arginine level to NOS for nitric oxide generation. Therefore; it inhibits nitric oxide-mediated arteriolar dilation in ischemia/reperfusion.

Insulin sensitivity is associated with acetylcholine-stimulated vasodilation in healthy population, thus insulin has an major role in the initial processes of endothelial function impairment [20]. It is fact that insulin resistance begins before the occurrence of obvious type 2 diabetes mellitus and is related with elevated plasma von Willebrand factor **(vWF)** level and endothelin in patients with obesity [21].

The insulin vasculature action might be reduced in insulin resistant situations including obesity [21], impaired glucose tolerance, type 2 diabetes mellitus and hypertension [22]. A recent study [23] showed that free fatty acid, which serum level was elevated in metabolic

syndrome, stimulates insulin resistance, inhibits activation of endothelial nitric oxide synthase and accordingly induces endothelial function impairment in cultured endothelial cells. Another study [24] supported that hypothesis in type 2 DM without complications by connecting insulin resistance with cellular glucose disposal faults.

Insulin Resistance-Mediated Vasodilation Impairment

A trial investigated the impact of hyper insulinemia on function of endothelium and demonstrated the tumor necrosis factor-α role in insulin-induced function of endothelium in humans [25] The inhibition of the forearm blood flow response to acetylcholine by tumor necrosis factor-α and the larger inhibitory effect of tumor necrosis factor-α during insulin infusion were found in the trial. The results indicate a major role of tumor necrosis factor-α in insulin-mediated impairment of endothelial function. Tumor necrosis factor-α also plays a role on signaling of intracellular insulin in skeletal muscle and fat that are insulin sensitive tissues. It inhibits activity of kinase in the initial part of the insulin signaling pathway [26,27].

An anologous signaling pathway causes to produce nitric oxide in vascular endothelium [28], which is necessary for insulin-induced vasodilation [29].

In a rat model of obesity-related insulin resistance impairment of vascular insulin signaling was reported [30]. Nitric oxide production and insulin signaling are inhibited by a short term exposure to tumor necrosis factor-α in cultured endothelial cells.

Peroxisome Proliferator-Activated Receptor-γ and The Improvement of Insulin Resistance

A member of the nuclear receptor family called as peroxisome proliferator-activated receptor-γ is a ligand-activated transcription factor [31]. The peroxisome proliferator-activated receptor-γ sets glucose and lipid metabolism. It is the target of insulin-sensitizers like thiazolidinediones [32].

Plasma levels of insulin and glucose are decreased by thiazolidinediones that stimulates long-term peroxisome proliferator-activated receptor-γ activation in patients with type 2 DM. The reduction of plasma levels of insulin and glucose provides recovery of vascular function impairment [33]. peroxisome proliferator-activated receptor-γ is also demonstrated in endothelial and vascular smooth muscle cells [34,35]. peroxisome proliferator-activated receptor-γ activators may use another mechanism to protect vascular function in diabetes mellitus, which is irrelevant to lipid and carbohydrate metabolits [36]. In additon, peroxisome proliferator-activated receptor-γ increases the release of nitric oxide in pulmonary artery of porcine and endothelial cells of human umbilical vein in culture [37]. In-vivo human studies showed that peroxisome proliferator-activated receptor-γ antagonists also ensured good outcomes. Coronary vasomotor alterations were healed by thiazolidinediones in Mexican American patients with insulin resistance [38]. Both myocardial glucose uptake and myocardial blood flow measured

by positron emission tomography were significantly improved by the addition of pioglitazone to traditional lipid-lowering treatment in insulin-resistant patients that had familial combined hyperlipidemia [39].

The Effects of Inflammation

Insulin resistance and diabetes mellitus in inflammatory diseases

Inflammation plays an important role in a lot of cardiovascular pathologies such as insulin resistance, endothelial dysfunction, metabolic syndrome and diabetes mellitus. The markers of inflammation including interleukin- 6 (IL-6), C reactive protein **(CRP)**, and tumor necrosis factor-α are associated with the mechanisms of insulin resistance. Tumor necrosis factor-α is one of the major inflammatory mediators released during various inflammatory situations [25] and can trigger a whole spectrum of inflammatory cytokines varying from interleukins to interferons. Many trials have investigated the predictive potentials of these cytokines in the evaluation of diabetes risk.

A recent study [40] demonstrated that the levels of IL-6, CRP and tumor necrosis factor-α are increased in the patients with insulin resistance syndrome. In another study [41] it was found similar associations among healthy middle-aged females. The insulin resistance and atherosclerosis study was performed on 1008 non-diabetic subjects without history of coronary artery disease [42]. It was found that CRP levels were not dependent of insulin resistance measured via a frequently sampled intravenous glucose tolerance test. Elevated CRP levels were directly connected with an enhancement of a number of components of the metabolic syndrome. These investigations propose that an increased acute phase reaction is related with insulin resistance and may induce the advancement of type 2 diabetes mellitus. These epidemiological data is supported by experimental observations that show the effects of hyperglycemia on several proinflammatory cytokines such as tumor necrosis factor-α and IL-6, both of which are partly released from fat tissues. In trials with rodents, glucose-stimulated insulin release from isolated pancreatic β cells is modified by IL-6 and it also decreases glycogen synthesis of hepatocytes in culture [43,44]. In humans, the exogenous implementation of recombinant IL-6 stimulates dose-dependent hyperglycemia and concordant increment in glucagons circulating levels [45].

Insulin resistance may be induced by tumor necrosis factor-α with various mechanisms such as direct inhibitory effects on the insulin receptor, insulin receptor substrates and the glucose transporter protein GLUT4 [46].

It has been found that tumor necrosis-α alters the intracellular insulin signaling in adipose tissue, skeletal muscle, and other insulin-responsive tissues via blocking kinase activity in the proximal part of the insulin-signaling pathway [26,27]. An analogous signaling pathway in vascular endothelium causes formation of nitric oxide, which is involved in insulin-induced vasodilation [28,29]. A study [30] showed in a comparable rat model that vascular signaling is

compromised. Insulin signaling and nitric oxide generation were inhibited by 10 min of exposure to tumor necrosis factor -α in cultured endothelial cells.

Tumor necrosis factor -α also plays a role in the coronary microvascular function impairment, and was firstly defined in the reperfusion-injury order via using a murine genetic model (Tumor necrosis factor-α overexpression mice, Tumor necrosis factor-α ++/++) [47]. A recent trial also represented that tumor necrosis factor-α also tended to be associated with coronary microvascular function impairment in not only overt diabetes but also pre-diabetic metabolic syndrome. It was also showed in an animal model of metabolic syndrome that over expression of could cause coronary endothelial dysfunction [48].

Is anti-inflammatory treatment necessary for diabetes therapies?

The prospective data is not enough for evaluation of the association between subclinical chronic inflammation and the type 2 DM incidence in spite of significant evidence. The Atherosclerosis Risk Communities Study showed that markers of inflammation including fibrinogen, white blood cell count and low serum albumin [49] and inflammation-associated hemostasis factors like vWF and factor VIII [50] were related with the risk of type 2 DM. On the other hand, these associations generally disappeared after adjustment for obesity. Pradhan et al. [51] also demonstrated the relation between high levels of CRP and interleukin-6 and the risk of type 2 DM in healthy middle-aged females. In the Women's Health Study, it was shown that increased CRP levels raised the risk of DM four times inthe study population who were followed for four years, through family history of DM, age-matched analyses on obesity control and other clinical risk factors.

The data from recent trials strengthens the connection between inflammation, atherosclerosis and diabetogenesis. Two large intervention trials of angiotensin-converting enzyme **(ACE)** inhibitors for the prevention of cardiovascular disease demonstrated that treatment with captopril and ramipril [52] significantly decreased the incidence of type 2 DM. Pravastatin using for the prevention of coronary artery disease was also related with a 30% decline in the type 2 diabetes risk [53].

It was hypothesized that these effects are strongly associated with decrement in subclinical inflammation. Angiotensin II stimulates IL-6 expression from both smooth muscle cells and macrophages, and is located in human atheroma with IL-6 [54]. Moreover, it has been found that long-term ACE-inhibition reduces CRP levels in patients with coronary artery disease [55]. The statin therapy also decreases CRP levels and help recovery of endothelial function [56].

The Effects of Oxidative Stress

Increment in the steady state levels of reactive oxygen species is described as oxidative stress. It commonly occurs when free radical formation enhances and/or antioxidant defense mechanisms reduce. Oxidative stress constitutes the final pathway for development of endothelial dysfunction in patients with diabetes mellitus. The mitochondria, xanthine oxidase, and NAD(P)H oxidase (NADPHO) are some of the intracellular sources for the generation of oxygen free radicals.

Mechanisms Reducing Antioxidant Mechanisms

Elevated blood glucose levels initate glycation and inactivation of antioxidant proteins including Cu/Zn superoxide **(SO)** dismutase. It causes inactivation or decline of antioxidant defense of these proteins [57]. Experimental studies demonstrated that concentrations of antioxidants like vitamin E, SO dismutase and catalase were decreased in streptozotocin-induced diabetic rats [58]. For instance, glutathione activity, which is effective to capture free radicals, is decreased when the NAD(P)H consumption rises [59]. The trials showed that the normal activities of SO dismutase (which captures O2.) and catalase (H2O2 inhibitor) protect the hyperglycemic patients against endothelial dysfunction.

The Effects of Increased Generation of Oxygen Free Radicals

A lot of trials propose that enhanced SO production mainly causes deficiency of nitric oxide in the diabetic patients vessels. NAD(P)H-dependent oxidases[60,61], xanthine [62] and mitochondrial oxidase, lipoxygenase and nitric oxide syntahse [63] are major sources of vascular SO formation. The production of reactive oxygen species at the transport chain level of mitochondria and oxidative stress are increased by hyperglycemia. A recent study [64] suggested that the one of the therapeutic goal in patients with DM may be oxidative activity of mitochondrium. At the same time, it has been found that the major source of SO formation is NAD(P)HO in some animal models of vascular disease such as DM [65]. NAD(P)HO activation and reactive oxygen species generation are stimulated by the increment in tumor necrosing factor -α expression, which causes endothelial function impairment in type 2 DM [66].

Enhanced SO generation mechanisms in human DM have been identified by Guzik et al. [67]. It was shown that vascular release of SO in diabetic patients is increased compared to SO release in normal population. They proved that endothelium is a obvious participant to whole SO formation in vessels. However; in arteries of non-diabetic patients, endothelium removal caused a significant increment in SO release. It promotes that endothelium plays a role in reduction of vascular SO release via formation of nitric oxide in those vessels. Moreover, the reduction of SO release because of endothelium removal in arterial segments from patients with DM indicates the major role of the endothelium in SO formation. The inhibiton of nitric oxide synthase in vasculature structure of diabetic patients also reduces SO release, which promotes that the net contribution of nitric oxide synthase activity in those vasculature structures is SO formation rather than NO generation. Moreover, they reported that sepiapterin (a tetrahydrobiopterin precursor) decreases SO formation in vascular structures of patients with DM. Therefore, the most important source of SO generation is NAD(P)HO in DM and the SO anion tends to decrease nitric oxide activity by direct scavenging. In patients with DM, the major source of SO is the endothelium owing to significant loss of endothelial nitric oxide synthase function, and an alteration from nitric oxide formation to SO generation. Actually, peroxynitrite, generated from nitric oxide and SO, directly oxidizes tetrahydrobiopterin to dihydrobiopterin. Dihydrobiopterin

is a biopterin that doesn't promote endothelial nitric oxide synthase enzymatic activity [68]. There are evidences proposing that competition between dihydrobiopterin and tetrahydrobiopterin for endothelial nitric oxide synthase binding may enhance nitric oxide synthase uncoupling. In addition, upregulation of vascular SO formation by NAD(P)HO may in turn lead to endothelial nitric oxide synthase uncoupling owing to oxidation of tetrahydrobiopterin, decreasing nitric oxide generation and further increasing endothelial SO formation. In an *in vitro* study , [69] it was reported that administration of the NAD(P)HO inhibitor apocynin repaired flow-induced vasodilation of coronary arterioles in mice with type 2 DM, proposing that NAD(P)HO is tend to be the major source of the increased SO generation in coronary microvascular structures. The data from another trial promotes the role of NAD(P)HO [70] by showing that endothelial function impairment in atherosclerosis is mediated, at least partially, through the interaction of oxidizing low density lipoproteins with its receptor, Lectin-like oxidized low-density lipoprotein receptor-1. This interaction subsequently causes endothelial production of SO by activation of NAD(P)HO. Recent studies have demonstrated different sources of SO anions [71,72]. They have suggested that an raised level of mitochondrial SO generated pending the high rate of glucose metabolism, may be liable for all high glucose associated processes. Bagi et al. [73] noticed that hyperglycemia-induced SO generation is decreased by either 2-DG (a competitive inhibitor of glycolysis) or the thenoyltrifluoroacetone availability (inhibitor of the mitochondrial complex II) in carotid arteries. In skeletal muscle arterioles, 2-DG is able to prevent the impairment of flow-induced dilation pending hyperglycemia. In addition, the thenoyltrifluoroacetone availability substantially alleviates the hyperglycemia-induced decrement in flow-mediated arteriolar dilation. These mechanisms reveal that an increased glucose metabolism (glycolysis and mitochondrial utilization) seems to cause more generation of SO in mitochondria of skeletal muscle arterioles pending hyperglycemia.

MECHANISMS THAT CAUSES CORONARY ARTERIOLAR VASOMOTION IMPAIRMENT

Myogenic Response

Microvscular structures can respond to transmural pressure alterations by dilation or constriction. Not only subendocardial arterioles but also subepicardial arterioles are able to give active myogenic responses. In a recent study, [74] it was shown that subepicardial arterioles represented enhanced constriction at high pressures and greater dilation responses at lower pressure. Alterations in local regulative mechanisms including myogenic response to pressure, have been suggested to conduce to the reduced dilatation of skeletal vessels in patients with type 2 DM. Therefore; myogenic reactivity affects coronary vascular resistance, increased myogenic tone may adversely influence arteriolar vasodilator function.

Endothelium-Dependent Nitric Oxide Mediated- Dilation

The intraluminal blood flow is one of the major local *in vivo* regulatory mechanism that affects arteriolar diameter. Increment in intraluminal blood flow stimulates endothelium-dependent vasodilation by releasing of vasodilator molecules like nitric oxide [75].

Frisbee et al. demonstrated that *in vivo* FMD in microvessels of skeletal muscle was significantly decreased compared to healthy control in obese Zucker rat with type 2 DM [76]. Another study [73] showed that acute hyperglycemia causes the decrement of nitric oxide-dependent FMD microvessels of skeletal muscle in healthy rats; transient increase in blood glucose level tended to reveal raised generation of SO which decreased the nitric oxide availability and the level of the nitric oxide synthase cofactor, tetrahydrobiopterin, thus revealing a decrement in flow-stimulated dilation of arterioles.

In diabetic animals it was shown that [77] in an *in vivo* beating heart, epicardial coronary arteriolar dilatator response to acetylcholine, the endothelium-dependent vasodilator, was decreased, when responses to sodium nitroprusside and adenosine were not impaired. Bagi et al. [73] demonstrated that in coronary arterioles of mice with DM, nitric oxide mediated flow and agonist-stimulated dilation was diminished.

Hypoxia-Induced Vasodilation

Hypoxia prominently stimulates endothelium-related vasodilation. Some animal trials have demonstrated that ATP-sensitive potassium channels **(KATP)** have an essential role in mediating vasodilation in both conduit and resistance arteries [78]. Miura et al. [79] defined the vasodilator response to hypoxia and the role of ATP-sensitive potassium channels in coronary arteries of patients with DM. They showed that opening of ATP-sensitive potassium channels causes hypoxia-induced vasodilation. The vasodilator response to both hypoxia and ATP-sensitive potassium channels excitation is disturbed in coronary microvessels of patients with type 1 and type 2 DM. The impairment mechanism of the vasodilation is not properly explained. The vasodilation impairment tends to be associated with only ATP-sensitive potassium channels, because no decrement is represented in relaxation of vascular smooth muscle cell following a reduction in intracellular Ca2+ concentration either by cGMP formation traceless to nitric oxide (sodium nitroprusside) or by hyperpolarization of membrane via activation of Ca2+-activated K+ channel. Accordingly, the decreased hypoxia-induced coronary arteriolar vasodilation causes the myocardial perfusion impairment in DM.

In a recent study it was demonstrated that in human coronary arterioles, DM inhibits endothelium-dependent dilatation,which the activation of small/ intermediate conductance of calcium-activated potassium channels mediate by clearly reducing in currents of endothelial the small/ intermediate conductance of calcium-activated potassium channels. The investigators concluded that endothelial dysfunction in patients with DM may be associated with inactivation of endothelial the small/ intermediate conductance of calcium-activated potassium channels channels [80].

Vasodilation during Increased Metabolic Requirement

Coronary arterioles are liable for setting of myocardial oxygen consumption and coronary blood flow [81], even though the related mechanism is not explained properly. Blood flow in coronary arterioles is able to enhace five to six-fold from basal values [82]. The primary mechanism of oxygen delivery to the myocardium pending higher demand is vasodilation of coronary arterioles.

Microvascular dilation varies considering the vessel size in the course of increased myocardial oxygen demand [81]. The microvascular dilation is inversely correlated to basal coronary diameter. In a study [83] it was noticed that nitric oxide contributes in coronary microvascular dilation when metabolic demand rises via rapid atrial pacing. In another study [84] it was shown that nitric oxide synthase inhibiton may prevent coronary microvascular dilation when myocardial oxygen demand enhances via a dobutamine and pacing control. On the other hand, some authors noticed that the increment in coronary blood flow related with enhanced myocardial oxygen demandis prevented by glibenclamide.It was shown that glibenclamide may play a role for ATP-sensitive potassium channels, which mediates faster coronary blood flow when oxygen demand is increased [85]. In a study on hyperglycemic dogs [86] it was reported that coronary blood flow is disturbed during enhanced myocardial oxygen demand. It has been showed that endothelium mediated dilation was only impaired pending hyperglycemia because only acetylcholine-mediated dilation is impaired while dilatory response is not altered pending administration of the nitric oxide-donor, sodium nitroprusside. It was also noticed that both endothelium-derived vasoconstrictors including prostaglandin H2, thromboxane A and and free radicals tends to cause dilation impairment pending hyperglycemia because reversal of either mechanism allows normal vasodilation to increase the metabolic consumption. Xu et al. [87] also verified that endothelial function impairment in atherosclerosis is intervened, at least in part, through the interaction of oxidizing low density lipoproteins with its receptor, lectin- like oxidized low-density lipoprotein receptor-1, which is liable for endothelial generation of SO radicals by activation of NAD(P) HO. The results of this study conduces to the advancement of novel adjunctive therapies using anti- oxidizing low density lipoproteins and/or anti lectin-like oxidized low-density lipoprotein receptor-1 antibodies or soluble receptors to prevent endothelial function impairment caused by atherosclerosis.

Recent studies has shown that coronary microvascular dysfuction is associated with worse cardiovascular outcomes [88-90]. Dikic et al. [88] demonstrated that coronary flow reserve that reflects coronary microvascular function acquired by transthoracic Doppler echocardiography ensure independent prognostic data for cardiovascular outcomes with calcium score obtained by multislice computed tomography in asymptomatic DM patients.

Kawata et al. reported that coronary flow reserve obtained by transthoracic Doppler echocardiography maintains independent prognostic data in asymptomatic patients with type 2 diabetes without obvious CAD [89].

Cardiovascular atherosclerosis is the most common cause of the mortality in patients with Diabetes Mellitus and coronary microcirculatory endothelial dysfunction initiates atherosclerosis in patients with diabetes mellitus. Recent studies reported that coronary microcirculatory endothelial dysfunction could be detected with non- invasive methods in asymptomatic diabetic patients. Therefore; coronary microcirculatory endothelial dysfunction should be defined by non-invasive techniques and should be taken early precautions to decrease cardiovascular mortality in diabetic patients.

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