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Original Article

Upregulation of SCUBE2 expression in dyslipidemic type 2 diabetes mellitus is associated with endothelin-1

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a major health problem for morbidity and mortality world-wide due to diabetic vascular complication. Following T2DM, dyslipidemia is known well for the main reason of vascular complication leading to atherosclerosis and impaired life expectancy in diabetes. Thus, a new prediction marker in T2DM could help prevent the progression disease despite of metabolic control. Signal peptide—CUB-EGF like containing protein 2 (SCUBE2), has been detected in vascular endothelium and was affected by cytokines. Recently, SCUBE2 was reported to increase in atherosclerotic human coronary artery, involving vascular smooth muscle cells (VSMCs) and macrophages. The aims of this study were to examine the expression level of SCUBE2 in T2DM patients with dyslipidemia and its correlation with endothelial dysfunction marker, endothelin-1 (ET-1) in this group. This study design was cross sectional control study, recruited 28 patients diagnosed as T2DM who were found with dyslipidemia and 15 healthy control subjects. Our results showed that T2DM patients showed higher LDL cholesterol, triglycerides, and ET-1 expression level compared to healthy subjects. Further, we found that SCUBE2 had strong correlation with ET-1 in these dyslipidemic T2DM patients. In conclusion, our study confirmed first that SCUBE2 was upregulated in T2DM with dyslipidemia. Moreover, Pearson correlation analysis of ET-1 and SCUBE2 in this group showed high correlation $r = 0.797$, $P < 0.001$, suggesting that SCUBE2 may plausible target in vascular function changes in dyslipidemic T2DM. Improving our exploration of these findings may lead to uncover SCUBE2 involvement in diabetic vascular complication in T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is widely known endocrine-metabolic disorder that associated with vascular endothelial and platelet function alterations precede atherosclerosis [1,2]. Endothelial dysfunction has received higher attention to contribute to vascular complication in T2DM. Both insulin resistance and dyslipidemia are more likely to link with early endothelial dysfunction. Cholesterol, especially low density lipoprotein (LDL) cholesterol and triglyceride have been considered as major risk factor for atherosclerosis susceptibility in T2DM. Typically small dense - LDL, and elevated triglyceride retained in intima and attract monocytes which differentiate into macrophages. Monocyte-derived macrophages released cytokines and chemokines further recruit immune cells cause endothelial dysfunction. However, impairment of endothelial function prior to hyperglycemia also induces oxidative stress, alteration of NOS expression and NO production, thus amplifies the production of vasoconstrictor endothelin-1 (ET-1) and further evokes the expression of proatherogenic gene expression. In addition, elevated ET-1 level induced from glucose metabolism changes also stimulate platelet aggregation, vascular smooth muscle cell (VSMC) proliferation and migration, and promote the progression of atherosclerosis [3,4]. Therefore, molecular

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mechanism of vascular dysfunction in T2DM remains to be elucidated. Hence the discovery of new molecule would help to tailor the preventive or therapeutic approaches to delay the diabetic vascular complication.

Signal peptide – CUB – EGF domain containing proteins 2 (SCUBE 2) is a secreted protein and a second family member of membrane-anchored protein which consists of 9 copies of EGF like repeats, spacer region, 3 cystein-rich region, and 1 CUB domain at the carboxyl terminus. SCUBE2 is expressed in vascular endothelium which is known to maintain pathophysiologic process of angiogenesis, inflammation and vascular diseases [5]. In vasculature, SCUBE2 detected in endothelial cells, vascular smooth muscle cells, and monocyte derived-macrophages. This gene also showed responsiveness to pro-inflammatory stimulation such as interleukin-1β (IL-1β) and tumor necroting factor (TNF)-α. Previous study reported that SCUBE2 was detected in diffuse intimal thickening (DIT) and advanced atherosclerotic plaque in human coronary artery. Immunohistochemistry from serial section of human coronary artery confirmed that SCUBE2 was distributed in vascular smooth muscle cells in DIT and strongly co-localized with macrophage in plaque lesion [6]. Moreover, upregulation of SCUBE2 induced vascular endothelial growth factor (VEGF) 2 signaling by binding to VEGF receptor (VEGFR) 2 and further induced angiogenesis [7].

Many studies suggested SCUBE2 expression in endothelial cell may play a crucial role in vascular functional changes. Based on several lines those evidences, current study aims to explore the expression of SCUBE2 in diabetes mellitus type-2. Hyperglycemia in T2DM has been postulated involved in endothelial cell dysfunction preceded vascular damage leading to progressed vascular complication in T2DM. Instead of hyperglycemia, T2DM also changed normal lipid profile into dyslipidemia in which both hyperglycemia and dyslipidemia orchestrated for macrovascular and microvascular complication in T2DM. Furthermore, we investigated the correlation between SCUBE2 and ET-1 expression, an endothelial dysfunction marker in this group.

2. Materials and methods

2.1. Patients and study design

This is a cross-sectional control study. We enrolled 15 healthy subjects as controls and 28 patients aged 40–60 years, diagnosed as T2DM according to American Diabetes Association (ADA) criteria which random plasma glucose (RPG) was greater than or equal to 200 mg/dL. Individuals with retinopathy, nephropathy, and cardiovascular disorders due to diabetic complication are excluded. Healthy subjects served as control subjects with no clinical diseases whose were undergoing medical check-up. Informed consents were obtained from all subjects and the protocol of the study was approved by the Ethics Committee of Faculty of Medicine, Andalas University.

2.2. Total cholesterol, LDL, trygliceride, and HDL

Serum from all subject were examined for lipid profile in Health Laboratory Office of West Sumatra, Indonesia.

2.3. RNA isolation and quantitative RT-PCR

Sodium Ethylene Diamine Tetraacetic Acid (EDTA) blood samples were collected from all subjects via venipuncture and processed with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) for RNA isolation and stored at −80 °C until further use. cDNA synthesis was performed with iSCRIPT cDNA synthesis kit (Bio-Rad, USA). SCUBE2 and ET-1 were confirmed using SsoFast Evagreen Supermix (Bio-Rad, USA) with Bio-Rad CFX96™ Real-Time PCR System. Primer for SCUBE2 as forward: AGA CCC CAG AAGCTT GGA ATA; SCUBE2 primer reverse, TCC CCT CCA CAT CTT CTG TTT; ET-1: primer forward, GCCTTGGAGAATTTACTTCC; ET-1 primer reverse, AAATTCCAGCACTTCTTGTC, and reference gene, GAPDH forward: ATG GGT GTG AAC CAT GAG AAG TA, reverse: GGC AGT GAT GGC ATG GAC.

2.4. Statistical analysis

Relative expressions of SCUBE2 and ET-1 in DMT2 patients were calculated by the 2-ΔΔCt formula and were expressed as mean ± SEM, with statistical significance $P < 0.05$. Furthermore, Pearson correlation and significance analysis between SCUBE2 and ET-1 were conducted by SPSS software with P value < 0.05 was considered statistically significant.

3. Results

3.1. Lipid profile of participants

Fifteen healthy controls and twenty eight T2DM patients were recruited for further random plasma glucose. Dyslipidemia has been known as a key feature of T2DM. Lipoproteins levels and ratio between them specifically change preceding diabetes [8]. Our study showed significantly higher total cholesterol, triglyceride, and LDL-C in T2DM subjects compared to healthy controls (Table .1). Total cholesterol/HDL ratio and LDL-C/HDL-C ratio also found higher in T2DM compared to healthy subjects, suggested for coronary heart disease (CHD) risk in T2DM as previously reported [9].

3.2. Increase ET-1 expression

Endothelin-1 (ET-1) has been known as a key factor in mediating vascular dysfunction and further induces several biochemical changes in the target organs contributing to disease progression [10,11]. Twenty eight T2DM patients participated in our study shown 3-fold higher expression of ET-1 compared to healthy controls (Fig. 1).

Table 1
Lipid profile of participants. We found that T2DM patients had dyslipidemia. Markedly higher triglyceride level in T2DM compared to healthy controls.

	Healthy controls	Type-2 Diabetes Mellitus	P value
Total Cholesterol (mg/dL)	184.07 ± 5.95	244.82 ± 7.205	<0.001*
Triglyceride (mg/dL)	163.36 ± 4.15	274.93 ± 24.12	<0.001*
HDL-C (mg/dL)	63.86 ± 0.94	60.54 ± 2.06	0.27
LDL-C (mg/dL)	87.43 ± 6.97	129.29 ± 5.94	<0.001*
Total CHO/HDL-C Ratio	2.90 ± 0.17	4.15 ± 0.12	<0.001*
LDL-C/HDL-C ratio	1.39 ± 0.12	2.23 ± 0.15	<0.001*

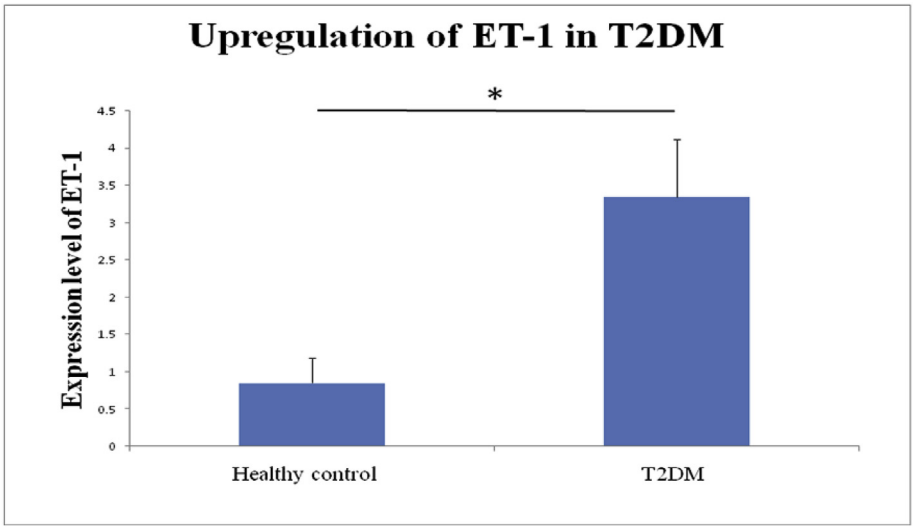

Fig. 1. Upregulation of ET-1 in T2DM. ET-1 alteration contributes to endothelial dysfunction in T2DM. ET-1 expression in T2DM patients showed higher compared to healthy controls.

3.3. SCUBE2 expression in T2DM

Previous study showed that SCUBE2 is expressed in vascular endothelium and its expression level was affected by proinflammatory cytokines [5]. Moreover, we observed SCUBE2 expression in T2DM patients. Our study showed an upregulation of SCUBE2 expression in T2DM participants (Fig. 2).

3.4. Correlation of ET-1 and SCUBE2

ET-1 and SCUBE2 were both expressed in vascular endothelium. Further, correlation of ET-1 and SCUBE2 in T2DM were assessed with Pearson correlation analyses. Upregulation of SCUBE2 is positively related with higher expression of ET-1 in T2DM patients implying for SCUBE2 involvement in vascular dysfunction in diabetes ($r = 0.797$; $P < 0.001$) (Fig. 3).

4. Discussion

In our study, we first confirmed that SCUBE2 expression was increased in T2DM patients with dyslipidemia and had a strong correlation with increased of ET-1 in this group. Our data showed T2DM patients had significantly higher lipid profiles compared to healthy controls. Dyslipidemia is associated with higher risk for cardiovascular diseases in T2DM. It has been postulated that dyslipidemia is a main contributor to atherosclerosis susceptibility in T2DM. High triglycerides, high LDL and insulin resistance amplify vascular function alterations further activate adhesion molecules and proinflammatory pathways. Conceptually, endothelial cell (EC) was the earliest cell underwent functional changes and primary target of tissue damage due to chronic hyperglycemia. ET-1 is known to augment with higher vascular endothelial growth factor (VEGF) mRNA expression in hyperglycemia, lead to increase vascular permeability as a feature for diabetic microangiopathy. Thus, increased production and activity of ET-1 contributed to vasoconstriction at early onset of diabetes and in long term, increased extracellular matrix production, urged a higher risk for diabetic complication [10–12].

SCUBE2 has previously been reported, expressed ubiquitously in vascularized tissues and detected in vascular endothelium. In vitro

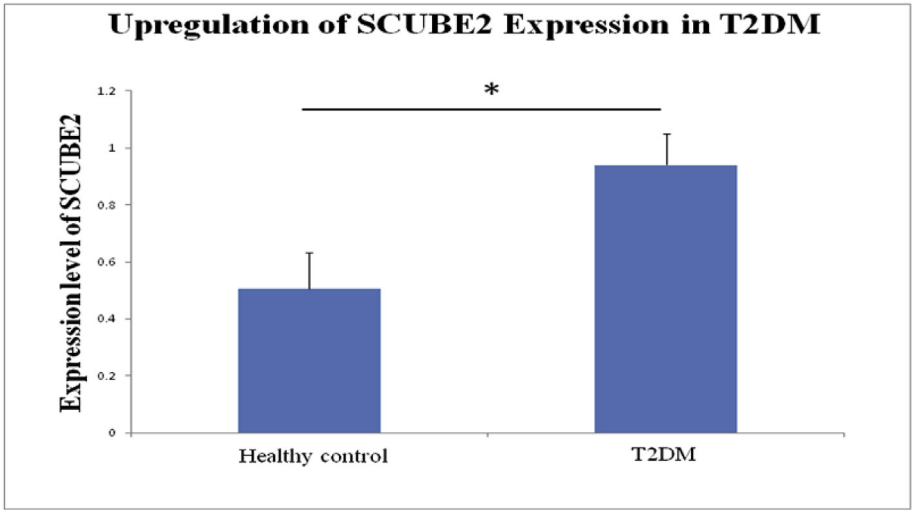

Fig. 2. Upregulation of SCUBE2 Expression in T2DM. SCUBE2 expression level showed 2-fold higher than healthy control subjects.

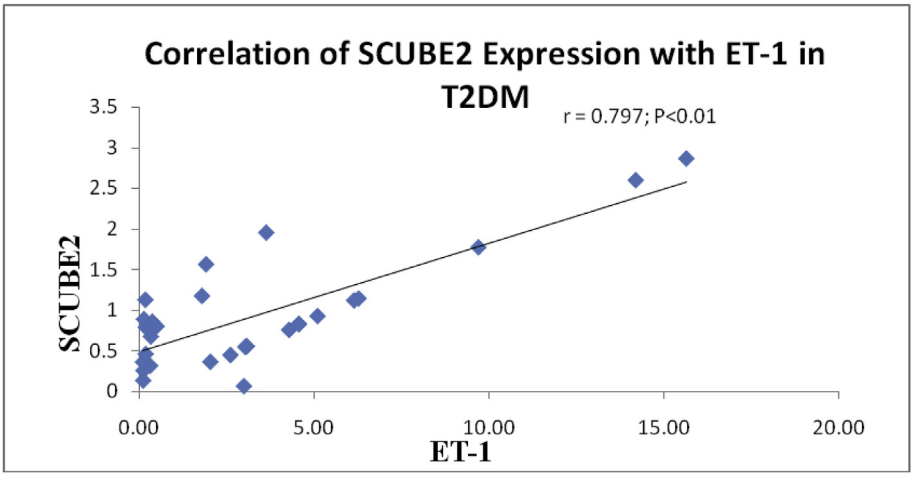

Fig. 3. Strong correlation of SCUBE2 and ET-1 in T2DM. Positive and strong correlation is found between SCUBE2 and ET-1 in T2DM patients.

study with human umbilical vein endothelial cells (HUVECs) showed that SCUBE2 expression is affected with proinflammatory cytokines, IL-1β and TNF-α [5]. Furthermore, in vivo study of vascular dysfunction in mice, 2 weeks after carotid artery ligation showed an increased mRNA level of SCUBE2 and markedly increased in LDLr−/− mice after 8 weeks fed with high fat diet as well. Moreover, SCUBE2 revealed in diffuse intimal thickening (DIT) of human coronary artery, where at this stage, SCUBE2 co-localized with vascular smooth muscle cells (VSMCs). As atherosclerotic plaque progressed, SCUBE2 expressed both in VSMCs and macrophages, implying for its role in inflammation leading to vascular dysfunction [6].

Recently reported, in vitro study with human umbilical vein endothelial cell (HUVEC), SCUBE2 expression increased in hypoxia condition. Interestingly, SCUBE2 directly bound to vascular endothelial growth factor receptor 2 (VEGFR2) which is mediated by hypoxia-inducible-factor-1α (HIF-1α) thus stimulated EC proliferation and induced angiogenesis [7,13]. It has been reported that HIF-1α induced angiogenesis in the presence of glucose. Exposure of glucose to cultured EC for 72 h showed increased glycolytic extracellular acidification rate and glycolytic capacity under the shear stress condition thus induced HIF-1α. The influence of HIF-1α–dependent glycolysis promotes EC proliferation and inflammation leading to atherosclerosis initiation [14].

In summary, we report for the first time that SCUBE2 expression upregulated in T2DM with dyslipidemia and had strong correlation with ET-1. SCUBE2 may provide a therapeutic target to prevent diabetic vascular complication. Many evidences reported that SCUBE2 expression in vascular endothelium may play a role in inflammation and other vascular function changes, thus the underlying mechanism linking SCUBE2 to other vascular inflammatory molecules in T2DM are further to be elucidated.

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