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Glycemic control as the main determinant factor of serum VEGF levels in type 2 diabetes mellitus patients

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Running head: Glycemic control determinant of VEGF in type 2 DM

Abstract

Introduction. Diabetes mellitus (DM) is a main endocrine disorder that may cause vascular complications as the disease progresses. Vascular endothelial growth factor (VEGF) has been linked to the development of micro and macrovascular diabetic complications. This study aimed to assess several factors including blood pressure, body mass index, lipid profile, kidney function, and glycemic control that may provide the rise of serum VEGF levels in type 2 DM subjects.

Methods. This cross-sectional study was carried out among 65 type 2 DM subjects. Systole, diastole, mean arterial pressure (MAP), and body mass index (BMI) were measured. The levels of serum VEGF were measured by Enzyme-linked immunosorbent assay (ELISA), Hemoglobin A1c (HbA1c) levels were measured by latex agglutination inhibition test, while serum glucose, lipid profiles, urea, and creatinine levels were tested by enzymatic photometric method.

Results. The levels of serum VEGF had a significant correlation with BMI ($p = 0.001$, $r = 0.397$), fasting plasma glucose (FPG) ($p = 0.001$, $r = 0.418$), HbA1c ($p < 0.001$, $r = 0.600$), systole ($p = 0.001$), $r = 0.397$), diastole ($p = 0.021$, $r = 0.286$), and MAP ($p = 0.001$, $r = 0.403$). Further multivariate linear regression analysis revealed that HbA1c logarithm (log) was the determinant factor of VEGF levels ($p < 0.001$, $\beta = 0.631$, Adjusted $R^2 = 38.9\%$).

Conclusion. HbA1c is the main determinant factor of serum VEGF levels among type 2 DM patients.

Keywords: VEGF, HbA1c, BMI, Blood Pressure, Lipid, Type 2 DM

What is new/what is important: Glycemic control, BMI, and blood pressure have a significant association with serum VEGF levels in type 2 DM. Among all, glycemic control (HbA1c) remains the main and only determinant factor of serum VEGF levels.

INTRODUCTION

The prevalence of type 2 DM has increased globally among developed and developing countries in the last three decades [1]. Chronic diabetes patients may develop complications, including microvascular and macrovascular ones, leading to elevated morbidity and mortality risks. Nephropathy, neuropathy, and retinopathy are the common complications of microvascular disease, while peripheral artery disease, stroke, and cardiovascular disease are major complications of macrovascular disease [2]. Diabetic complications develop due to several mechanisms involving the role of growth factors, including VEGF, insulin-like growth factor (IGF), transforming growth factor-β (TGF-β), and others [3].

VEGF is a family of platelet-derived growth factors (PDGF) that has an essential part in angiogenesis and vasculogenesis [4, 5]. Prediabetes and type 2 DM subjects have higher serum VEGF levels compared to normal subjects [6]. VEGF is also linked to several diabetic complications pathogenesis, including retinopathy and nephropathy [7]. It is reported that proliferative diabetic retinopathy subjects have higher serum VEGF levels compared to non-proliferative ones, in addition, nephropathy subjects with macroalbuminuria have higher VEGF levels compared to microalbuminuria subjects [8]. Serum VEGF levels are increased gradually along

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with the retinopathy progression in diabetic patients [9].

Serum VEGF levels have been shown to be higher in diabetic patients, however, only a few researches investigate the determinant factors of VEGF levels in diabetes. Therefore, in the current study, we evaluated the blood pressure, body mass index, age, lipid profile, kidney function, and glycemic control as determinant factors of serum VEGF levels in diabetic patients.

MATERIAL AND METHODS

STUDY POPULATION

This cross-sectional study was performed from March to April 2019. The research subjects were type 2 DM patients who went to the Internal Medicine Metabolic Endocrine Polyclinic at dr. Wahidin Sudirohusodo, Makassar, Indonesia. The inclusion criteria were people with type 2 DM aged over 18 years who agreed to participate in the research. The exclusion criteria were malignancy and having undergone hemodialysis. A total of 65 type 2 DM subjects were included in this research. Diabetes mellitus diagnosis was made according to the American Diabetes Association (ADA) 2019 criteria [10]. The ethical recommendation was gained from the Health Research Ethics Committee, Medical Faculty, University of Hasanuddin, Makassar, Indonesia, Number: 190/UN464531/PP36/2019.

LABORATORY PROCEDURE

Height (m) and body weight (Kg) were evaluated for the research subjects, and BMI was quantified using the formula body weight/(height)^2. Blood pressure was evaluated from the patient's arm after resting for 15 minutes using a Riester mercury sphygmomanometer (Germany). Systolic and diastolic blood pressure were recorded, and MAP was determined using the following calculation: MAP = 1/3 (systolic + 2x diastolic).

Blood sampling was carried out in the morning after a fasting period of 8–12 hours using a vacutainer. Whole blood was taken with a tube containing ethylenediamine tetraacetic acid (EDTA) anticoagulant for HbA1c examination, blood samples were also taken with a tube without anticoagulant, then centrifuged to obtain serum. Total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting plasma glucose (FPG), urea, and creatinine were tested using serum sample. HbA1c and all chemical chemistry tests were performed using Abx Pentra 400, Horiba, USA. The rest of the serum samples were kept at –20^{o}C in aliquots and then used for serum VEGF testing using the ELISA method (Bioassay Technology Laboratory, China). The limit of detection for the VEFG test was 1.52 ng/L, the limit of quantification was 900 ng/L, and the intra-assay coefficient variation was 2.7%. A microplate reader (Type 357, Thermo Fisher Scientific, Shanghai, China) was used to measure the absorbance of the ELISA test.

Measurements of BMI and blood pressure were carried out at the Internal Medicine Metabolic Endocrine Polyclinic, while clinical chemistry parameters were measured at the Central Laboratory of Dr. Wahidin Sudirohusodo, Makassar, Indonesia. The serum VEGF test was carried out at the Hasanuddin University Hospital Research Laboratory Unit.

STATISTICAL ANALYSIS

The Kolmogorov-Smirnov test was used to determine the normality of the data distribution. The correlation between VEGF levels and other parameters was tested with the Spearman test because VEGF was not distributed normally. Variables that had a correlation with VEGF levels (p-value < 0.25), were then analyzed in the multivariate linear regression. Variables that were not normally distributed were normalized in logarithmic form. The Statistical Package for the Social Sciences, Version 21.0 (SPSS Inc, Chicago, IL, USA) software was used to conduct the statistical analyses.

RESULTS

A total of 65 type 2 DM subjects, consisting of 33 (50.8%) female and 32 (49.2%) male subjects, were recruited in this research. The mean age of the research subjects was 56.35 ± 9.96 years. 29 (44.6%) subjects were controlled diabetes patients (HbA1c < 7%) while 36 (55.4%) subjects were uncontrolled ones (HbA1c $\geq$ 7%). Parameters that were normally distributed included MAP, total cholesterol, HDL-cholesterol, LDL-cholesterol, and age, while VEGF, HbA1c, urea, creatinine, triglycerides, systolic, diastolic blood pressure, and BMI were not distributed normally. The basic subject's features are pointed out in Table 1.

The relationship between serum VEGF and other parameters is shown in Table 2. It appeared that VEGF had a significant correlation with BMI,

FPG, HbA1c, systolic, diastolic blood pressure, and MAP ($p < 0.05$).

HbA1c was included in the multivariate analysis as a glycemic control marker because it had a stronger correlation power than FPG. MAP was also included as a blood pressure marker representing systolic and diastolic because it had a stronger correlation power. BMI and total cholesterol were also included in the analysis. Parameters that were not normally distributed were transformed into logarithmic form.

Table 3, after multivariate linear regression, showed that HbA1c was the only parameter that could explain 38.6% of serum VEGF levels.

Table 1

Features of subjects

Variables	Mean ± SD	Median
Age (Years)	56.35 ± 9.96	58
BMI (kg/m2)	25.34 ± 3.10	24.78
FPG (mg/dL)	167.23 ± 74.20	148
HbA1c (%)	7.61 ± 1.85	7.2
Urea (mg/dL)	33.86 ± 18.82	27
Creatinine (mg/dL)	1.14 ± 0.82	0.9
Total Cholesterol (mg/dL)	201.57 ± 46.34	197
HDL-Cholesterol (mg/dL)	47.29 ± 9.74	47
LDL-Cholesterol (mg/dL)	126.00 ± 38.37	125
Triglycerides (mg/dL)	151.17 ± 70.43	140
VEGF (ng/L)	473.85 ± 421.44	244.38
Systole (mmHg)	131.23 ± 15.26	130
Diastole (mmHg)	85.38 ± 7.92	90
MAP (mmHg)	100.67 ± 9.32	100

Table 2

Correlation between serum VEGF levels with the glycemic index, body mass index, kidney function, lipid profiles and blood pressure in type 2 DM patients

Variables	VEGF	
	r	p*
Age	–0.370	0.768
BMI	0.397	0.001
FPG	0.418	0.001
HbA1c	0.600	< 0.001
Urea	–0.044	0.728
Creatinine	–0.190	0.883
Total Cholesterol	0.166	0.185
HDL-Cholesterol	0.196	0.612
LDL-Cholesterol	0.078	0.537
Triglycerides	0.044	0.730
Systole	0.397	0.001
Diastole	0.286	0.021
MAP	0.403	0.001

* Spearman Test

Table 3

Multiple linear regression analysis between serum VEGF and body mass index, total cholesterol, and HbA1c in type 2 DM patients

	Variables	β	p
Model 1	Log HbA1c	0.661	< 0.001
Adjusted R2 = 37.5%	Log BMI	0.109	0.350
	Total Cholesterol	–0.085	0.443
	MAP	–0.074	0.583
Model 2	Log HbA1c	0.609	< 0.001
Adjusted R2 = 38.2%	Log BMI	0.114	0.326
	MAP	–0.084	0.442
Model 3	Log HbA1c	0.586	< 0.001
Adjusted R2 = 38.6%	Log BMI	0.094	0.405
Model 4	Log HbA1c	0.631	< 0.001
Adjusted R2 = 38.9%			

DISCUSSION

This study analyzed the effect of various parameters, including glycemic control, age, lipid profile, kidney function, blood pressure, and BMI, on serum VEGF levels among type 2 DM subjects. It was shown that serum VEGF levels had a significant positive correlation with BMI, glycemic control (HbA1c, FPG), and blood pressure (systole, diastole, MAP).

In this study, BMI was found to have a positive relationship with VEGF levels ($p = 0.001$, $r = 0.397$). BMI is one of the parameters commonly used to define obesity. Subjects with obesity will have a higher BMI. Obese subjects have more adipose tissue, including white adipose tissue (WAT). WAT has an important feature in the storage of lipids and energy, besides that, it also acts as a tissue that produces various cytokines, hormones, and growth factors including VEGF [11]. VEGF is expressed by adipose tissue and is essential for triggering neovascularization in adipose tissue [12]. Increased serum VEGF levels were also found in the obese healthy young men population compared to the low and normal-weight ones, and VEGF levels increased with increasing BMI [13].

Serum VEGF was found to have a positive correlation with glycemic control, both with FPG ($r = 0.418$, $p = 0.001$) and HbA1c ($r = 0.600$, $p < 0.001$). This finding is in concordance with the study reported by Zhang *et al.*, which reported that VEGF correlated with fasting blood glucose ($r = 0.483$, $p < 0.001$) and HbA1c ($r = 0.531$, $p < 0.001$) levels in healthy people and Type 2 DM subjects. [14], while another study reported the opposite, which finding no significant correlation between HbA1c and FPG with VEGF ($p > 0.05$) [15]. Chronic increases in blood glucose levels in diabetes mellitus subjects can trigger an increase in VEGF production through various pathways. Hyperglycemia in diabetes will produce advanced glycation end products (AGEs), which trigger VEGF production and release. Increased synthesis of reactive oxygen species (ROS) in diabetes and angiotensin II (Ang II) in diabetic nephropathy also stimulate the increased synthesis of VEGF [16].

Serum VEGF also correlated with blood pressure, including systolic ($r = 0.397$, $p = 0.001$), diastolic ($r = 0.286$, $p = 0.021$), and MAP ($r = 0.403$, $p = 0.001$). A similar finding was also found by Zorena *et al.*, who reported that serum VEGF levels in type 1 DM children and adolescents with hypertension were higher than the subjects without hypertension, which had a positive correlation with systole ($r = 0.4$, $p = 0.0004$) and diastole ($r = 0.2$, $p = 0.041$) [17]. The role of hypertension in increasing VEGF levels in DM patients is still unclear. Possibly, this can be caused by a complex process in diabetes. Prolonged and chronic hyperglycemia causes the formation of AGEs, which trigger an increase in VEGF production and can trigger hypertension indirectly. The interaction between VEGF and its receptors on the vascular endothelium can trigger a complex process that results in fibrosis, vascular stiffness, and impaired blood flow, which in turn can lead to hypertension. Oxidative stress, mitochondrial dysfunction, and renin-angiotensin-aldosterone system activation in diabetes can trigger vascular stiffness and lead to hypertension [17, 18].

Although total cholesterol was not significantly correlated with VEGF in this study, it was included in the multiple linear regression analysis ($p < 0.25$). Mondal *et al.* reported that total cholesterol levels had a significant association with plasma VEGF levels in diabetic retinopathy subjects ($r = 0.524$, $p = 0.014$). This suggests that increased blood lipid levels might have a role in endothelial dysfunction and stimulate VEGF production and release [19]. Zhang *et al.* also reported that levels of serum VEGF were increased in the DM group with high total cholesterol compared to those with normal total cholesterol [20].

Multiple linear regression analyses including HbA1c, BMI, total cholesterol, and MAP parameters showed that HbA1c as a glycemic control parameter was the only variable that played a role in explaining 38.9% of the variability in serum VEGF levels. This could be because blood pressure and lipid profile are related to glycemic control and may not have a direct effect on VEGF levels. Zorena *et al.* reported that systolic blood pressure, HbA1c, and diabetes duration significantly contributed to explaining 30% of the variation in serum VEGF in type 1 DM children and adolescent patients [17]. The other study reported that among type 2 DM patients, HbA1c levels and the ratio of type 1 and type 2 helper T cells (Th1/Th2) contributed to 33.2% of VEGF level variation [14]. This difference could be due to differences in the type of diabetes population and differences in age among the subjects in our study.

This research is one of the few studies that report the role of blood pressure, body mass index, age, lipid profile, kidney function, and glycemic control on serum VEGF levels among type 2 DM subjects. In conclusion, we revealed that HbA1c is

the main determinant factor of VEGF levels in type 2 DM subjects and may explain 38.9% of the variability of serum VEGF levels. The limitation of this study is that the current study design cannot explain the causality between variables. We also did not analyze the duration of diabetes because of the lack of accurate data on exactly when subjects had diabetes due to late diagnosis in developing countries. The other limitation was that the diabetes complications were not evaluated in this study, and thus their effect on VEGF levels could not be determined. The effects of oral and insulin medications were also not evaluated because several subjects did not have regular treatments.

Introducere: Diabetul Zaharat (DZ) este o boală enodcrină asociată cu complicaţii vasculare. VEGF a fost legat de dezvoltarea complicaţiilor micro şi macrovasculare. Scopul studiului a fost de a evalua mai mulţi factori asociaţi cu creşterea nivelurilor serice ale VEGF la pacienţii cu diabet zaharat tip 2.

Metode: A fost realizat un studiu transversal pe 65 de pacienţi cu diabet zaharat tip 2, fiindu-le măsurate BMI şi valorile tensionale. Nivelurile serice ale VEGF au fost evaluate prin ELISA. Nivelul hemoglobinei glicozilate a fost evaluate prin test de aglutinare la latex iar analizele uzuale prin tehnici fotometrice.

Rezultate: Nivelurile serice ale VEGF s-au corelat cu BMI ($p = 0.001$, $r = 0.397$), glycemia a jeun ($p = 0.001$, $r = 0.418$), HbA1c ($p < 0.001$, $r = 0.600$), tensiunea arterială sistolică ($p = 0.001$), $r = 0.397$), diastolică ($p = 0.021$, $r = 0.286$) şi medie ($p = 0.001$, $r = 0.403$). Analiza de regresie liniară multivariate a arătat ca nivelul HbA1c s-a asociat independent cu VEGF circulant ($p < 0.001$, $\beta = 0.631$, R^2 ajustat = 38.9%).

Concluzii: HbA1c este principalul factor determinant pentru nivelurile serice ale VEGF la pacienţii cu DZ tip 2.

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