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The Effect of Hypoxia Inducible Factor -1 Alpha and Vascular Endothelial Growth Factor Level in Type 2 Diabetes Microvascular Complications and Development

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ABSTRACT

Background: Angiogenesis in diabetic patients is often caused by hyperglycemia induced by hypoxia **Objective:** The aim of this study was to analyze the serum level of Hypoxia Inducible Factor -1α (HIF-1α) and Vascular Endothelial Growth Factor (VEGF) between March until Desember 2020. **Methods:** This is a cross-sectional analytic methods, 135 patients with Type 2 Diabetes 48 samples with Microvascular complication and 87 samples with non-microvascular complication were recruited from the various primary health care centers in Medan city and surrounding areas in North Sumatera. VEGF levels and HIF-1α tested were done with ELISA methods in the laboratory of Medical Faculty, Universitas Sumatera Utara. Statistical analysis was performed using the IBM SPSS Statistics version 24. The significance level was set up to 0.005. **Results**: The median HIF-1 levels in patients with microvascular complications were lower than those without microvascular complications, with a range of HIF-1α values in non-complicated samples (0.02-13.96) ng/ml and a range of HIF-1α values in vascular complications (0.52- 8.87) mg/dL. There was a significant difference in HIF-1α levels in patients with Type-2 DM with complications compared to those without complications ($p<0.05$). Median VEGF levels were higher in complicated Type-2 DM. There was no difference in VEGF levels in patients with Type-2 DM with complications compared to those without complications ($p > 0.005$). **Conclusion:** HIF-1α and VEGF levels showed the development in vascularity. With the higher level of HIF-1α, an increase in VEGF levels were found, indicating the angiogenesis is occurring. Although complications have not yet occurred, it is predicted that high VEGF values will cause vascular complications in the future.

Keywords: type 2 diabetes mellitus, blood sugar level, Hba1c, lipid profile, VEGF, HIF-1α .

1. BACKGROUND

The prevalence of Diabetes mellitus will increase rapidly and grow faster worldwide, estimating the IDF will increase from 425 million people worldwide in 2017, to 629 million in 2045 (1). Diabetes and its associated complications are a fairly large societal problem, where they may cause high mortality and also health rates (2). Hyperglycemia is a major factor causing endothelial dysfunction in patients with diabetes mellitus as a major determinant of the occurrence of chronic diabetes complications (3).

Chronic hyperglycemia can lead to disruption of oxygen homeostasis resulting in tissue hypoxia (4). During this hypoxia, the hypoxia-inducible factor (HIF)-1α is the core regulatory factor of adaptive responses. Meanwhile the Vascular endothelial growth factor (VEGF) is multi - tasking cytokine known to increase vascular permeability and vasodilatation and which stimulates differentiation, survival, migration, proliferation (5, 6), tubulogenic and vascular permeability in endothelial cells. The expression of VEGF can be induced by hypoxia through HIF-1 (hypoxia-inducible factor-1), as well as by IGF-1 and TGF-β1 (7). Moreover, there is evidence that VEGF is involved in the pathogenesis of cancer, arteriosclerosis, obesity, and diabetes mellitus-related complications such as diabetic retinopathy. The synthesis and secretion

of VEGF is affected by several variables in vitro, VEGF is up-regulated by hypoxia as well as by hyper- and hypoglycemia (8). The persistent elevation of HIF-1α level enhances body glycolysis and erythrocytosis, weakens mitochondrial metabolism and causes blood thickening thereby increasing VEGF and protein in cells due to hypoxia and glucose significantly blunting hypoxic VEGF regulation and the occurrence of microvascular complications in diabetes mellitus (9, 10).

2. OBJECTIVE

The aim of the study were threefold: a) to analyze the level of hypoxia inducible factor -1 alpha and Vascular Endothelial Growth Factor at type 2 diabetes mellitus with microvascular complication; b) to analyze the level of hypoxia inducible factor -1 alpha and Vascular Endothelial Growth Factor at type 2 diabetes mellitus without microvascular complication; c) to compare the level of hypoxia inducible factor -1 alpha and Vascular Endothelial Growth Factor at type 2 diabetes mellitus with and without microvascular complication.

3. PATIENTS AND METHODS

Participants

This study was a cross-sectional analytic methods carried out in primary health care centers in January until Desember 2020 in Medan city and surrounding areas in North Sumatera, Indonesia. Of 135 patients diabetes 48 with microvascular complication and 87 without complication. All the samples were examined the examination laboratory, such as the blood-sugar levels (BSL), glycated hemoglobin (HbA1c) levels, lipid profiles such as cholesterol, LDL, HDL, Triglycerides in the Paramita Laboratory Clinic, and examination of the VEGF levels and Hypoxia-Inducible Factor -1α with ELISA methods in the Integrated laboratory in Medical Faculty, Universitas Sumatera Utara. The inclusion criteria of the samples were all the patients diagnosed with type 2 diabetes mellitus, both the sexes, while the exclusion criteria of the samples were patients with type 1 diabetes mellitus and severe disease.

Procedure and Ethical considerations

The Ethical Committee of Universitas Sumatera Utara approved of the study protocol, with number 90/KEP/USU/2020. Additionally, the study was conducted after review and written approval by the Administrational and Scientific Society of primary health care centers in North Sumatera, Indonesia. The researcher informed each participant about the purpose of the study. Furthermore, all participants were informed of their rights to refuse or to discontinue their participation, according to the ethical standards of the Helsinki Declaration of 1983. Participation in the study was contingent on individual verbal consent.

Statistical analysis

The data were analyzed statistically via the SPSS software version 24.0 (SPSS Inc., Chicago, Illinois). All the variables in this sample of the study were tested by Shapiro–Wilk, the normal distribution variables ($p > 0.005$) were tested by parametric test, but the abnormal distribution variables ($p < 0.005$) were tested by Not Parametric test, Mann-Whitney test.

4. RESULTS

Demographic and Clinical Characteristics

We evaluated clinical and laboratory findings in 135 patients with Type 2 Diabetes Mellitus. Of the total number of subjects, 32.6% (44) were males, and 67.4% (91) of the subjects were females. Microvascular complication was found in 48 samples (20 males and 28 females), and 87 samples were found to have non-microvascular complication (24 males and 63 females). For the microvascular-complication group, the average age was 58 years old with the interval of 44 -79 years, whereas 56 years old was the median age for non-microvascular samples with the interval of 35-78 years. The minimum BMI of the population at microvascular complication group was 17.63 kg/m2, and for the non-microvascular group, a maximum of BMI was 46.44 kg/m2 with a median of BMI 24.25 kg/m2. The minimum BMI of non-microvascular group was 18.21 kg/m2 and the maximum BMI 46.44 kg/m2. As it is displayed in the characteristics table, there is no difference in ages, BMI, blood pressure systolic and diastolic, FBS and Hba1C. However, there are differences in the duration of diabetes for microvascular complications in Type-2 DM

	Samples	N	Median	P value
Age (years)	Microvascular Complication	48	58(44-79)	0.346
	Non-Microvascular Complication	87	56 (35-78)	
BMI (kg/m^2)	Microvascular Complication	48	24.25 (17.63-46.44)	0.05
	Non-Microvascular Complication	87	25.26 (18.21-46.44)	
Systole (mmHg)	Microvascular Complication	48	156 (110-209)	0.069
	Non-Microvascular Complication	87	140 (98-216)	
Diastole (mmHg)	Microvascular Complication	48	84 (68-111)	0.539
	Non-Microvascular Complication	87	84 (60-113)	
FBS (mg/dL)	Microvascular Complication	48	250 (73-610)	0.074
	Non-Microvascular Complication	87	203 (80-610)	
Hba1C (%)	Microvascular Complication	48	9.2 (4.7-15.20)	0.204
	Non-Microvascular Complication	87	8.2 (5-13.40)	
Duration of Illness (years)	Microvascular Complication	48	7.5 (1-30)	0.00

Table 1. Data Characteristic of the samples (N = 135)

patients. The type-2 DM patients with microvascular complications experienced longer disease than those without complications, with a p value of <0.05. Hence, there was a difference in the length of time suffering from diabetes mellitus for the presence of microvascular complications compared to those who have not experienced microvascular complications (Table 1).

Based on the Table 1, it was found that the mean cholesterol level in patients with and without the microvascular complications were found to have a slightly higher by SD value (50.19), the mean value was slightly higher in non-complications. Nevertheless, the statistical analysis showed that there was no a significant difference (p value = 0.468). The other markers, such as the LDL, HDL and TG levels, were found to have no significant difference in both microvascular complications and non-complicated patients (Table 2). The VEGF levels that we encountered in this study had a higher median value in the non-complicated microvascular Type-2 DM patients compared to those with microvascular complications, with a p value (> 0.05). This means that there was no significant difference in VEGF levels in patients with Type-2 DM with complications and without microvascular complications. The median HIF-1 levels in patients with microvascular complications were lower than those without microvascular complications, with a range of HIF-1α values in non-complicated samples (0.02-13.96) mg/dL and a range of HIF-1α values in vascular complications (0. 52- 8.87) mg/dL. There was a significant difference in HIF-1α levels in patients with Type-2 DM with complications compared to those without complications (p<0.05) (Table 3).

	Samples	N	Mean ±SD	Median	P value
Cholesterol (mg/dL)	Microvascular Complication	48	208.13 ±50.19		0.468
	Non-Microvascular Complication	87	213.29 ±42.28		
LDL (mg/dL)	Microvascular Complication	48		113.50 (64-213)	0.196
	Non-Microvascular Complication	87		126 (50-259)	
HDL (mg/dL)	Microvascular Complication	48		47 (24-77)	0.987
	Non-Microvascular Complication	87		46 (25-77)	
TG (mg/dL)	Microvascular Complication	48		209.50 (83-662)	0.594
	Non-Microvascular Complication	87		192 (49-1157)	

Table 2.The Marker Metabolic of the samplesa

	Samples	N	Median	P value
VEGF (mg/dL)	Microvascular Complication	48	3163.03 (111.64-3274.70)	0.144
	Non-Microvascular Complication	87	75159.89 (262-75421.89)	
HIF-1α	Microvascular Complication	48	8.35 (0.52-8.87)	0.015
(mg/dL)	Non-Microvascular Complication	87	13.94 (0.02-13.96)	

Table 3. Level VEGF and HIF-1α of the samples

5. DISCUSSION

Diabetes mellitus is a metabolic disorder that is caused by a chronic hyperglycemia. In this study, the youngest sample was found to be 35 years old, and the longest duration of suffering from diabetes mellitus in the both samples was 30 years in the patients with no complications (p>0.05). There was a significant difference duration of illness with complication compared to those without complication (p<0.05), however; no significant differences were occurred in term of ages particularly for the type-2 DM patients with complication compared those without complication (p>0.05). It has been reported that the duration of suffering Type-2 DM was related to the complication, but not to the age (10). This study has found that no significance differences were found the average BMI value, the blood pressure, the FBS and the Hba1c in both patient group (Table samples). Based on our results, both of the groups (with and without complication) that the highest FBS value was 610 mg/dl. Thus, the hyperglycemia could be a risk factor that damage the endothelial as well as the blood vessels, while at the same time would secrete cytokines (11).

The VEGF is a growth factor that can induce angiogenesis in vascular endothelial cells, and it is a significant regulator of angiogenesis in both physiological and pathological conditions (12). The VEGF is also a key factor in the maintenance of normal endothelial function under physiological conditions, however abnormally high VEGF concentrations will cause aberrant angiogenesis (13).

In cultured endothelial cells, the VEGF has been proven to be induced by the elevated levels of glucose and advanced glycation end-products (14) and a study has found that VEGF was involved in the pathogenesis of diabetic complications (15). Our findings have found that the median VEGF level was higher in the patients without microvascular complications group compared to those with complications. However, statistically speaking, the analysis found no any differences in the VEGF levels in both groups. Another study has also found that plasma VEGF levels were reported to be higher in diabetic patients than in healthy control individuals (16). Moreover, it has been reported that VEGF levels in plasma were positively correlated with fasting blood glucose level, glycosylated hemoglobin (HbA1c) level, and via the multiple linear regression analysis showed that HbA1c ratio were the independent predictors of VEGF levels in Type-2 DM patients (17). Another study has also shown that there was an increase in serum VEGF levels in the group of samples with Insulin Glucose Tol-

erance (IGT) and the Type-2 Diabetes mellitus groups compared to those in the healthy sample group (18). These higher VEGF levels in patients without microvascular complications group could be an indicator that endothelial angiogenesis is still ongoing and carries a risk of microvascular complications. In a diabetic retinopathy which is a microvascular complication, an increase in the VEGF levels is a result of a reaction to retinal hypoxia or ischemia which is a response to maintain endothelial function and circulation as a result of the loss of pericytes and acellular capillaries (19).

In our study, we found a significant difference in HIF-1α levels in the microvascular complication group with the non-vascular complication group ($p<0.005$). However, it was found that the median HIF-1 al alpha level in the complication group was lower than the non-complicated group (Table 3). This indicates that the response of HIF-1 in the non-complicated group to tissue hypoxia is higher and may lead to a complication process due to the HIF-1 functions in maintaining molecular oxygen in a homeostatic state in the tissue. The oxygen is a key substrate for energy production in mitochondria and also a number of pathways for biochemical reactions in cells (20). In diabetes mellitus, there is a dysregulation of HIF-1α signaling resulting in impaired adaptive responses to hypoxia, which contributes to the development of diabetes and its complications (21). Stability and function of HIF-1 through a mechanism that is dependent or independent of the Prolyl hydroxylase domain protein (PHD) contributes to the development and also complications of diabetes mellitus. In this study, high levels of HIF-1α in the non-complicated group contributed to the occurrence of complications. As it is explained that a chronic hyperglycemia causes tissue hypoxia due to poor circulation so that HIF-1α directly, the condition of hyperglycemia may induce the expression of a large number of genes whose products modulate vascular function and angiogenesis in different cell types. Such VEGF is an important angiogenic factor in the initiation stage of vascular branching (22). Therefore, this shows that HIF-1α and VEGF are closely related to the occurrence of disease complications in diabetes mellitus (23).

Moreover, the chronic hyperglycemia also causes a dysfunction in endothelial cell and neovascularization defects induced by hypoxia in skeletal muscle, myocardium, skin and nerves. This is due the ischemia response that forms inadequate collateral blood vessels, thus; a compensation for decreased neovascularization elsewhere implies to a complication. The hyperglycemia-induced by hypoxia will induce VEGF which is the most potent proangiogenic growth factor, and plays a key role in angiogenesis process. Clinically, diabetes is associated with the failure of various organs, especially blood vessels which results in many complications over time.

The presence of diabetes complications can be in the form of microvascular and macrovascular. In this study VEGF levels were found to be higher in patients without microvascular complications group compared to those in vascular complications group. This illustrates that vascular complications are no longer occurred in the angiogenesis process, where the VEGF levels are no longer formed. Meanwhile, in the non-complicated group, the angiogenesis may still occur and VEGF could be still formed as the VEGF also acts as homeostasis. Various studies have stated that VEGF was involved in the pathogenesis of complications in diabetes mellitus, signaling the VEGF production which may cause microvascular complications. Moreover, there are other factors that can affect VEGF production, including hypoxia, gender, smoking, increased levels of lipids in the blood, inflammatory status, and activated stress axis. All of them can influence the VEGF synthesis and secretion; among them, the main physiological stimulus for VEGF expression is cellular hypoxia.

6. CONCLUSION

HIF-1α levels indicate a large area of hypoxia, meaning that the higher HIF-1α levels, the wider the hypoxic area. The VEGF level is an indicator of the presence a vascularized state due to the induction of HIF-1α. In conclusion, both the HIF-1α and VEGF levels can be used as an indicator that shows the severity of diabetes mellitus with a complication.

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