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Short Communication

Assessment of Hypoxia-Inducible Factor-1*α* and Vascular Endothelial Growth Factor Levels in Type 2 Diabetes Mellitus Patients in North Sumatera, Indonesia

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ABSTRACT

Chronic hyperglycemia in type 2 diabetes mellitus causes endothelial cell dysfunction vascularization disorder and due to hypoxic condition, marked by hypoxia inducible factor-1 α (HIF-1α) and promote neovascularization and induce the secretion of vascular endothelial growth factor (VEGF). This study was aimed at evaluating the correlation between the HIF-1α and VEGF levels in type 2 diabetes mellitus patients. A cross-sectional analytic method was employed in the research. Eighty-nine patients with type 2 diabetes mellitus from various public health clinics in Medan, North Sumatera, Indonesia were recruited. Body mass index, blood pressure, disease duration, family history, and medical treatment were recorded. Also, blood samples were collected from the type 2 diabetes mellitus patients for laboratory analysis. The levels of blood sugar, glycosylated hemoglobin, HIF-1α, and VEGF were evaluated. The results revealed that the minimum HIF-1α level was 0.52 µL/dL and the maximum was 13.45 µL/dL, with a mean value of 1.93 µL/dL and SD of 2.46 µL/dL. Similarly, the lowest VEGF level was 111.64 µL/dL, while the highest was 22052.61 µg/dL, with a mean of 931.49 µg/dL and SD of 2405.95 µg/dL. The finding of this study indicated that there was a significant ($p<0.005$) correlation between the HIF-1α and VEGF levels in the patients with type 2 diabetes mellitus.

Keywords: Blood sugar levels, Diabetes mellitus type 2, Hba1c, Hypoxia-inducible factor 1α, Vascular endothelial growth factor.

Introduction

Type 2 diabetes mellitus is a disorder of metabolism characterized by chronic hyperglycemia, resulting from dysfunctional endothelial.[1,2] Hyperglycemia causes hypoxia in mitochondrial cells because it increases oxygen consumption.[3] It is the primary stimulus for hypoxia-inducible factor (HIF)-1α protein regulation. HIF-1α is a protein complex that is activated by low oxygen pressure. This protein plays a role in various physiological processes such as angiogenesis, erythropoiesis, and cellular metabolism to increase oxygen delivery to the tissues.[4] Hyperglycemia and hypoxia are the results of the inadequate response of the tissues to lower oxygen tension, and they are suggested to play essential pathophysiological roles in the complications of diabetes.[5] Adaptive responses of cells to hypoxia are mediated by HIF-1α, and it is a primary regulator of vascular endothelial growth factor (VEGF) expression.[6] Studies have found that VEGF is involved in the pathogenesis of diabetic complications.[7] HIF-1α stimulates the transcription of more than 60 proteins, including VEGF and erythropoietin. These protein products increase oxygen availability by promoting erythropoiesis and angiogenesis, which activate the genes involved in glucose transport and metabolism.

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In addition, hyperglycemia promotes VEGF signaling, resulting in diabetic microvascular complications.[8,9] However, other factors, such as hypoxia, gender, smoking, elevated levels of blood lipids, inflammatory status, and activated stress axes may affect the synthesis and secretion of VEGF. Among these factors, the significant physiological stimulus for VEGF expression is the cellular hypoxia.[10] HIF-1*α* is a high oxygen-sensitive monitor of regulatory protein in the body.[11]

This study was conducted to evaluate the correlation between the hypoxia-inducible factor-1α and the vascular endothelial growth factor levels in type 2 diabetes mellitus patients who were attending primary health care centers in and around Medan, North Sumatra, Indonesia.

Materials and Methods

Study population and ethical approval

Eighty-nine patients with type 2 diabetes mellitus were recruited from the primary health services in and around Medan, North Sumatra, Indonesia, from May to July 2020. All patients of both sexes diagnosed with type 2 diabetes mellitus with or without complications were included in the study, while type 1 diabetes mellitus and severe disease were excluded. The study included diabetic patients who were taking oral hypoglycemic drugs, managing their diabetes with diet, or using insulin for glycemic control. Ethical permission from the Institutional Review Committee was obtained with approval number: 90/KEP/USU/2020. The patients were informed of the study's detail, and written consent was obtained from each patient before participation. Due to the occurrence of the COVID 19 pandemic in the country, personal protective equipment was used to prevent COVID-19 transmission, and all patients wore face masks when visiting the clinic.

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Measurements of body mass index and blood pressure
The subject's height and weight were measured while standing up and wearing light clothing. The body mass index (BMI) was calculated by multiplying the weight (kg) by the square of the height (meters). After the subject had been seated for at least five minutes, the blood pressure values were obtained as the average of two measurements. Also, disease duration, family history, and medical treatment were recorded.

Collection of blood samples
Before the laboratory examination, the subjects were required to fast overnight to provide a blood specimen. Blood samples were collected from 89 type 2 diabetes mellitus patients using a syringe and the blood samples were transferred immediately to the clinical laboratory to determine fasting blood sugar, glycosylated hemoglobin, and lipid profiles.

Laboratory analysis
The blood sugar levels (BSLs) and glycosylated hemoglobin were evaluated by the hexokinase and HPLC methods, respectively. The enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum levels of HIF-1α (Cayman Chemical Co, Ann Arbor, Michigan, USA). The human HIF-1α antibody was used to pre-coat the plate. HIF-1α from the sample was placed into the wells, where it was bound by antibodies and biotinylated human HIF-1α. After that, the substrate solution was added, and the colour developed in proportion to how much human HIF-1α was present. The process was stopped by adding an acidic stop solution and measuring the absorbance at 450 nm. The evaluation of vascular endothelial growth factor levels was determined based on the serum obtained from the blood samples, which were allowed to clot for 10-20 minutes at room temperature. To make a standard stock solution of 3200 ng/L, the reagents were placed at room temperature and rearranged with standard 120 L (6400 ng/L) and standard diluent 120 L. Before diluting, the standard was allowed to stand for 15 minutes and gently stirred. Standard points were made using the standard stock solution (3200 ng/L) and diluted 1:2 with standard diluent to provide solutions of 1600, 800, 400, and 200 ng/L. Standard diluents function as standard zero (0 ng/L).

Statistical analysis
SPSS version 24.0 (SPSS Inc., Chicago, Illinois) statistical software was used for the statistical analysis. All the variables in the study were tested by Shapiro–Wilk. The normal distribution variables ($p > 0.005$) were tested by parametric test, while the abnormal distribution variables ($p < 0.005$) were tested by non-parametric test.

Results and Discussion

The study involved 89 patients with type 2 diabetes mellitus. Of the total number of subjects, 22.5% (20) were males, while 77.5% (69) of the subjects were females. The minimum and maximum ages were 35 and 79, respectively. The minimum BMI was 17.63 kg/m^2, while the maximum was 46.44 kg/m^2, with a mean value of 26.29 kg/m^2. The minimum abdominal circumference was 98 cm, and the maximum was 216 cm, with a mean value of 92.69 cm and SD of 10.47 cm. The highest systolic and diastolic BP were 98 and 60 mmHg, respectively, while the lowest were 216 and 113 mmHg with mean values 150.96 and 87.75 mmHg, and SD of 22.05 and 10.44 mmHg, respectively (Table 1). The BSL ranged from 73 to 610 mg/dL, with a mean of 283.55 mg/dL and a SD of 137.43 mg/dL. The Hba1C value ranged from 4.7 to 14.7%, with a mean of 9.1% and a SD of 2.72%. The minimum HIF-1α level was 0.52 µL/dL and the maximum was 13.45 µL/dL, with a mean value of 1.93 µL/dL and SD of 2.46 µL/dL. The lowest level of VEGF was 111.64 µL/dL, and the highest was 22,052.61 µg/dL, with a mean of 931.49 µg/dL and a SD of 2,405.95 µg/dL. More so, intra- and inter-assay coefficients of the variation for HIF-1α were < 8 and <10%, respectively (Table 2).
The study evaluated the levels of HIF-1α and VEGF in type 2 diabetes mellitus patients. The study showed that there was a significant positive correlation between HIF -1α and VEGF levels. There was a rise in the concentration of HIF-1α as the concentration of VEGF increased. The complication of diabetes is closely related to the HIF-1α and VEGF concentrations.[12] Prolonged hyperglycemia enhances the synthesis and secretion of VEGF, which is transcriptionally regulated by HIF-1α, as the significant growth factor in mediating vascular leakage and neovascularization.[13]

Table 1: Characteristics of recruited subjects for the study

Characteristic	Mean	Median	Minimum	Maximum	SD
Age (years)	55.20	54	35	79	8.92
Duration of illness (years)	4.44	3	1	18	4.25
BMI (kg/m^2)	26.29	24.56	17.63	46.44	5.61
Abdominal Circumference (cm)	92.69	91.00	68	121	10.47
Blood Pressure (Systolic) mmHg	150.96	150	98	216	22.05
Blood Pressure (Diastolic) mmHg	87.75	86	60	113	10.44

Table 2: Levels of clinical and marker metabolites

Marker metabolic	N	Minimum	Maximum	Mean	SD
Blood Sugar Level (mg/dL)	89	73	610	137.43	137.43
Hba1c (%)	89	4.70	14.70	2.72	2.72
Cholesterol (mg/dL)	89	136	335	42.06	42.06
LDL (mg/dL)	89	51	249	34.7	34.7
HDL (mg/dL)	89	24	77	11.68	11.68
TG (mg/dL)	89	77	708	124.04	124.04
VEGF (mg/dL)	89	111.64	22052.61	2405.95	2405.95
HIF-1α (mg/dL)	89	0.52	13.45	2.46	2.46

Jiang *et al*. showed that HIF-1α regulates VEGF expression and the higher levels of HIF-1α and VEGF contribute to the pathogenesis of diabetic micro-angiopathy. Serum levels of VEGF and HIF-1α were significantly higher in diabetic patients than in the controls.[14]. Wang discovered that HIF-1α was up-regulated in diabetic nephropathy patients, and the patients with a large amount of albuminuria showed the highest expression of HIF-1α.[15] HIF-1α is a key oxygen sensor and mediator of angiogenesis,[16] and it acts by binding a hypoxia-response element in the VEGF promoter to up-regulate VEGF expression.[17] Other researchers found no significant relationship between HIF-1α, FBS, and HbA1c ($p > 0.005$) in type 2 diabetes mellitus with or without malignancy ($p > 0.005$).[18] However, the results of this study showed that both groups had relatively small levels of HIF-1α and also high levels in the serum. HIF-1α is a significant factor that regulates oxygen homeostasis and plays a crucial role in many physiological and pathological processes, with more than 100 genes under its control.[20] Diabetes is a significant risk factor for cardiovascular disease, and HIF-1α has also been closely associated with it. Also, hypoxia has been found to have a prominent effect on all diabetic complications.[21]

Conclusion

The findings of this study revealed a significant correlation between HIF-1α and VEGF levels in patients with type 2 diabetics. An increase in HIF-1α level resulted in high VEGF levels.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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