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Assessment of Vascular Endothelial Growth Factor level and Vascular Endothelial Growth Factor Receptor-2 in patients with un-controlled type 2 diabetes mellitus

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ABSTRACT— Late microvascular problems such retinopathy, neuropathy, and diabetic nephropathy are frequently brought on by uncompensated chronic hyperglycemia. In the pathophysiology of diabetes mellitus, the vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are of critical importance. The aim of this study to assessment level of VEGFA and VEGFR-2 in a group of patients with un-controlled type 2 diabetes mellitus (T2DM) and compare it with apparently healthy non diabetic subjects and study the correlation with other factors. There were 180 participants in the research study,70 control persons and 110 T2DM patients. Glycated hemoglobin (HbA1c) and fasting plasma glucose (FPG) values were calculated in order to evaluate glycemic management. In addition, serum cholesterol, triglyceride, HDL and LDL were calculated by COBAS INTEGRA 400 plus. VEGF-A, VEGFR-2 levels were estimated using enzyme-linked immunosorbent assay(ELISA) kit. The levels of VEGF-A and VEGFR2 in the patients group and in the normal control found to differ significantly ($p < 0.001$). Significant positive correlation was seen between the level of VEGF-A and glucose, duration of disease. Weak non-significant positive correlation was also found between VEGFR2 and serum glucose ($p=0.09$). Patients with uncontrolled diabetes produce VEGF-A and its receptor 2 concentrations that are comparable to those of apparently healthy people, which may be a sign that insufficient glucose management accelerates the development of vascular problems. The absence of correlation between VEGF-A, VEGFR2 and lipid abnormalities demonstrate that lipid problems related to diabetes could not affect how angiogenesis is regulated.

KEYWORDS: Diabetes mellitus Type 2, VEGF-A, VEGFR-2, un-controlled hyperglycemia

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder with a variety of causes. Persistent hyperglycemia is accompanied with unpleasant effects on carbohydrate, fat, and protein metabolism, which are caused by defects in insulin release, insulin activity, or both [29].

Type 2 diabetes mellitus(T2DM) is a complex condition brought on by a confluence of genetic and environmental variables. Excessive calorie consumption and a sedentary lifestyle that leads to obesity are two environmental factors that lead to the development of T2DM. There are monogenic and polygenic types of T2DM, and some monogenic forms necessitate a unique therapeutic approach. Uncontrolled hyperglycemia is linked to problems in the eyes, nerves, kidneys, and heart, lowering both quality of life and life expectancy [18]. According to [11], increased hepatic glucose synthesis, insulin resistance in adipose tissues, and malfunction of pancreatic beta cells all contribute to type 2 diabetes.

VEGF is a potent angiogenic agent involved in both physiological and pathological neovascularization. It is a multifunctional growth factor active in embryonic development [13], [8], [12]. Vascular permeability factor (VEGF), also known as the VPF, is a vital and necessary growth factor for vascular endothelial cells and has a variety of functions in the development of the cardiovascular system, the central nervous system, and carcinogenesis.

The endothelial growth factor family (VEGFs), which now has seven recognized members with regulatory functions in various vascular systems, includes the VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PIGF [20].

It works biologically by interacting with certain (VEGF-R) receptors, a subgroup of the receptor tyrosine kinase (RTK) family [20]; the fetal liver kinase (VEGFR-2/KDR/Flk-1), the fms-like tyrosine kinase Flt-1 (VEGFR-1/Flt-1), and Flk-4. (VEGFR-3), which promotes the growth of blood vessels (VEGF-A, VEGF-B, VEGF-C and VEGF-E) and lymphangiogenesis (VEGF-C and VEGF-D) [9], [14].

The VEGF/VEGFR system is a crucial target for pro - angiogenic therapies in ischemic disorders and neurodegeneration as well as antitumor therapy in cancer [30].

One important element in the pathophysiology of diabetic retinopathy(DR) is vascular endothelial growth factor (VEGF). The blood-retina barrier breakdown and the multiplication of retinal neovessels that cause severe visual impairments in DR patients are specifically caused by prolonged expression and production of VEGF in the retina [27].

In most forms of renal illness, irregular VEGF-A expression in the kidney has been commonly described. [17]. When mature podocytes penetrate the glomerular basement membrane (GBM) to communicate with VEGFR-2 on the glomerular endothelial cells (GEnCs), they primarily express large quantities of VEGF-A, which is a crucial regulator of proper function inside the glomerulus [10].

In the early stages of diabetic peripheral neuropathy (DPN), the affected endothelium may respond to hypoxia and oxidative stress by producing more VEGF. Meantime, ischemia responses to nerve fibers will cause the production of VEGF to rise in an effort to preserve the nerve and maintain the integrity of the axon or myelin. This occurrence is referred to as the dual impact of VEGF [7]. The VEGF will aggravate the damage to the nerve fibers by thickening the basal membrane and increasing vascular permeability [31].

The protein kinase C pathway also causes damage from hyperglycemia in tissues that are predisposed to difficulties. High glucose levels promote diacylglycerol (DAG), which in turn activates PKC. Increased PKC-isoform synthesis has been associated with the development of diabetic complications such retinopathy, nephropathy, and cardiovascular disease, as well as with the angiogenic protein PAI-1, vascular endothelial growth factor (VEGF), NF-B, and TGF- upregulation [24].

The aim of this study is to detect the level of VEGFA and VEGFR-2 in patients with un-controlled type 2 diabetes mellitus and to compare it with serum level of VEGFA and VEGFR-2 in normal individuals, and to find the correlation between level of VEGFA, VEGFR-2 and FPG, HbA1c, as well as lipid profile.

2. Materials and Method

The patients for this study, which was conducted between February 2022 to July 2022, were chosen from Specialist unit for Diabetes and Endocrinology in AL-Batool Teaching Hospital for Obstetrics and

pediatrics, Baquba, Diyala. One hundred eighty (180) subjects were involved in this study. They are subdivided in to two groups,110 patients with diabetes mellitus type 2 as (G1), and 70 apparently healthy subjects as control group (G2). The age of patients and control between 40-70 years. The mean age of duration of type 2 diabetes mellitus in patient's subjects was 8.8±4.1 years.

Patients with type 2 diabetes mellitus diagnosed and classified by physician according to ADA criteria [3] by estimate FPG and HbA1c.

Exclusion criteria were Type 1 diabetes mellitus, Pregnant women, subjects with Hashimoto's thyroiditis and SLE, patients with liver disease, renal disease, recent history of cardiovascular disorder, hypertension and neurological disease.

After 8 to 10 hours of fasting, intravenous blood samples were taken from patients and control participants by puncturing veins with disposable 5ml syringes. One ml was deposited into EDTA tubes for measurement of HbA1c, and the remaining 4 ml was slowly forced into disposable test tube containing separating gel. The serum separated from the blood to calculate the value of cholesterol, triglyceride, HDL and LDL and fasting plasma glucose using fully automated COBAS INTEGRA 400 plus instrument (Roche Diagnostics) before frozen the serum. The remaining serum frozen at about -20°C until used for analysis of VEGF-A and VEGFR-2 by ELISA technique using (MyBioSource kits).

A two-tailed independent samples t-test was used to compare continuous variable means between patients and control groups. The Spearman correlation test was used to test the correlation among VEGF-A, VEGFR-2, Duration, Glucose, HbA1c, Cholesterol, Triglyceride, HDL, and LDL. Cohen's standard was used to figure out how strong the relationships were.

3. Results

Table (1) and a bat blot of the means figure (1) showed that the mean serum concentration of VEGF-A, VEGFR-2 for patients with T2DM were significantly higher ($p < 0.001$) in comparison with control group.

The fasting serum glucose, HbA1c, cholesterol, triglyceride, HDL and LDL were significantly higher in comparison with control group.

Table (1) mean values of the study parameters comparison between patients and control

Variables	groups	**Mean**± SD	P value
VEGF-A pg/ml	Control(n=70)	172.02± 24.67	< **0.001**
	Patients(n=110)	754.83± 209.60	
VEGFR-2pg/ml	Control	205.39± 29.16	< **0.001**
	patients	736.16± 156.56	
Glucose	Control	101.71± 9.26	< **0.001**
	patients	275.58± 75.19	

HbA1c	Control	5.41± 0.51	**< 0.001**
	patients	10.47± 2.00	
Cholesterol	Control	164.71± 28.31	**< 0.001**
	patients	196.60± 48.39	
Triglyceride	Control	121.86± 39.66	**< 0.001**
	patients	241.11± 108.94	
HDL	Control	43.29± 7.53	**0.0019**
	patients	39.27± 8.74	
LDL	Control	107.63± 26.17	**0.037**
	patients	118.77± 38.87	

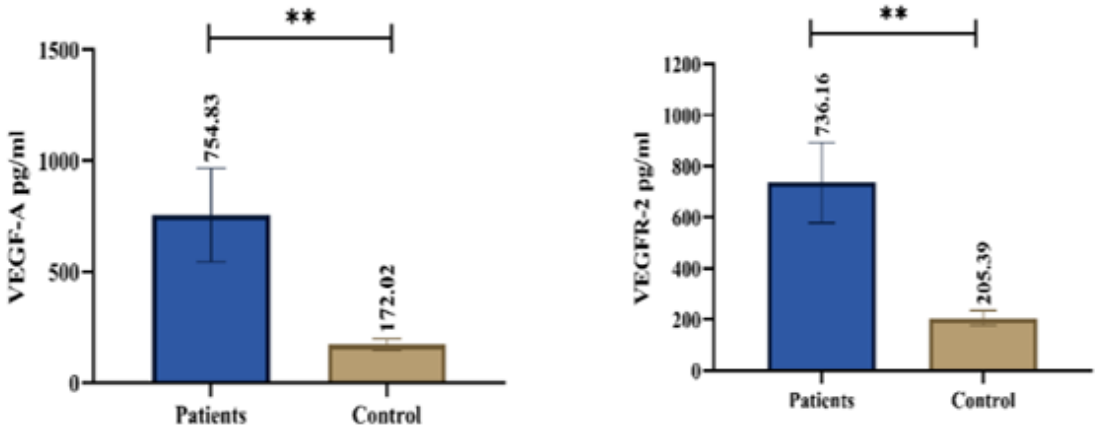

Figure (1) comparison between patients and control in level of VEGF-A and VEGFR-2

Table (2) presents the correlation coefficients (r) stratified by its P value.

As shown in table (2), serum VEGFA demonstrated significant positive correlation with glucose level in serum of patients with diabetes mellitus (R = 0.21 and p = 0.03). Weak non-significant positive correlation between VEGFR-2 and glucose was found at p = 0.09.

Positive correlation was seen between VEGFA and duration of disease (R= 0.19 and P = 0.045), while no significant correlation between VEGFA, VEGFR2 and cholesterol, HDL, LDL triglyceride and HbA1c (p > 0.05).

Table (2) Spearman Correlation Results among study markers and (Glucose, HbA1c, cholestrol, triglyceride, HDL, LDL, duration of disease) in patients' group.

	Chol	HDL	LDL	TG	Glucose	HbA1c	Duration of disease

VEGFA pg/ml	r	0.01	0.01	0.02	-0.04	**0.21**	0.12	**0.19**
	p	0.91	0.94	0.87	0.66	**0.03***	0.22	**0.045***
VEGFR-2 pg/ml	r	-0.13	0.11	-0.08	-0.07	0.16	0.08	0.13
	p	0.16	0.26	0.44	0.45	0.09	0.40	0.17

4. Discussions

In the present study, we compared serum level of VEGFA, VEGFR2 in patients with T2DM with heathy control and found that level of VEGFA, VEGFR2 were highly significant increase ($p < 0.001$) in patients in compassion with healthy persons.

Many studies have found that VEGF is involved in the pathogenesis of diabetic complications [1], hyperglycemia-induced diacylglycerol, a lipid molecule, activates protein kinase C in the vascular tissues, and then turn promotes VEGF signaling, resulting in diabetic microvascular complications [28]. But still, the other factors, including hypoxia, gender, smoking, elevated levels of blood lipids, inflammatory status, and activated stress axes, may affect the synthesis and secretion of VEGF; among them, the significant physiological stimulus for VEGF expression is the cellular hypoxia [19], [21].

Individuals with type-2 diabetes mellitus (T2DM) are often at high risk for microvascular complications, including diabetic retinopathy (DR), diabetic nephropathy (DN), and diabetic peripheral neuropathy (DPN) due to diabetic microvascular dysfunction [6]. Angiogenesis, as an essential biological process, involves the progression of diabetic microvascular complications. Meanwhile, vascular endothelial growth factor (VEGF) is the most potent proangiogenic growth factor that increases vascular permeability in vivo and activates endothelial cells in vitro [5]. In previous study, Plasma VEGF levels were reported to be higher in diabetic patients than in healthy control individuals [2].

Study was done by [22] in contrast with the present finding who stated that a significant difference was not observed between the concentrations of VEGF-A, VEGFR1 or VEGFR2 in the control group and the patients group. This may suggest that appropriate glucose control in diabetes can delay the onset of vascular diseases and poor glycemic management with its metabolic complications might be regarded important causes in increased VEGF expression [23].

These results could harmonize with findings from other studies that found in type 2 patients, a significant positive connection between VEGF and fasting plasma glucose (FPG) has been seen [4].

The significant positive correlation which was found between VEGFA and duration of disease in addition to FPG confirm that VEGFA and its receptor 2 have important role to increase complication may appear in patients with type 2 diabetes mellitus.

The current study confirmed the previous study that found no significant relationships between VEGF and TG, TC, HDL-C, LDL-C (Sun et al,2019). The absence of correlation between VEGFA, VEGFR2 and lipid abnormalities demonstrate that the regulation of angiogenesis may not be influenced by lipid imbalances seen in diabetes.

5. Conclusion

According to the findings of this study, there is a connection between circulating VEGFA, VEGFR-2 levels and T2DM, with patients having higher levels of VEGFA and VEGFR-2 than non-diabetic people.

Inadequate glucose control and its metabolic effects might be regarded as significant variables that contribute to enhanced expression of VEGFA and its receptors in type 2 diabetes. Proper glycemic management significantly lowers the risk of vascular problems connected to increased amounts of VEGF.

Further study may be recommended with larger sample size to confirm the results in this research and provide appropriate assistance to prevent the complication may have appeared in T2DM patients.

Ethical Clearance

All of the subjects' groups received information regarding the study's purpose and methodology. They provided informed permission for study participation, and the study was authorized by Research Committee of Diyala Health Department-Training and Human Development Center.

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