Evaluation of VEGFR1 and VEGFR2 levels in Iraqi patients with DFU

Hajir Khalil Ismail[1]*, Salma Abdul-rudha Abass[2], Isam Noori Salman[3]
[1]Department of Chemistry, University of Al-Mustansiriyah, College of Science, Baghdad,
[3]National Diabetes Center, University of Mustansiriyah, Baghdad, Iraq
hajir_92@yahoo.com

Abstract
Diabetes mellitus causes diabetic foot ulcers, "which are the leading cause of non-traumatic lower extremity amputations (up to 88 %)". We aimed to show the serum levels of VEGFR1 and VEGFR2 in T2DM patients with and without DFU. A total of 90 subject including patients who have T2DM (N=30), with DFU (N=30), and healthy subjects (N=30) were enrolled in this study. The serum levels of vascular endothelial growth factor receptor (VEGFR1 and VEGFR2) were determined by enzyme-linked immunosorbent assay (ELISA) method. When comparing the DFU group to the T2DM and control groups, the serum level of VEGFR1 was significantly lower in the DFU group (P= 0.0024 and P= 0.0026, respectively). There was a significant decrease in DFU in serum level of VEGFR2 when comparing to control group (P=0.0313). Positive correlations were found between the levels of glucose and VEGFR1, in the T2DM group, also a positive correlations were found between VEGFR2, and glucose levels, in the DFU group. We conclude that concentrations of VEGFR 1 and 2 in DFU patients are lower than those of control subjects, it could indicate that damage to the endothelial vessels lead to decreased concentration of these receptors in the DFU group. The positive correlation between glucose and VEGFR2 levels suggests that glucose abnormalities seen in diabetics may play a role in angiogenesis modulation.
Keywords: VEGFR1; VEGFR2; Iraqi patients; Nursing; DFU

Introduction

Diabetes mellitus (DM) is a "chronic metabolic disease marked by insulin resistance (IR) or insulin insufficiency"[1]. The long-term consequences of diabetes have been clearly reported, and they affect various organs systems if left untreated, the uncontrolled hyperglycemia can cause angiogenesis abnormalities, resulting in variable levels of impairment and long-term problems defined as microvascular such as "neuropathy, retinopathy , and nephropathy"and macrovascular "coronary heart disease, stroke and peripheral vascular disease"[2-5].

Diabetes affects approximately 1.4 million Iraqis. In Iraq, reported

T2DM prevalence is found to range from (8.5) % to (13.9) % (Abusaib et al., 2020). In the southern Iraqi city of Basrah, a local study of approximately 5400 persons reported a 17.9% age adjusted prevalence of diabetes in people aged 19 to 94 years.[6]

One of the most painful effects of diabetes is diabetic foot ulcers (DFU), with potentially fatal consequences. DFU that is not treated properly can result in osteomyelitis, gangrene, and limb amputation[7]. An ulcer develops as a result of tissue damage or death, which is associated with the severity of peripheral vascular disease in the lower limb and may be accompanied by infections[8].

Angiogenic factors are released in abundance, like VEGF, causes an angiogenic response. VEGFA is known to produce by endothelial cells (ECs), megakaryocytes, and leukocytes[9]. "Angiogenesis" is required for the growth and survival of solid tumors, and VEGF is the primary inducer of tumor-associated "angiogenesis"[10, 11]. VEGF is found in the majority of malignant epithelial tumor cells, Furthermore, the levels of VEGFA expressions in tumor tissues has been found to have predictive value[12].

Diabetes-related processes such as hyperglycemia, insulin resistance, dyslipidemia, hypertension, obesity, and decreased (NO) production reduce flow of the blood in the vessels and result in hypoxic tissue. Hypoxia triggers angiogenesis and the activation of a number of genes, including VEGF and VEGFR2, which may have an impact on the diabetes complications development due to their roles[13].

VEGFA is a heparin-bound glycoprotein of 45 kDa that binds to the (VEGFR1) with high affinity and kinase insert domain receptor (VEGFR2) receptors[11]. The family of VEGF (growth factors) includes five secreted growth factors: (VEGFA, B, C, D and placental growth factor (PlGF), these ligands bind to surface receptors: VEGFR1, VEGFR2, and VEGFR3[14]. To promote angiogenesis, VEGF induces the generation of new vessels through binding to the VEGFR2 [15]. There are two isoforms of VEGFR-2: soluble VEGFR2 (sVEGFR2) and membrane VEGFR2. VEGF predominantly promote angiogenesis by attaching to the membranous VEGFR2 receptor[16]. The binding of VEGF to sVEGFR2 in

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wound fluid reduces VEGF availability for binding to membrane VEGFR2, resulting in decreased angiogenic effects of the VEGF[17].

Human VEGFR1 is "a mature 180 kDa glycoprotein with 1312 amino acids that is found in the endothelium, neural, epithelial tissues and immune cells". VEGFR1 binds to all VEGFA, VEGFB, and PIGF isoforms. VEGFA binds to VEGFR1 with a much higher affinity (Kd) of 2–10 picomolar (pM) than VEGFR2[18].

VEGFA/VEGFR1 signal transduction were eventually discovered to be involved in the creation of neovessels in a variety of diseases, including cancer[19]. The VEGF receptors (1 and 2) are exclusively found in the ECs. VEGFR1 appears to be a mediator of vascular hyperpermeability, while VEGFR2 appears to take a role in angiogenesis[4].

Human VEGFR2 mature protein is "a 200–230 kDa glycoprotein found in both vascular and lymphatic ECs"[20]. VEGFR2 activation is the primary mediator of VEGFA signaling. VEGFA binding can stimulate the production of VEGFR1 and VEGFR2 heterodimers, despite the fact that VEGFR2 homodimers are involved in functional regulation[21].

According to the literature review, the expression of VEGF and VEGFR2 increases significantly during the progression of pathogenic disorders like "diabetic retinopathy and nephropathy"[22, 23].

2. Materials and methods

2.1 subjects

The groups of the study comprised of T2DM (N=30), and DFU (N=30) who were recruited from National Diabetes Centre/ AL-Mustansiriyh University and Specialist Centre for Endocrinology and Diabetes in Baghdad, Iraq. Healthy control subjects (N=30) (who are mostly blood donors and National Diabetes Centre internal staffs).

2.2 sample collection

Venous blood were withdrawn by vein puncture then separated in the plain tube of polyethylene to obtain serum for VEGFR1 and VEGFR2 determination.

2.3 VEGFR1 and VEGFR2 level Evaluation

The enzyme-linked immunosorbent assay (ELISA) was used to determine the serum VEGFR1 and VEGFR2 levels using (Al-shkairate establishment for medical supply. Amman, Jordan), according to the manufacturer's instruction.

2.4 statistical analysis

The statistical package SPSS26 were used to analyse the results. For normally distributed data, ANOVA was used to compute mean SD for comparison among several groups using Tukey's post hoc test. The (Spearman R coefficient) was used to determine the degree of correlation between the parameters studied, with $P<0.05$ being considered significant.

3. Results

Table (I) shows glucose, VEGFR 1 and 2 concentrations in the study group compared with the control group. Analysis results indicated that there was a highly significant increase in serum glucose level in non DFU, DFU when compared to healthy control group ($p<0.001$). When compared to T2DM and the control group, the serum level of VEGFR1 in DFU was significantly lower (P= 0.0024 and P= 0.0026, respectively). When compared to the healthy control group, there was a significant decrease in DFU of VEGFR2 serum levels (P=0.0313). Positive correlations were found between the levels of VEGFR1, and glucose, in the T2DM group, also a positive correlations were found between VEGFR2, and glucose levels in the DFU group as shown in table (II).

Table I: Concentrations of Glucose, VEGFR (1 and 2) in the studied groups.

parameters	Group	N	Mean	±SD	P-Value
VEGFR1 (pg/ml)	Control	30	754.4	491.2	0.0026** (DFU vs. Control) 0.0024**(T2DM vs.& D.F) NS (T2DP vs. control)
	DFU	30	381.2	279.5	
	T2DM	30	763.3	462.1	
VEGFR2 (pg/ml)	Control	30	2789	539.3	0.0313*(DFU vs. control) NS(DFU vs. T2DM) NS (T2DP vs. control)
	DFU	30	2438	548.9	
	T2DM	30	2708	465.8	
Glucose (mg/dl)	Control	30	87.96	26.18	<0.001**(DFU vs. control) <0.001**(T2DM vs. Control) NS (DFU vs. T2DM)
	DFU	30	204.2	84.82	
	T2DM	30	231.4	99.82	

Table II: The spearman R correlation coefficients of VEGFR (1 and 2) with the Glucose.

parameter	glucose			
	DFU		T2DM	
	R	P	R	P
VEGFR1	0.1383	0.4662	0.4335	0.0212*
VEGFR2	0.5692	0.0010	0.1602	0.4154

Discussion

The most well-known factor involved in angiogenesis is VEGF, which has been associated to persistent hyperglycemia and its effects. VEGFA stimulates the differentiation, proliferation,

and ECs survival. It is also utilized to promote endothelial-dependent vasodilation, improve permeability, and participate in the remodeling of the cellular interstitial matrix[22].

Evidence for VEGF and its two receptors playing a role in the etiology of DFU is more widespread among the VEGF family, VEGFR1 (mediated signaling participates in critical action by increasing vascular permeability), while VEGFR2 which are known as transmembrane proteins [24].

This is the first study that we are known to determine the correlation between VEGFR1, VEGFR2 and glucose in DFU Iraqi patients. In our study, a highly significant decrease were found between the VEGFR1 concentration in the group of DFU compared to T2DM and control groups. Hyperglycemia-induced pathological mechanisms also affect the expressions of VEGFA and its receptors VEGFR1, VEGFR2[25]. We thought that an increase in VEGF concentration owing to hyperglycemia caused it to bind to receptors in the cell membrane, resulting in VEGF occupation of these receptors and hence a decrease in VEGFR1 concentration in DFU compared to healthy control and T2DM groups, also it could indicate that damage to the endothelial vessels lead to decreased concentration of these receptors in the DFU group. This also shows that the decrease in VEGFR1 concentration may be the cause of the delay in wound healing in the DFU group of patients, even if the amount of VEGF increased.

Placental VEGFR1 immunoreactivity was high in the clinical diabetes groups, normoglycemic and gestational DM but low in the hyperglycemic group, according to Pietro et al[26]. However, some studies hold the opposite opinions. According to Erturk et al., the average sVEGFR1 levels in the diabetes mellitus group were significantly higher than those in the healthy control (0.106 ±0.052 and 0.073± 0.049, respectively; p = 0.005)[24].

VEGFR1 is mostly found in vascular ECs, macrophages, monocytes, renal mesangial cells and placental trophoblasts. A receptor is found within the gene's promoter on (hypoxia-dependent sequence) "hypoxia responsive elements (HRE)". This receptor's activation by its molecule is described by a "poor response"[27, 28]. VEGFR2 is a key VEGF-A signaling receptor that mediates the majority of its biological actions in ECs. VEGFR-1's function is less clear, however it is assumed to control VEGFR2. Another role of VEGFR1 may be to act as a "decoy receptor", preventing VEGFA from attaching to VEGFR2[14]. The previous finding support our results that the low concentration of VEGFR1 it will be followed by a lack of VEGFR2 concentration through modulating it.

Hyperglycemia is a toxin to the endothelium because it increases oxidative stress and inhibits NO bioavailability, as well as advanced glycation end products formations. The high concentration of glucose in the blood, in addition to lowering the availability of NO, stimulate the production of vasoconstrictor molecules, particularly endothelin1[29].

VEGFR2 is a type of VEGF receptor that is principally responsible for the receptor's biological response. The principal mechanism that activates the vascular endothelium is VEGF activation via VEGFR 2, and the amount of VEGFA is significantly linked to the concentration of VEGFR2[3].

There was a significant decrease in DFU in serum level of VEGFR2 when compared to control group (p=0.0313), in the present study. This decrease in VEGFR2 in serum level of DFU leads to impair the pro-angiogenic properties of ECs.

Warren et al demonstrated that hyperglycemia-induced reactive oxygen species enhanced ligand-independent activation of VEGFR2. This signalling via VEGFR2 took place within the Golgi appartus, As a result, VEGFR2 availability at the cell surface decreases with time. As a consequence, despite no change in transcript abundance, EC responses to "exogenous VEGF" in a mouses model of DM were reduced due to "a particular VEGFR2 deficiency" at the cell surface[30]. The previous finding support our results since we found a decrease in VEGFR2 concentration in DFU group.

[31]discovered contrasting results in the expressions of VEGFA, (angiopoietin 1 and 2), and its receptors in human placental of control and patients with good-controlled diabetes type1. VEGFR2 levels were significantly higher in diabetic patients with retinopathy or diabetic nephropathy than in healthy people[31]. A study by Cooper et al investigated the changes in VEGFA and its receptor gene expression in diabetes caused by streptozotocin. VEGFA and its receptor (VEGFR2) levels were measured three (short term) and 32 (long term) weeks after a pharmaceutical substance was administered. After three weeks of streptozotocin treatment, the mRNA levels of VEGF and VEGFR2 were increased, and a twofold increase in VEGFR-2 gene expression with short-term diabetes as compared to the control rats (Cooper et al., 1999). According to Lebok et al. reduced or missing VEGFR1 has been linked to tumor growth, fast cell proliferation, and shorter survival in individuals with breast cancer[32].

According to the current findings, there is a positive correlation between VEGFR1 and glucose levels in T2DM patients, as well as a positive correlation between VEGFR2 and glucose levels in DFU patients, implying that glucose irregularities in diabetes may play a role in the modulation of angiogenesis.

The limited sample size of this study is one limitation that may impair the dependability of the results. Because of regional variances, the results may not be applicable to other populations. To

improve our findings, more well-designed studies with a larger sample size would be required.

Conclusion

VEGFR1 and VEGFR2 concentrations in DFU patients are lower than in healthy control, it could indicate that endothelial vascular cell injury leads to lowered concentrations of these receptors in this group of patients, also we thought that an increase in VEGF concentration owing to hyperglycemia caused it to bind to receptors in the cell membrane, resulting in VEGF occupation of these receptors and hence a decrease in concentration of VEGFR1 in DFU patients group compared to the control and T2DM groups. Positive correlation between (glucose and VEGFR1) levels in T2DM patients, also positive correlation were noted between (VEGFR2 and glucose) levels in DFU group suggests that glucose irregularities in diabetes may be involved in angiogenesis modulation. This study confirmed that the low concentration of VEGFR1 it will be followed by a lack of VEGFR2 concentration through modulating it.

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