

Research Article



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Evaluation of plasma VEGF and sVEGFR-1 levels in patients with diabetes mellitus receiving insulin treatment

İnsülin tedavisi alan Diabetes Mellitus'lu hastalarda plazma VEGF ve sVEGFR-1 düzeylerinin değerlendirilmesi

<https://doi.org/10.1515/tjb-2017-0348>

Received December 1, 2017; accepted April 3, 2018; previously published online January 19, 2019

Abstract

Background: Diabetes mellitus (DM) is a multifactorial chronic disease, in which patients need to be treated with insulin in some conditions. Capillary growth is regulated by growth factors like vascular endothelial growth factor (VEGF) and endogenous inhibitors such as the splice variant of VEGF receptor-1 (sVEGFR-1). We aimed to show the levels and the clinical significance of VEGF, sVEGFR-1 in patients with DM on insulin treatment.

Materials and methods: A total of 83 subjects consisting of patients with the diagnosis of DM (n=47) and healthy control (n=36) were included the study. Plasma levels of VEGF and sVEGFR-1, were measured using the enzyme-linked immunosorbent assay method.

Results: The average sVEGFR-1 levels of DM group was significantly higher than the control group (0.106 ± 0.052 and 0.073 ± 0.049 , respectively; $p=0.005$). Significantly lower

sVEGFR-1 levels were determined in patients receiving metformin vs. without metformin using (0.065 ± 0.016 and 0.118 ± 0.053 , respectively; $p=0.001$).

Conclusion: This is the first study evaluating and demonstrating the importance of plasma VEGF and sVEGFR-1 levels together in DM patients receiving insulin. Using metformin may have positive effect on angiogenesis in DM. Further studies are required to understand these effects.

Keywords: Diabetes mellitus; Insulin; VEGF; sVEGFR-1; Metformin.

Öz

Amaç: Diabetes mellitus (DM), bazı durumlarda insülin ile tedavi edilmesi gereken multifaktöryel kronik bir hastalıktır. Kapiller büyüme, vasküler endotelial büyüme faktörü (VEGF) gibi büyüme faktörleri ve çözünebilir VEGF reseptörü-1 (sVEGFR-1) gibi endojen inhibitörler tarafından düzenlenir. Bu çalışmamızda insülin tedavisi alan DM hastalarında VEGF, sVEGFR-1'in klinik önemini ve düzeylerini göstermeyi amaçladık.

Gereç ve Yöntem: Çalışmaya DM tanısı alan (n=47) ve sağlıklı kontrol (n=36) olan toplam 83 olgu dahil edildi. VEGF ve sVEGFR-1'in plazma seviyeleri, enzimle bağlı immünosorbent analiz yöntemi kullanılarak ölçüldü.

Bulgular: DM grubunun ortalama sVEGFR-1 düzeyleri kontrol grubundan anlamlı derecede yüksekti (sırasıyla 0.106 ± 0.052 ve 0.073 ± 0.049 ; $p=0.005$). Metformin kullanan hastaların ortalama sVEGFR-1 düzeyleri metformin kullanmayan hastalardan anlamlı derecede düşük saptandı (0.065 ± 0.016 ve 0.118 ± 0.053 , $p=0.001$).

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Sonuç: Çalışmamız, insulin tedavisi alan DM hastalarında plazma VEGF ve sVEGFR-1 düzeylerinin önemini gösteren ilk çalışmadır. Metformin kullanılması DM’de anjiyogenez üzerinde olumlu bir etkiye sahip olabilir. Bu etkileri anlamak için ileri çalışmalar gereklidir.

Anahtar kelimeler: Diabetes mellitus; İnsülin; VEGF; sVEGFR-1; Metformin.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by insulin resistance or accompanied by relative insufficient insulin [1]. The adverse long-term effects of DM have been well described and involve many organ systems and if left untreated, uncontrolled hyperglycemia may lead to abnormalities of angiogenesis and can lead to varying degrees of disability and long-term complications classified as micro-vascular (such as retinopathy, nephropathy, and neuropathy) and macrovascular (coronary heart disease, peripheral vascular disease and stroke) [2, 3]. Failure to achieve adequate glycemic control despite dietary and oral antidiabetic treatments or in cases such as type 1 DM treatment leads to insulin therapy. Epidemiological studies indicate that DM can accelerate atherosclerotic processes and increase the incidence of cardiovascular events and strokes [4]. In a study evaluating the management of DM and DM policies in Turkey, cardiovascular complications were seen the most as the rate of 32.6% [5].

Angiogenic molecules and its receptors act critical roles in endothelial development, survival transcriptional factors, microvascular permeability and pathological angiogenesis [6]. Vascular endothelial growth factor (VEGF) has been known for a long time as an angiogenic molecule which both promotes blood vessel dilation and induces new blood vessel formation. Mainly VEGF receptors are VEGF receptor-1 (VEGFR-1) and VEGFR-2 which are known as transmembrane proteins. VEGFR-1 mediated signaling participate crucial action by enhancing the vascular permeability [7]. Soluble VEGFR-1 (sVEGFR-1) is produced by alternative splicing of VEGFR-1 mRNA and play a role as a decoy protein. Current studies suggest that sVEGFR-1 may be regarded as an angiogenic inhibitor (a negative regulator of VEGF) [8, 9]. As indicated before, diabetic microvascular complications are considered to be influenced by angiogenic and anti-angiogenic factors. Sufficiently releasing of sVEGFR-1 can be important in terms of prevention of exaggerated angiogenesis and the VEGF–sVEGFR-1 mechanism is crucial to the physiological homeostasis of vasculature and modulation

of pro- and anti-angiogenesis in patients with DM [8]. However, studies reporting the association among circulating VEGF and sVEGFR-1 levels and clinical information of patients with DM patients receiving insulin treatment are very limited and the role of different concentrations of VEGF and sVEGFR-1 in the process of angiogenesis is still unknown [10, 11]. From this point of view, we hypothesized that increased sVEGFR-1 levels may contribute to the impaired angiogenesis in DM patients on insulin treatment and aimed to assess the impact of DM on the serum levels of VEGF (proangiogenic factor) and sVEGFR-1 (an angiogenesis inhibitor) in patients with DM on insulin treatment. In this respect, we think that this study will eliminate a physiopathological unknown in DM patients and will shed light on future studies.

Materials and methods

Subjects

The protocol of this study was approved by the Ethics Committee of Gulhane Faculty of Medicine Hospital, Ankara, Turkey; which was conducted according to the Helsinki II Declaration and informed consent was obtained from each individual.

A total of 83 subjects consisting of patients with a diagnosis of DM on insulin treatment ($n=45$) and healthy ($n=34$) in Gulhane Education and Research Hospital, Ankara, Turkey were included in this cross-sectional study. All patients underwent a medical examination and a structured interview to investigate any family history of vascular and cancer diseases, smoking and use of medications. Body mass index (BMI, kg/m^2) was calculated as weight (in kilograms) divided by height (in meters) squared and used as an index of body fat. The patients with infection, acute or chronic inflammatory disease, high erythrocyte sedimentation rate (>20 mm/h) or C-reactive protein (CRP) (>5 mg/L), have or suspected malignancy, heart failure, chronic obstructive pulmonary disease were excluded from the study.

Sample collection and laboratory measurements

Blood samples of each subject were collected after an overnight fasting and serum samples were separated by centrifugation at 2000 g for 10 min. Subsequently, the analysis of the biochemical parameters were performed

without freezing while 2 mL of serum samples were aliquoted and immediately frozen at -80°C for future analyses of VEGF and sVEGFR-1 until examination.

Total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting blood glucose (FBG), postprandial plasma glucose (PPG) and total protein levels were also measured by the enzymatic and colorimetric methods with Olympus AU5800 (Beckman Coulter, USA) using reagents from Olympus Diagnostics. Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's formula [12]. CRP level was determined in serum by immunoturbidimetric fixed rate method by Olympus AU-5800 autoanalyzer (Beckman Coulter, USA). HbA_{1c} levels were measured by Premier Hb9210 analyzer (Trinity Biotech, Bray, Ireland/Kansas City, MO, USA) using a boronate affinity chromatography-based high-performance liquid chromatography system for the measurement of glycated hemoglobin. Glomerular filtration rate (GFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation [13].

Serum VEGF and sVEGFR-1 levels were measured using quantitative ELISA kits (Hangzhou Eastbiopharm Co., Ltd, China). Measurements were carried out using ELISA plate reader Bio-Tek Synergy HT (Biotek Instruments Inc., Winooski, VT, USA). Intra-assay CV and inter-assay CV were $<10\%$ and $<12\%$, respectively, while the minimum detectable dose of was less than 20 ng/mL.

Statistical analyses

Clinical features, history of drug use and laboratory results of patients were compared by VEGF and sVEGFR-1 levels.

The Statistical Package for Social Science v 16.0 software package (SPSS, Chicago, IL, USA) was used to conduct the statistical analyses. For the descriptive statistics, discontinuous variables were shown as numbers and percentages (%); continuous variables were shown as the mean $\pm$ standard deviation or median (minimum, maximum) as appropriate. Normality of the data was evaluated with the Kolmogorov Smirnov test or Shapiro Wilk tests.

Comparisons between two groups were assessed for continuous variables with the independent samples t-test and Mann-Whitney U-test, as appropriate. Correlations were performed by the Pearson's correlation test. Additionally logistic regression analysis was performed considering the accompanying complications and used medications. All of the reported p-values were two-tailed, and those less than 0.05 were considered to be statistically significant.

Results

The differences between patients with DM receiving insulin treatment and the control group were analyzed and demographics and clinical characteristics of the study population together with the laboratory findings are presented in Table 1. According to this, the average age of patients with DM and controls were similar. There was also no statistically significant difference between groups in terms of BMI, AST, ALT, total protein, total cholesterol, triglyceride, HDL-C and LDL-C levels while serum sVEGFR-1, white blood cell, HbA_{1c}, FBG, PPG and CRP levels were significantly higher in patients with DM than controls, as expected ($p < 0.005$ for all). In addition the average GFR levels was significantly lower in DM patients than control subjects ($p \leq 0.001$) (Table 1).

In correlation analysis sVEGFR-1 and GFR levels were negatively correlated in DM patients ($r = -0.453$, $p = 0.01$) (Table 2).

Significantly higher levels of sVEGFR-1 also was observed among DM patients with β -blocker therapy than the DM patients without β -blocker therapy (0.131 ± 0.049 ; 0.095 ± 0.050 , respectively; $p = 0.012$) while significantly lower sVEGFR-1 levels were determined in DM patients receiving metformin therapy vs. without therapy (0.065 ± 0.016 vs. 0.111 ± 0.053 , respectively; $p = 0.001$) (Table 3). Logistic regression analysis was performed considering the accompanying complications and used medications. The equation did not give a significant variable among the parameters shown in Table 3 ($p > 0.05$).

The VEGF levels of diabetic patients with end stage renal disease (ESRD) were significantly lower than the patients without ESRD diabetic patients (0.107 ± 0.112 , 0.412 ± 0.223 , respectively; $p = 0.004$) while the sVEGFR-1 levels of ESRD diabetic patients were significantly higher than the diabetic patients without ESRD (0.192 ± 0.055 , 0.096 ± 0.042 , respectively; $p = 0.002$) (Table 3).

Discussion

As far as we know, this is the first study evaluating and demonstrating the importance of plasma VEGF and sVEGFR-1 levels together in DM patients receiving insulin treatment. Serum sVEGFR-1 levels were significantly higher in patients with DM than controls. In addition, significantly higher levels of sVEGFR-1 were observed among DM patients with β -blocker therapy than without therapy while significantly lower sVEGFR-1 levels were determined in patients receiving metformin therapy vs. without therapy.

Table 1: Comparison of anthropometric and laboratory features of patients with diabetes mellitus and controls.

	DM (n = 45)	Control (n = 34)	p-Value
Gender (M/F)	14/31	14/20	0.354
Age (years)	68.9 ± 9.2	69.6 ± 10.6	0.779
BMI (kg/m ²)	30.4 (21.6–39.5)	27.6 (22.1–42.9)	0.285
VEGF	0.337 (0.054–1.006)	0.335 (0.055–0.913)	0.991
sVEGFR-1	0.092 (0.019–0.223)	0.061 (0.017–0.248)	<0.001
WBC (10 ³ /μL)	7.5 ± 2.9	5.9 ± 1.4	0.002
Platelet (10 ³ /μL)	263 (112–400)	280 (162–390)	0.219
GFR (mL/min/1.73 m ²)	54.2 ± 25.9	76.4 ± 16.3	<0.001
AST (U/L)	21.3 ± 8.6	23.5 ± 6.2	0.225
ALT (U/L)	17 (5–50)	19 (6–40)	0.622
Total Protein (g/dL)	7.4 ± 1.4	7.8 ± 0.9	0.089
HbA _{1c} (%)	9.1 ± 2.6	5.3 ± 0.3	<0.001
FPG (mg/dL)	190.4 ± 86.1	93.8 ± 8.7	<0.001
PPG (mg/dL)	231.9 ± 101.8	126.2 ± 16.7	<0.001
Triglyceride (mg/dL)	141 (51–617)	150 (34–330)	0.741
T. Cholesterol (mg/dL)	182 (86–301)	196.5 (70–287)	0.205
LDL-C (mg/dL)	110 ± 40	112 ± 35	0.852
HDL-C (mg/dL)	42 ± 10	46 ± 11	0.136
ESR (mm/h)	15 (4–98)	13 (3–92)	0.392
hsCRP (mg/L)	3 (1–9.1)	1.2 (2–8.3)	<0.001

Data are expressed as the mean ± SD or median (minimum, maximum) as appropriate. (Normality of distribution was tested using Kolmogorov Smirnov or Shapiro Wilk tests where applicable).

p-Values were calculated using independent-sample t-test or Mann-Whitney U-test. BMI, body mass index; VEGF, vascular endothelial growth factor; sVEGFR-1, soluble vascular endothelial growth factor receptor 1; WBC, white blood cells; GFR, glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA_{1c}, Hemoglobin A_{1c}; FPG, fasting blood glucose; PPG, postprandial plasma glucose; T. Cholesterol, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ESR, erythrocyte sedimentation rate; hsCRP, high sensitive C-reactive protein.

As previously demonstrated by numerous studies, VEGF and its receptors promote angiogenesis, while sVEGFR-1, as a decoy receptor for VEGF, inhibits angiogenesis [14]. Our findings indicating up-regulation of serum sVEGFR-1 levels reveal that sVEGFR-1 has important

physiological effects on the inhibition mechanisms of angiogenesis as a ligand trap in diabetics and are consistent with the studies evaluating tissue levels in animals [14–16]. However, the VEGF levels in our patient group did not differ from the control group despite the change in the sVEGFR-1 receptor levels. According to some of the literature VEGF levels of DM patients were significantly higher than the control patients [17, 18].

In contrast to these literatures we have found the VEGF levels were not significantly higher than the control groups. In a crosssectional control study by Sun et al. showed that serum VEGF levels did not differ between the type 2 DM patients and the healthy controls which was found similar to our result [19]. Arnon et al. demonstrated that patients with type 2 DM with no-retinopathy and with non-proliferative retinopathy had higher levels of VEGF, which decreased in patients with diabetic proliferative retinopathy which is more severe condition as unexpected [20]. With those in mind we think that VEGF levels in DM patients receiving insulin treatment may not be significantly higher than the controls. The major difference between plasma and serum is the contribution of VEGF released from platelets and leukocytes in serum. The many points associated with analyzing circulating VEGF are described in a review by Jelkmann and demonstrated

Table 2: Correlation between serum VEGF, sVEGFR-1 levels and laboratory parameters in DM group (n = 45).

	VEGF		sVEGFR-1	
	r	p-Value	r	p-Value
VEGF	–	–	–0.231	0.126
sVEGFR-1	–0.231	0.126	–	–
WBC	–0.058	0.704	0.298	0.047
GFR	0.216	0.154	–0.466	0.001
HbA _{1c}	0.044	0.776	–0.034	0.826
FPG	–0.015	0.920	–0.273	0.069
PPG	–0.007	0.963	–0.194	0.201
hsCRP	–0.131	0.389	–0.153	0.317

Correlation analysis among variables was performed by using Pearson's correlation test. A p-value of <0.05 was considered significant. VEGF, vascular endothelial growth factor; sVEGFR-1, soluble vascular endothelial growth factor receptor 1; WBC, white blood cells; GFR, glomerular filtration rate; HbA_{1c}, Hemoglobin A_{1c}; FPG, fasting blood glucose; PPG, postprandial plasma glucose; CRP, C-reactive protein.

Table 3: Comparison of VEGF, sVEGFR-1 levels and clinical information of patients with DM.

		VEGF			sVEGFR-1		
		Mean $\pm$ SD	p^1	p^2	Mean $\pm$ SD	p^1	p^2
Control	34	0.335 (0.055–0.913)			0.061 (0.017–0.248)		
CAD (–)	34	0.335 (0.024–1.006)	0.497	–0.633	0.104 $\pm$ 0.050	0.802	–0.037
CAD (+)	11	0.315 (0.086–0.920)			0.113 $\pm$ 0.060		
HT (–)	21	0.346 (0.024–1.006)	0.907	–0.977	0.098 $\pm$ 0.050	0.296	–0.002
HT (+)	24	0.304 (0.027–0.920)			0.112 $\pm$ 0.054		
HF (–)	25	0.351 (0.055–1.006)	0.211	–0.447	0.081 $\pm$ 0.031	<0.001	–<0.001
HF (+)	20	0.303 (0.024–0.920)			0.137 $\pm$ 0.057		
ESRD (–)	40	0.351 (0.024–1.006)	0.004	–0.005	0.096 $\pm$ 0.042	0.002	–0.001
ESRD (+)	5	0.078 (0.027–0.303)			0.192 $\pm$ 0.055		
CKD (–)	35	0.320 (0.024–1.006)	0.264	–0.324	0.099 $\pm$ 0.050	0.122	–0.002
CKD (+)	10	0.503 (0.086–0.920)			0.130 $\pm$ 0.054		
Retinopathy (–)	25	0.328 (0.027–1.006)	0.780	–0.932	0.074 (0.017–0.248)	0.606	–0.019
Retinopathy (+)	20	0.337 (0.024–0.839)			0.081 (0.040–0.274)		
Nephropathy (–)	36	0.324 (0.024–1.006)	0.871	–0.955	0.100 $\pm$ 0.050	0.199	–0.004
Nephropathy (+)	9	0.073 (0.017–0.274)			0.129 $\pm$ 0.058		
ACE (–)	39	0.349 (0.024–0.920)	0.786	–0.829	0.108 $\pm$ 0.054	0.444	–0.265
ACE (+)	6	0.299 (0.195–1.006)			0.090 $\pm$ 0.039		
ARB (–)	34	0.320 (0.024–1.006)	0.183	–0.303	0.106 $\pm$ 0.054	0.970	–0.014
ARB (+)	11	0.477 (0.181–0.638)			0.105 $\pm$ 0.049		
ASA (–)	33	0.328 (0.024–0.920)	0.826	–0.766	0.106 $\pm$ 0.055	0.845	–0.012
ASA (+)	12	0.360 (0.086–1.006)			0.104 $\pm$ 0.043		
Enoxaparin (–)	39	0.328 (0.024–1.006)	0.588	–0.666	0.102 $\pm$ 0.050	0.264	–0.026
Enoxaparin (+)	6	0.453 (0.027–0.920)			0.129 $\pm$ 0.061		
β -Blocker (–)	31	0.343 (0.024–1.006)	0.816	–0.897	0.095 $\pm$ 0.050	0.012	–0.001
β -Blocker (+)	14	0.309 (0.027–0.920)			0.131 $\pm$ 0.049		
CCB (–)	37	0.321 (0.024–0.913)	0.282	–0.361	0.075 (0.017–0.274)	0.610	–0.132
CCB (+)	8	0.576 (0.027–1.006)			0.074 (0.047–0.204)		
Insulin glargine (–)	13	0.328 (0.024–0.913)	0.372	–0.530	0.065 (0.017–0.274)	0.953	–0.002
Insulin glargine (+)	32	0.337 (0.078–1.006)			0.100 (0.040–0.223)		
Insulin lispro (–)	37	0.321 (0.027–1.006)	0.270	–0.386	0.106 $\pm$ 0.056	0.713	–0.008
Insulin lispro (+)	8	0.523 (0.024–0.839)			0.105 $\pm$ 0.028		
Insulin aspart (–)	30	0.320 (0.024–1.006)	0.479	–0.475	0.095 $\pm$ 0.041	0.082	–0.001
Insulin aspart (+)	15	0.429 (0.043–0.920)			0.127 $\pm$ 0.065		
Insulin regular (–)	26	0.354 (0.024–0.920)	0.093	–0.371	0.117 $\pm$ 0.056	0.077	–0.068
Insulin regular (+)	19	0.272 (0.027–1.006)			0.090 $\pm$ 0.041		
Metformin (–)	34	0.335 (0.024–0.920)	0.366	–0.459	0.118 $\pm$ 0.053	0.001	–0.716
Metformin (+)	11	0.320 (0.208–1.006)			0.065 $\pm$ 0.016		
Statin (–)	35	0.321 (0.024–0.920)	0.168	–0.241	0.105 $\pm$ 0.053	0.785	–0.030
Statin (+)	10	0.458 (0.181–1.006)			0.107 $\pm$ 0.052		

Normality of distribution was tested using Kolmogorov Smirnov or Shapiro Wilk tests where applicable. p^1 : Comparison of the accompanied diseases or medications used in DM group, p^2 : Comparison with control group. DM, diabetes mellitus; CAD, coronary artery disease; HT, hypertension; HF, heart failure; CKD, chronic kidney disease; ACE, angiotensin-converting-enzyme; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; CCB, calcium channel blocker.

that VEGF should be analyzed in plasma [21]. Also, the use of anticoagulant and antiaggregant when collecting the plasma can affect the results [22]. Also In addition, not only the anticoagulants and antiaggregants but also the analyzing center, centrifuge, and analyzing method have been found to be independent confounders for the measurement of circulating plasma levels of VEGF [23]. With that in mind it is because of that difficult to compare different studies reporting on levels of VEGF in the blood

since there are a lot of aspects that can influence the results. Apart from the VEGF resistance encountered in patients with DM, our findings reveal the importance of targeting sVEGFR-1 to avoid common microvascular complications and modulate angiogenesis in diabetes. No significant difference was found in the multiple regression analysis. Due to the fact that reason sVEGFR-1 was found not to be an independent risk factor for DM and its complications. Considering the physiopathology of angiogenesis

in DM patients receiving insulin treatment, the statistical analyses revealed that sVEGFR-1 may be included in this process.

The negative correlation between GFR and serum sVEGFR-1 levels and the increase in sVEGFR-1 levels with the development of renal failure in diabetic patients reveal that this pathology is based on the inhibition of angiogenesis. In our subgroup analyzes, serum levels of VEGF were decreased while sVEGFR-1 values were increased in diabetic patients with ESRD. It is well known diabetic nephropathy is a poor prognostic factor in patients with DM and circulating VEGF and its soluble receptor sVEGFR-1 levels are possibly associated with the emergence of this pathology.

In the comparison of VEGF, sVEGFR-1 levels and medication status of patients with DM, we observed that the average level of serum sVEGFR-1 levels in patients using β -blockers was significantly higher. There are studies reporting that β -blockers suppress the angiogenesis in the literature [24, 25]. In a study by Ding et al., propranolol was reported to inhibit human umbilical vascular endothelial cell proliferation and migration, as well as the VEGF-induced activation of VEGFR-2 [24]. In another recent study, the effect of carvedilol has been revealed as inhibiting VEGF-induced angiogenesis by blocking the VEGF/VEGFR-Src-ERK-mediated pathway [25]. In addition, in a study investigating whether β -blockers could be used in combination with chemotherapy for the treatment of cancer, Pasquier et al. found that some β -blockers (i.e. propranolol, carvedilol, and nebivolol) were consistently able to inhibit angiogenesis in vitro and potentiate the anti-angiogenic and anti-proliferative effects of chemotherapy agents [26]. These findings support our results indicating the inhibitory effect of β -blocker usage on angiogenesis in diabetic patients.

It is conceivable that the combination of angiogenesis inhibitors and metformin may be effective for arresting the carcinogenic process [27]. It has been shown that metformin also significantly decreases the levels of VEGF and there is a study revealing the reduction of inflammatory angiogenesis by metformin in a murine sponge model [28]. Ersoy et al. indicated that, metformin addition had beneficial effect on VEGF and PAI-1 levels in obese type 2 diabetic patients under medical nutrition treatment + regular exercise program, independent from its favorable effects on BMI and glycemic control in contrast to the literatures above [29]. In our study we found average VEGF levels of patients with DM using metformin was higher than the patients not using metformin. A further study with more participants may reveal a significant result.

In the current study, lower sVEGFR-1 levels were determined in DM patients receiving metformin therapy vs. without therapy. This emphasizes the efficacy of metformin in lowering anti-angiogenic factors particularly in DM patients receiving insulin treatment. Consistent with these studies and based on significantly lower sVEGFR-1 levels in DM patients receiving metformin therapy vs. without therapy in our patient group, it can be suggested that the use of metformin may improve angiogenesis by reducing sVEGFR-1 levels. On the other hand, in the same patient group, there was no effect of insulin administration on sVEGFR-1 levels.

In addition, serum sVEGFR-1 levels in patients receiving regular insulin therapy were decreased, however, this decrease was not significant compared to those who do not use. We think that significant results can be found in a study performed in a larger group of patients.

The present study has some limitations. Firstly, our study is limited to analysis of only one angiogenic molecule, VEGF and one of its soluble receptor sVEGFR-1, which makes it difficult to evaluate the imbalance of angiogenic/anti-angiogenic factors in patients with DM since other angiogenic factors have not been evaluated concurrently. However, we think that our study is meaningful and valuable with the reason that it has not been studied before in the literature and it is a clinical study. Finally, this study is based on a limited number of patients and thus cannot ascertain whether these findings apply to other patients with DM. Accordingly, larger clinical studies will be necessary for confirmation of these findings.

In conclusion, metformin may have a positive effect on angiogenesis in diabetic patients by decreasing and β -blockers may have a negative effect on angiogenesis via increasing sVEGFR-1 levels. Although no solid conclusion can be drawn from our study because of the small numbers of patients, it increases awareness about the physiopathological role of sVEGFR-1 in DM patients on insulin treatment and the need of further studies.

Conflict of interest statement: None.

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