ORIGINAL ARTICLE

Aqueous concentrations of VEGF and soluble VEGF receptor-1 in diabetic retinopathy patients

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Background: The aim of this study was to simultaneously measure the concentrations of vascular endothelial growth factor (VEGF) and soluble VEGF receptor-1 (sVEGFR-1, also known as sFlt-1) in the aqueous humor of patients with non-proliferative diabetic retinopathy (NPDR) and to investigate whether aqueous levels of vascular endothelial growth factor (VEGF) and VEGFR-1 are related to diabetic macular edema. **Materials and Methods:** Aqueous humor was collected from 27 diabetic patients and 33 age- and sex-matched normoglycemic controls and analyzed for pro-angiogenic VEGF and angiogenic inhibitor VEGFR-1 by enzyme-linked immunosorbent assay (ELISA). The mean foveal thickness was measured by optical coherence tomography (OCT). **Results:** There was no significant difference in the aqueous levels of VEGF in patients with NPDR compared with control subjects ($P > 0.05$), while the NPDR patients had significantly lower sVEGFR-1 in their aqueous humor. Furthermore, a significant ($P < 0.01$) positive correlation was observed between VEGF/sVEGFR-1 concentration and the mean foveal thickness measured on OCT. **Conclusion:** The results suggest that decreased chelating effect of sVEGFR-1 may be the preliminary event allowing VEGF to activate the proangiogenic endothelial cell state and to induce permeability. The imbalance between angiogenic agent (VEGF) and the antiangiogenic factors (sFlt-1), which is disturbed in the diabetic state, may determine the fate of diabetic macular edema.

Key words: Diabetic macular edema, diabetic retinopathy, neovascularization, soluble vascular endothelial growth factor receptor-1, vascular endothelial growth factor

INTRODUCTION

Nearly half of the world's diabetic people have certain degree of diabetic retinopathy,[1] and diabetic macular edema (DME) is the most frequent cause of visual impairment in these patients.[2]

The early stage of the disease, termed non-proliferative diabetic retinopathy (NPDR), is associated with the swelling of the retina ensuing from the leakage and accumulation of extracellular fluid and proteins in the macula. Exudation arises from structural changes in the endothelium of retinal vasculature that lead to the breakdown of the blood–retina barrier (BRB) and a rise in vascular permeability.[3-5] These processes may cause reversible decrease in visual acuity at first, but over time the injured neurons die due to excess interstitial fluid and permanent visual loss occurs.[5] Characterization of the underlying pathophysiological mechanism involved in this process has resulted in the recognition of the angiogenic growth factor and vascular permeability factor/vascular endothelial growth factor (VEGF) as a key molecule in the retinal microvascular complications of diabetes.[6]

In the normal healthy adult eye, active angiogenesis does not occur despite the presence of proangiogenic agents in these healthy tissues. This is achieved by the predominant presence of antiangiogenic molecules, a number of which have been isolated from the eye.[7] One such important factor is soluble VEGF receptor-1 (sVEGFR-1, also known as sFlt-1). sVEGFR-1 has attracted considerable attention for its potential clinical application as an inhibitor of angiogenesis. sVEGFR-1 is the secreted extracellular domain of VEGF receptor 1, which lacks the membrane-proximal immunoglobulin-like domain, transmembrane-spanning region, and intracellular tyrosine-kinase domain.[8] Its angiostatic effects are exerted via two inhibitory mechanisms: by sequestering VEGF, and inactive heterodimer formation with two membrane-spanning isoforms of the VEGF receptor, VEGFR-1 and VEGFR-2.[9]

In DME, the non-perfused hypoxic retina produces angiogenic growth factors such as VEGF, which is a

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potent inducer of vascular permeability and angiogenesis.[3] There are evidences that hypoxia can increase sVEGFR-1 expression.[10] However, the mechanism that regulates the balance between VEGF and sVEGFR-1 in the endothelial microenvironment and the role of sVEGFR-1 in development of retinopathy remains unknown.

The aim of this study was to investigate whether patients with DME have imbalanced VEGF and sVEGFR-1 expression, and whether this ratio correlated with changes in macular thickness and edema.

MATERIALS AND METHODS

This nonrandomized, observational, case control study was approved by the ethical committee of Isfahan University of Medical Sciences. It conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000). Written informed consent was obtained from all patients participating in the study and patient anonymity had been preserved. Inclusion criteria for diabetic group were patients with NPDR without any previous retinal laser therapy. Patients who had any ocular disease except cataract were excluded especially if macula was involved. Furthermore, the patients were excluded if there was any complication during cataract surgery. At the screening visit, a comprehensive ophthalmic evaluation was performed and included a medical history, blood pressure (BP) measurement, applanation tonometry, slit-lamp examination, dilated fundus biomicroscopy, and ophthalmoscopy, as well as optical coherence tomography (OCT) evaluation (OCT/SLO, model 2006; Ophthalmic Technologies Inc., Ontario, Canada). Central macular thickness (CMT) was defined as the average thickness of a central macular area 500 μm in diameter centered on the patient's foveola. Total macular volume (TMV) values were automatically generated by built-in OCT software. The severity of diabetic retinopathy was diagnosed at the preoperative visit using slit-lamp funduscopic biomicroscopy and classified as no retinopathy, mild NPDR, moderate NPDR, and severe NPDR based on the International Clinical Diabetic Retinopathy Disease Severity Scale.[11]

The patients were chosen by convenience sampling. All patients visiting the Isfahan central eye clinic, Iran, from January 2009 to September 2010 were enrolled in this study according to the inclusion criteria and on a voluntary basis.

Aqueous samples were obtained from 27 diabetic patients (6 patients participated with 2 eyes) immediately before the intravitreal injection of bevacizumab. Thirty-three nondiabetic patients participated as controls and their aqueous fluids were acquired at the time of cataract surgery. Anterior chamber paracentesis was performed and 50–100 μl of aqueous humor was withdrawn using a tuberculin syringe attached to a 30-gauge needle.

The first group patients were type 2 diabetics with DME meeting the criteria of the NPDR according to Early Treatment Diabetic Retinopathy Study (ETDRS) report. Twenty-seven eyes representing 21 individuals had persistent DME for an average of 13 months. No eyes had ocular hypertension, vitreous hemorrhage, or previous macular laser therapy.

VEGF and VEGFR-1 in the aqueous samples were quantified using commercially available enzyme-linked immunosorbent assays (ELISAs) (R and D Systems, Minneapolis, USA) according to manufacturer's instruction. The VEGF ELISA has a detection limit of approximately 5 pg/ml, whereas the VEGFR-1 ELISA has a sensitivity of approximately 3.5 pg/ml.

All analyses were performed using SPSS software version 14. Comparisons of the aqueous VEGF and sVEGFR-1 levels were performed using the *t*-test. The Pearson correlation coefficient was used to examine for a correlation between aqueous sVEGFR-1 levels and mean CMT. Clinical outcomes and CMT were compared between the two groups using the *t*-test. $P < 0.05$ was considered significant. Tukey's *post-hoc* test was used to determine the significant differences in two groups of analytes.

RESULTS

NPDR patients' characteristics have been illustrated in Table 1. Also, 33 age- and sex-matched nondiabetic participants were recruited as the control group which included 14 males and 19 females. The mean of their ages was 68.16 ± 10.5 years.

There was no significant difference in the aqueous levels of VEGF between NPDR patients and the control group ($P = 0.2$) [Figure 1], but it had a significant correlation with macular thickness ($r = 0.51$, $P = 0.04$). It reveals that increase in VEGF concentration has more relevance with edematous changes in macula than retinal changes as NPDR in diabetic patients. The aqueous sVEGFR-1 concentration in control group was greater than in NPDR patients ($P = 0.03$) [Figure 1], but there was no correlation between sVEGFR-1and macular thickness. The correlation between VEGF/sVEGFR-1 and macular thickness was significant ($r = 0.46$, $P = 0.05$) which suggests that imbalance between VEGF and its soluble receptor results is the primary event in macular edematous change. Mean macular thickness in diabetic patients was 465.8 ± 193 μm.

Table 1: The basic characteristics of diabetic eyes with clinically significant macular edema

	Sex (m/f)	Age* [year]	Duration of DM*[year]	IOP* [mmHg]	VA* [logMAR]	Macular thickness [μm]*
Eyes with CSME	10/8	62.73 ± 9.3	16.07 ± 8.51	16.5 ± 4.16	0.38 ± 0.25	378.06 ± 123.68
Eyes with VS CSME	4/4	64.25 ± 4.4	14.25 ± 7.86	14.3 ± 2.92	0.32 ± 0.44	612.25 ± 212.55

VS CSME = very severe clinically significant macular edema, IOP = , VA = visual acuity, *Mean ± standard deviation

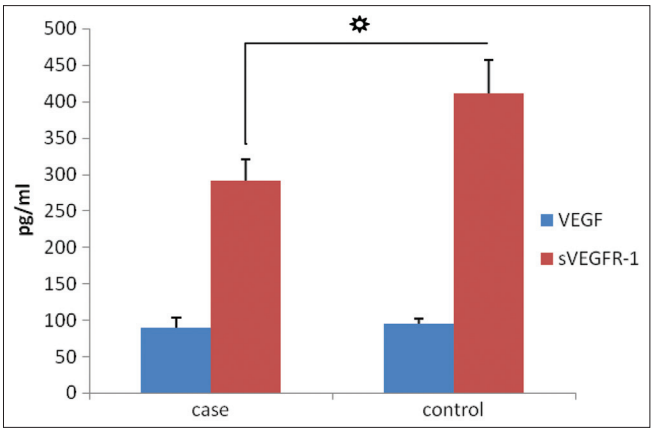

Figure 1: There was no significant difference in the aqueous levels of VEGF between patients with NPDR and control subjects ($P > 0.05$), while the NPDR patients had significantly lower sVEGFR-1 in their aqueous humor ($P < 0.05$)

DISCUSSION

In this study, we investigated whether the aqueous levels of VEGF and sVEGFR-1were related to vascular permeability and the severity of DME in NPDR patients. We obtained the following findings: (1) there was no significant difference in the aqueous fluid levels of VEGF in NPDR patients compared with nondiabetic patients, whereas that of sVEGFR-1 was significantly decreased in NPDR patients compared with nondiabetic patients; (2) there was no significant correlation between the aqueous level of sVEGFR-1 and mean CMT; but (3) VEGF/sVEGFR-1 had significant correlation with macular thickness.

VEGF is a potent vasopermeability factor[12] that may be involved in the pathogenesis of BRB breakdown in diabetes. Retinal VEGF levels are upregulated in diabetes, and this increase coincides with BRB breakdown in rodents and humans.[13] Several previous reports investigate the role of VEGF and some anti-antigenic molecules in increasing vascular permeability and angiogenesis in proliferative diabetic retinopathy (PDR) patients;[14-18] however, there are limited evidences about the role of anti-angiogenic molecules in NPDR patients. Since diabetic retinopathy is a multi-step process, it can be postulated that the unchanged level of VEGF in NPDR patients in our study may be related to early steps of this phenomena.

Similar to our results, Patel *et al*., in their elegant study, have shown for the first time that in patients with NPDR and macular edema, the sVEGFR-1 levels decreased compared to the levels in PDR patients, while the Pigment Epithelium Derived Factor (PEDF) concentrations were similar.[6] This suggests that decreased chelating effect of sVEGFR-1 may be the preliminary event allowing VEGF to activate the proangiogenic endothelial cell state and to induce permeability. They proposed that further decreased sVEGFR-1 concentration combined with a significant decrease in PEDF enables VEGF to produce the angiogenesis seen in active PDR.

Hazarika *et al*.[19] showed decreased expression of both full-length and soluble VEGFR-1 in diet-induced, type 2 diabetic (DM) mice. The endothelial cell is a major cell type that expresses[20] and deposits sVEGFR-1 in the adjacent extracellular matrix.[21] Endothelial-derived sVEGFR-1 sequesters exogenous VEGF,[22] suggesting a role as a natural inhibitor of paracrine VEGF signaling in endothelial cells to maintain normal vascular quiescence. Because VEGF-dependent activation of VEGF receptor-2 (VEGFR-2) on endothelial cells is an indispensible prerequisite for VEGF-driven angiogenesis,[23] paracrine VEGF needs to escape binding to sVEGFR-1 to bind to endothelial VEGFR-2 in the shift toward the proangiogenic state.[24] So, decreased VEGFR-1 may be the primary pathophysiological problem in diabetic retinopathy.

In conclusion, the unchanged level of VEGF in NPDR patients in our study, as well as decreased VEGFR-1 may be related to early steps of NPDR. Although the physiologic importance of maintaining normal vascular integrity is well known, our understanding of how vascular integrity is maintained and whether vascular permeability can be downregulated remains incomplete. The balance between angiogenic agent (VEGF) and the antiangiogenic factors (sFlt-1), which is disturbed in the diabetic state, and the degree to which the antiangiogenic agents are reduced may determine the level of diabetic retinopathy or maculopathy seen.

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