Concentration Gradient of Vascular Endothelial Growth Factor in the Vitreous of Eyes with Diabetic Macular Edema

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Purpose. Vascular endothelial growth factor (VEGF) concentrations in various regions of the vitreous were examined in patients with diabetic macular edema (DME) to explore the possibility of a concentration gradient in the vitreous.

Methods. Vitreous samples were collected during vitrectomy from 71 eyes of 71 patients with DME and without posterior vitreous detachment who had not undergone panretinal photocoagulation. Vitreous samples were collected from the premacular vitreous and mid-vitreous (group A, 35 eyes), and from the premacular vitreous and peripheral cortical vitreous (group B, 36 eyes). Mean foveal thickness was measured by optical coherence tomography (OCT). Ten eyes of 10 patients with stage 3 macular hole (MH) served as controls. Vitreous VEGF levels were measured by enzyme-linked immunosorbent assay.

Results. The VEGF concentration (mean ± SD; pg/mL) was significantly higher in the premacular vitreous (1386.2 ± 2134.1) than in the peripheral cortical vitreous (1169.7 ± 1840.3; $P = 0.0216$) and mid-vitreous (1080.9 ± 1534.1; $P = 0.0017$). The mean foveal thickness measured on OCT correlated significantly with VEGF concentrations in the premacular vitreous, peripheral cortical vitreous, and mid-vitreous ($R > 0.62$, $P < 0.0001$, for all). In controls, VEGF concentrations in the premacular vitreous, peripheral cortical vitreous and mid-vitreous were all below the detection limit (<20 pg/mL).

Conclusions. In DME, vitreous VEGF concentration correlates with mean foveal thickness measured on OCT. VEGF concentration was higher in premacular vitreous than in mid-vitreous and peripheral cortical vitreous, suggesting diffusion from the macular region to the periphery, and from the posterior to the anterior globe. (*Invest Ophthalmol Vis Sci.* 2009;50: 2953–2955) DOI:10.1167/iovs.08-2870

The vitreous body is composed of collagen and hyaluronic acid, with a high water content. Due to its structure, the migration of substances in the vitreous body is believed to be slow.[1] After intravenous injection of fluorescein, the dye diffuses from the retinal vessels into the vitreous. The presence of a concentration gradient in the vitreous has been reported.[2,3]

The development and progression of diabetic macular edema (DME) have been shown to be associated with several cytokines, including vascular endothelial growth factor (VEGF).[4–6] Aiello et al.[6] initially reported VEGF concentrations in the vitreous of patients with diabetic retinopathy and other retinal disorders, and they demonstrated higher concentrations of VEGF in vitreous fluid than in aqueous fluid in patients with proliferative diabetic retinopathy. Their study suggested a VEGF concentration gradient between the vitreous and anterior chamber. VEGF has a low molecular weight of 38 kDa, and is hydrophilic, water soluble, and diffusible. VEGF is continuously produced by the retina and diffuses into the vitreous in DME, as implied from the clinical study finding that chronic DME was controlled by intravitreal injection of a specific VEGF blocker and relapsed when the therapeutic effect disappeared.[7] Whether the VEGF concentration in the vitreous is homogeneous, or a concentration gradient exists, remains unknown. In DME, if retinal VEGF production is higher in the macular region than in the periphery, there may be a difference in the VEGF concentration of the preretinal vitreous. However, this possibility has not been examined clinically, partly because the vitreous is a gel-like structure and collection of samples from different regions is difficult.

In the present study, we measured the VEGF concentrations in different regions of the vitreous to examine whether a VEGF concentration gradient exists in the vitreous.

Methods

Seventy-one eyes of 71 patients with type 2 diabetes (ages 35 to 78 years, average 63.2 ± 9.0 years) with clinically significant DME, with neither posterior vitreous detachment nor vitreous hemorrhage, who underwent vitrectomy between April and October of 2007, were studied. None of the patients with DME had been treated with triamcinolone, bevacizumab, ranibizumab, or pegaptanib before vitrectomy. All surgeries were performed by one ophthalmic surgeon (HS) at the Surugadai Hospital of Nihon University. These patients with DME had a disease duration of 12.4 ± 5.8 years and hemoglobin A_{1c} of 7.3% ± 1.9%. The indications for vitrectomy in this study were diffuse macular edema and cystoid macular edema, evidence of a taut posterior hyaloid face, preoperative visual acuity less than 0.5 as measured with the standard Japanese decimal visual acuity chart, and no response to macular photocoagulation therapy. Exclusion criteria were prior ocular surgery except laser photocoagulation within 3 months; a history of ocular inflammation; proliferative diabetic retinopathies with fibrovascular proliferation causing traction retinal detachment; ophthalmic disorders associated with macular edema (such as uveitis and branch or central retinal vein occlusion); a history of panretinal photocoagulation, and rubeosis irides. Preoperative fundus color photography and fluorescein angiography were performed using a fundus camera (Topcon, Tokyo, Japan). Forty-six patients had diffuse macular edema and 25 had cystoid macular edema. Diffuse macular edema was diagnosed based on the detection of leakage from diffusely dilated retinal capillaries throughout the posterior pole. Cystoid macular edema was diagnosed based on a petaloid pattern of hyperfluorescence. Posterior vitreous detachment was diagnosed during surgery, by the presence of a Weiss ring. The mean foveal thickness was the mean thickness of the central 1-mm-diameter circle of nine macular zones measured by optical coherence tomography (OCT, Humphrey model 3000; Humphrey Instruments, San Leandro, CA), and the data with SD reported on the

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TABLE 1. Sites of Vitreous Sample Collection, VEGF Concentrations, and Relationships with Mean Foveal Thickness

Sites of Vitreous Sample Collection (number of eyes)	VEGF (pg/ml)	Mean Foveal Thickness (μm)	VEGF vs. Mean Foveal Thickness*
Premacular vitreous (71)	1386.2 ± 2134.1†‡	446.9 ± 159.9	$R = 0.654$ $P < 0.0001$
Peripheral cortical vitreous (35)	1169.7 ± 1840.3†		$R = 0.628$ $P < 0.0001$
Mid-vitreous (36)	1080.9 ± 1534.1‡		$R = 0.636$ $P < 0.0001$

Paired *t*-test: *Spearman's correlation coefficient; †$P = 0.0216$; ‡$P = 0.0017$.

device were used. The research was approved by the IRB at Nihon University, School of Medicine. Informed consent was obtained from each subject after an explanation of the purpose and potential adverse effects of the procedure were given. These patients were treated in accordance with the Declaration of Helsinki.

Vitrectomy was performed to improve visual acuity and decrease retinal thickness in the macula. First, trocars were inserted at three sites 4 mm from the limbus. Under noninfused conditions, a contact lens (Hoya, Tokyo, Japan) was placed on the cornea, and a 25-gauge cutter was inserted up to a point just anterior to the macular region using a light guide. The aspiration line of the cutter was connected to a 2.5-mL syringe. Because collection of a portion of the vitreous body would result in movement of the vitreous, the premacular vitreous, presumed to have a higher VEGF concentration, was collected first. The premacular vitreous was collected by placing the 25G cutter 1 mm anterior to the macular area. Since this region coincides with the vitreous pocket, the premacular vitreous sample was obtained by collecting the liquid in the vitreous pocket using aspiration alone. After flushing the aspiration line with air, the 25-gauge cutter was inserted into the mid-vitreous or the peripheral cortical vitreous, and the vitreous was excised and aspirated into another 2.5-mL syringe. The mid-vitreous sample was collected from the center of the vitreous approximately 10 mm from the retina. The peripheral cortical vitreous sample was collected from the retinal vascular arcade region at 1 mm anterior to the equatorial retina. Vitreous samples of 0.3 mL each were collected from the premacular vitreous and mid-vitreous in group A (35 eyes), and from the premacular vitreous and peripheral cortical vitreous in group B (36 eyes). Ten eyes of 10 patients (age 52 to 71 years, average 63.1 ± 6.9 years) with stage 3 macular hole (MH) served as controls. Vitreous samples were collected from the premacular vitreous and mid-vitreous of 5 eyes, and from the premacular vitreous and peripheral cortical vitreous of 5 eyes.

Infusion was started immediately after collection of the vitreous samples, cataract surgery was performed, and an intraocular lens was inserted. The vitrectomy was then performed.

Samples of vitreous were transferred into tubes and rapidly frozen at −70°C. The VEGF concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) for human VEGF (R&D Systems, Minneapolis, MN).

RESULTS

In patients with DME, the VEGF concentration (mean ± SD [range]) was significantly higher in the premacular vitreous (1386.2 ± 2134.1 [20–12,600] pg/mL) than in the peripheral cortical vitreous (1169.7 ± 1840.3 [20–9120] pg/mL; $P = 0.0216$) and mid-vitreous (1080.9 ± 1534.1 [20–5790] pg/mL; $P = 0.0017$).

Based on OCT findings, the mean foveal thickness (mean ± SD [range]) was 446.9 ± 159.9 [161–890] μm. A significant correlation was observed between mean foveal thickness measured on OCT and VEGF concentrations in premacular vitreous, peripheral cortical vitreous and mid-vitreous (Table 1 and Fig. 1).

In patients with MH, VEGF concentrations in the vitreous pocket, peripheral cortical vitreous, and mid-vitreous were all below the detection limit (<20 pg/mL).

There were no complications associated with the collection of vitreous samples.

DISCUSSION

Aiello et al.[6] measured VEGF concentrations in the anterior chamber and vitreous, and suggested VEGF diffusion from the vitreous toward the anterior chamber in patients with proliferative diabetic retinopathy. The present study demonstrated directly that VEGF concentration was higher in the premacular vitreous than in the mid-vitreous and peripheral cortical vitreous in eyes with DME. VEGF is produced mainly by retinal neurons and Müller cells, and is found in capillary pericytes and endothelial cells.[8,9] The VEGF produced in the retina diffuses into the vitreous. Therefore, it is reasonable to suggest that the VEGF concentration is higher in regions near the retina than in the mid-vitreous. A concentration gradient in the vitreous may exist in an anterior posterior as well as a radial direction.

To explore this issue further, we compared VEGF concentrations in the premacular vitreous and peripheral cortical vitreous in patients with DME, and found the level to be higher in the former. Higher VEGF expression in the macular than in the peripheral retina in DME may account for the higher VEGF concentration in the premacular vitreous than in the periph-

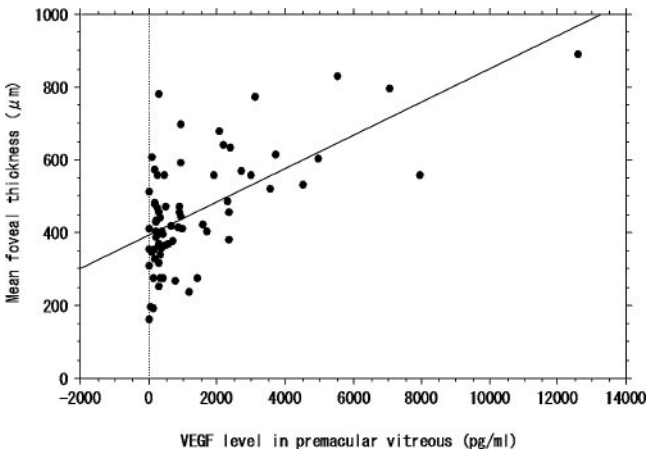

FIGURE 1. Correlation between mean foveal thickness measured on OCT and VEGF concentrations in premacular vitreous. Analysis was performed by Spearman's correlation coefficient method. $R = 0.654$, $P < 0.0001$.

eral cortical vitreous. Our results thus verified that a VEGF concentration gradient exists in the vitreous; the concentration was higher around the premacular region and lower in the center and peripheral regions. However, we cannot rule out the possibility that this finding may be influenced by the fact that there are normally more cells in the macular retina than in the peripheral retina.

Recent studies have shown that VEGF causes conformational changes of the tight junctions of retinal vascular endothelial cells and plays a major role in the elevated vascular permeability in diabetic eyes. The vitreous VEGF level correlates significantly with the severity of diabetic retinopathy. The vitreous VEGF level also correlates significantly with retinal thickness at the fovea.[10–12] Intraocular injections of ranibizumab significantly reduce foveal thickness and improve visual acuity in patients with DME, which demonstrates that VEGF is an important therapeutic target for DME.[13]

In this study, VEGF concentrations in the premacular vitreous, peripheral cortical vitreous, and mid-vitreous show strong correlation with the mean foveal thickness. While these findings demonstrate that VEGF levels in the vitreous are associated with the presence and severity of DME, they do not prove cause and effect. The role of VEGF in the production of diabetic macular edema can only be proven by interventional approaches, and hopefully a conclusion may be drawn when current clinical trials of anti-VEGF agents for diabetic macular edema are completed.

Worst[14] and Kishi et al.[15] reported the presence of a liquefied lacuna, termed the bursa premacularis or posterior precortical vitreous pocket, in the vitreous body anterior to the macular region. Spaide[16] measured the vitreous pocket by B-scan ultrasonography, and reported the horizontal diameter to be 10.3 mm, and the vertical diameter 11.1 mm. From these two-dimensional ultrasonographic measurements, the volume was estimated to be 0.6 to 0.7 mL. In the present study, since the premacular vitreous sample was collected from the vitreous pocket, we collected 0.3 mL of the liquid by aspiration alone. In contrast, mid-vitreous and peripheral cortical vitreous samples were collected by vitrectomy. Since the vitreous has a gel-like structure, selective sampling is possible to a certain extent by defining the method of collection and the site of collection.

While we restricted our subjects in the present study to patients without posterior vitreous detachment, vitreous syneresis or vitreous condensation occurs in some patients with DME. In addition, although the peripheral cortical vitreous or mid-vitreous sample was collected after the liquid in the premacular vitreous had been aspirated, movement of vitreous liquid is possible. This possibility can only be verified by animal experiments in which the eye can be removed for examination. Even considering the above factors, the VEGF concentration in the premacular vitreous is definitely higher than those in other sites of the vitreous in DME. However, the specificity of the VEGF assay needs to be validated, in particular with respect to related proteins, inactive forms, and metabolic products.

Patients with diabetes may manifest DME, proliferative diabetic retinopathy (PDR), or both. Inclusion of another control group consisting of patients with PDR but not DME in a study similar to the present work might provide more information on the role of VEGF in the development of DME. However, most patients do not have hemorrhage but do have traction retinal detachment and partial posterior vitreous detachment. In these patients, it is not possible to collect vitreous at different sites. Therefore, these patients were not included in the present study.

In conclusion, VEGF concentrations in the vitreous correlate with mean foveal thickness measured on OCT in patients with DME. In these patients, VEGF concentration was higher in the premacular vitreous than in the mid-vitreous and peripheral cortical vitreous, suggesting diffusion from the macular region to the periphery, and from the posterior forward to the anterior globe.

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