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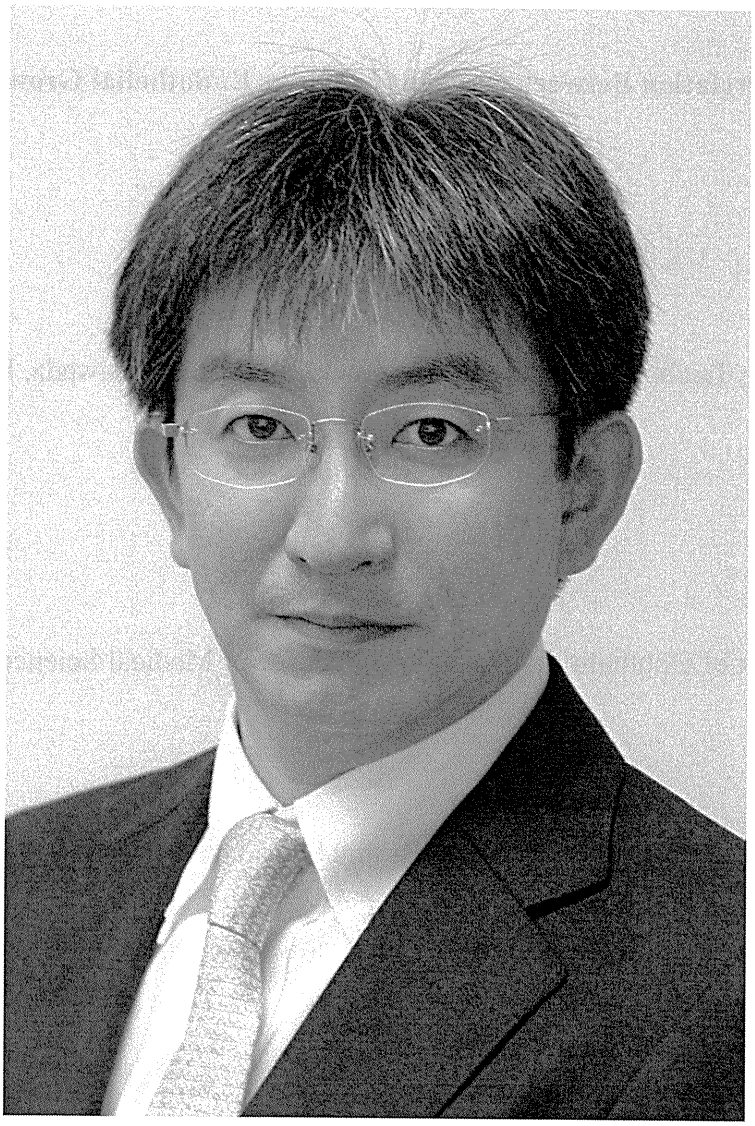

Biosketch

Yasushi Ikuno, MD, graduated from Osaka University Medical School, Japan, in 1990. He completed his residency and retinal fellowship program in Osaka University Hospital from 1990 to 1996. He became an assistant professor of Department of Ophthalmology, Osaka University Medical School in 1997 and has been working for more than 10 years. Currently he is an Associate Professor, and his main research interest is myopia-specific macular diseases and retinal/choroidal imaging.

Negative Correlation Between Aqueous Vascular Endothelial Growth Factor Levels and Axial Length

Aqueous VEGF levels and axial length

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Abstract

Purpose: The aim of this study was to evaluate the relationship between concentration of vascular endothelial growth factor (VEGF) in the aqueous humor and axial length.

Methods: Aqueous humor samples were obtained from 60 eyes of 60 patients without ocular diseases other than cataract. No patient with diabetes mellitus was included. The VEGF concentration in the aqueous humor was measured using an enzyme-linked immunosorbent assay.

Results: The VEGF concentrations in the aqueous humor samples ranged from 25 to 241 pg/mL (mean ± standard deviation [SD], 116.6 ± 46.7 pg/mL). The axial lengths ranged from 20.98 to 31.95 mm (mean ± SD, 24.09 ± 2.06 mm). The VEGF concentrations in the aqueous humor samples were correlated with axial length (Pearson product moment correlation test, $\rho = -0.373$; $P = 0.003$).

Conclusions: Concentration of VEGF in the aqueous humor is negatively correlated with axial length.

Keywords: vascular endothelial growth factor, aqueous humor, cataract, axial length

Introduction

Vascular endothelial growth factor (VEGF) is a pathogenic factor that affects the clinical condition in vitreoretinal diseases. The intraocular VEGF level is elevated in diabetic retinopathy, retinal vein occlusion, and retinopathy of prematurity [1-7]. Anti-VEGF drugs are widely used to treat retinal diseases such as age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), and macular edema secondary to retinal vein occlusion [8-19]. Some phenomena concerning VEGF remain puzzling, one of which is the lesser severity of diabetic retinopathy in patients with myopia than in patients with emmetropia or hypermetropia [20-22]. Another is the significantly lower VEGF concentration in the aqueous humor of eyes with myopic choroidal neovascularization (mCNV) [23, 24], although intravitreal injection of bevacizumab, an anti-VEGF antibody, is effective for treating mCNV [25-27]. The above-described phenomena seem to be ~~related~~ correlated with myopia or axial length.

Despite the attention that VEGF has been attracting, to the best of our knowledge, no reports have been published on the relationship between the aqueous VEGF level and the axial length of "normal" eyes. Therefore, we measured the VEGF concentration in the aqueous humor of patients without ocular diseases other than cataract and without diabetes mellitus and evaluated the relationship between the VEGF concentration and the axial

length.

Methods

In this prospective study, we measured the VEGF concentration in the aqueous humor of 60 eyes of 60 patients (20 men, 40 women) without ocular diseases other than cataract. We excluded patients with myopic changes such as staphyloma and myopic atrophy and patients with diabetes mellitus. The mean patient age was 72.1 years (range, 44-89). No ocular treatments including steroids and ocular surgery were administered before the cataract surgery.

Undiluted aqueous humor samples (0.2 mL) were obtained from the eyes of the patients immediately before the cataract surgery. All samples were collected using standard aseptic techniques that included the use of topical povidone-iodine and levofloxacin drops. The samples were stored in a freezer at −80°C until analysis.

The VEGF concentration in the aqueous humor was measured by an enzyme-linked immunosorbent assay for human VEGF (R&D Systems, Minneapolis, MN, USA). The primary antibody against VEGF detected 2 ($VEGF_{121}$ and $VEGF_{165}$) of the 4 VEGF isoforms [27]. The assay was performed according to the manufacturer's instructions. A standard curve was plotted from the measurements made with the standard

solution from 20 to 1000 pg/mL for VEGF, and the concentration of VEGF in the sample was determined.

The axial length was measured using the IOLMaster (Carl Zeiss Meditec, Jena, Germany).

The data were analyzed using SigmaStat software (version 3.1; Systat Software, Richmond, CA, USA) and expressed as the mean ± standard deviation (SD). An unpaired *t* test was used to evaluate the difference in the VEGF concentration of the aqueous humor samples between men and women. The Mann-Whitney test was used to evaluate the difference between men and women in axial lengths. The Pearson product moment correlation test was used to evaluate the correlation between the VEGF concentrations in the aqueous humor and age or axial length. A probability value less than 0.05 was considered statistically significant.

This study was approved by the institutional review board of Shiga University of Medical Science Hospital. All patients provided written informed consent, including consent to obtaining aqueous samples for measurement of the aqueous VEGF concentration.

Results

86 The VEGF concentrations in the aqueous humor of patients with cataract ranged from 25 to
87 241 pg/mL (mean ± SD, 116.6 ± 46.7 pg/mL). The axial lengths of the eyes with cataract
88 ranged from 20.98 to 31.95 mm (mean ± SD, 24.09 ± 2.06 mm).

89 The correlation between the VEGF concentration in the aqueous humor and age or
90 axial length was evaluated. The VEGF concentration in the aqueous humor was negatively
91 correlated with axial length in eyes with cataract (Pearson product moment correlation test,
92 $\rho = -0.373$; $P = 0.003$) (Figure 1). The regression line using the VEGF concentration as an
93 outcome variable (y) and the axial length as a predictor variable (x) was $y = -9.156x +$
94 337.226. The VEGF concentration in the aqueous humor was not significantly correlated
95 with age (Pearson product moment correlation test, $\rho = 0.173$; $P = 0.185$) (Figure 2). The
96 VEGF concentrations in the aqueous humor samples from men ranged from 25 to 241
97 pg/mL (mean ± SD, 108.5 ± 54.4 pg/mL) and in women from 31 to 228 pg/mL (mean ± SD,
98 120.7 ± 47.2 pg/mL). No significant difference was found between men and women in the
99 VEGF concentrations in the aqueous humor samples (unpaired *t* test, $P = 0.381$) (Figure 3),
100 nor in the axial lengths (Mann-Whitney test, $P = 0.185$).

101

102 **Discussion**

103 We measured the VEGF concentrations in the aqueous humor samples from patients

104 without ocular diseases other than cataract and without diabetes mellitus and found that the
105 VEGF concentration was negatively correlated with axial length.

106 Several explanations for the negative correlation between VEGF concentration in the
107 aqueous humor and axial length are possible, one of which is that the VEGF in the anterior
108 chamber and vitreous cavity might be diluted as a result of longer axial length and therefore,
109 greater intraocular volume.

110 To evaluate this explanation, regression analysis of the VEGF concentrations in eyes
111 with cataract in relation to axial length was performed, and we compared the value
112 according to the regression line with the value calculated by the dilution ratio. It may have
113 been better to evaluate the relationship between the VEGF concentration in aqueous humor
114 and intraocular volume. But it is difficult to measure the intraocular volume of each patient
115 correctly, whereas the methods to measure axial length are well established and widespread.
116 Therefore, we employed the axial length as the index of eyeball size. A significant negative
117 correlation was found between VEGF concentration and axial length. According to the top
118 regression line, ([VEGF concentration] = -9.156 [axial length] + 337.226), the adjusted
119 VEGF concentration was 154.1 pg/mL after substitution of 20 mm for the axial length and
120 62.5 pg/mL after substitution of 30 mm for the axial length. Because the circumferential
121 length of eyes is similar despite differences in the axial length between myopic eyes and

122 nonmyopic eyes except for the anterior segment, the intraocular volume might be assumed
123 to be linear to the axial length. Assuming the intraocular volume was linear to the axial
124 length, the dilution ratio of the VEGF concentration at 30 mm to that at 20 mm was 20 to
125 30. The VEGF concentration at 30 mm calculated by the dilution effect was 102.7 pg/mL.
126 This result is still higher than 62.5 pg/mL, the value obtained from the regression line. The
127 lower VEGF level in the aqueous humor samples from eyes with longer axial length is not
128 explained completely by the dilution effect resulting from longer axial length.
129 Another possible explanation is that VEGF production might decrease because the
130 retina is thinner with axial elongation [29] and retinal thinning might cause relatively
131 increased choroidal perfusion and decreased retinal hypoxia resulting in decreased VEGF
132 production derived from the retinal pigment epithelium [30].
133 There was no significant difference between men and women in the VEGF
134 concentrations in the aqueous humor samples and axial lengths in this study.
135 VEGF plays a key role in the progression of PDR [1]. The current study showed
136 that VEGF concentration was negatively correlated with axial length. The lower VEGF
137 concentration in aqueous humor samples of eyes with axial elongation might explain why
138 the severity of diabetic retinopathy in patients with myopia is less than that in patients with
139 emmetropia or hypermetropia.

This finding might contribute to an understanding of the pathogenesis of vitreoretinal disease concerning VEGF.

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LEGENDS

Fig. 1 Correlation between vascular endothelial growth factor (VEGF) concentrations in the aqueous humor samples and axial length. The VEGF concentration in the aqueous humor samples was negatively correlated with axial length in eyes with cataract (Pearson product moment correlation test, $\rho = -0.373$; $P = 0.003$)

Fig. 2 Correlation between vascular endothelial growth factor (VEGF) concentrations in the aqueous humor and age. No significant correlation between the VEGF concentrations in the aqueous humor and age was found (Pearson product moment correlation test, $\rho = 0.173$; $P = 0.185$)

Fig. 3 Vascular endothelial growth factor (VEGF) concentrations in the aqueous humor from men and women. No significant difference between men and women in the VEGF concentrations in the aqueous humor samples was found (t test, $P = 0.381$)

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Vascular endothelial growth factor in the aqueous humour in eyes with myopic choroidal neovascularization

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ABSTRACT.

Purpose: **To determine the concentration of vascular endothelial growth factor (VEGF) in the aqueous humour of eyes with myopic choroidal neovascularization (mCNV).**

Methods: **Aqueous humour samples were obtained from 21 eyes of 21 patients with mCNV and from 21 eyes of 21 patients with cataract without CNV or other ocular or systemic diseases (control group). The VEGF concentration in the aqueous humour was measured using an enzyme-linked immunosorbent assay.**

Results: **The VEGF concentrations in the aqueous humour of eyes with mCNV ranged from < 20.6 to 200 pg/ml (median 35 pg/ml). The concentrations in the control group ranged from 26 to 218 pg/ml (median 100 pg/ml). The difference between the two VEGF concentrations in the aqueous humour was significant ($p < 0.001$, Mann–Whitney rank sum test).**

Conclusion: **The VEGF concentration in the aqueous humour of patients with mCNV is lower than in normal controls. VEGF might localize in or around the CNV in eyes with mCNV.**

Key words: aqueous humour – bevacizumab – choroidal neovascularization – myopia – vascular endothelial growth factor

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Introduction

Vascular endothelium growth factor (VEGF) is thought to play a key role in the progression of choroidal neovascularization (CNV) associated with age-related macular degeneration (AMD) (Ishibashi et al. 1997; Kwak et al. 2000). Several anti-VEGF drugs have been used to treat CNV associated with AMD, and favourable results have been reported (Gragoudas et al. 2004; Avery et al. 2006; Rosenfeld et al. 2006).

VEGF also seems to play a key role in the progression of CNV secondary to pathological myopia. Anti-VEGF therapy has been reported to have a favourable effect on myopic CNV (mCNV). It was reported that intravitreal injection of bevacizumab (Avastin®; Genentech, South San Francisco, California, USA), a recombinant humanized monoclonal antibody against all VEGF isoforms (Ferrara 2004), improved the visual acuity (VA) and decreased the angiographic leakage in eyes with mCNV (Chan et al. 2009; Gharbiya et al. 2009; Ikuno et al. 2009). Intravitreal injection of ranibizumab (Lucentis; Novartis, Basel, Switzerland), a humanized antigen-binding portion of a murine anti-VEGF monoclonal antibody that has a mature high affinity to all VEGF isoforms, improved the VA and reduced the retinal thickness in eyes with mCNV (Konstantinidis et al. 2009). Further understanding of the role of VEGF in the pathogenesis of mCNV may aid current anti-VEGF treatment and combination therapy with photodynamic therapy (PDT). To study the relation between VEGF and mCNV, we obtained aqueous humour samples and measured the VEGF concentrations in the aqueous humour of patients with mCNV.

Materials and Methods

In this prospective comparative study, we determined the VEGF concentration in the aqueous humour of 21 patients (five men, 16 women) with mCNV. The mean patient age was 64.7 years (range 31–79 years). Aqueous samples from 21 patients (eight men, 13 women) with cataract who did not have CNV or other ocular or systemic diseases comprised the

Table 1. Clinical characteristics of patients with myopic choroidal neovascularization (mCNV) and controls with cataract.

	mCNV	Control	p-value
No. of patients	21	21	
Gender (female/male)	16/5	13/8	0.504
Age (mean ± SD)	64.7 ± 12.4	66.3 ± 9.6	0.693
Axial length (mm, mean ± SD)	29.50 ± 1.47	24.55 ± 2.27	< 0.001

SD, standard deviation.

control group. The mean patient age in the control group was 66.3 years (range 44–79 years) (Table 1).

Undiluted aqueous humour samples were obtained from the eyes of patients with mCNV just before intravitreal injection of 1.25 mg bevacizumab. Anterior-chamber paracentesis was performed before the intravitreal injection, because aspiration of the aqueous humour samples prevents a spike in intraocular pressure after bevacizumab (1.25 mg/0.05 ml) is injected intravitreally.

Undiluted aqueous humour samples were also obtained from the control eyes of the patients with a cataract and no CNV or other ocular disorders immediately before cataract surgery. All injections and sample collections were performed using a standard sterilization procedure that included the use of topical povidone-iodine and levofloxacin drops. No steroids were administrated to the cataract patients before cataract surgery. The samples were stored in a freezer at −80 °C until analysis.

The VEGF concentration in the aqueous humour was measured by enzyme-linked immunosorbent assay (ELISA) for human VEGF (R&D System, Minneapolis, Minnesota, USA). The primary antibody against VEGF detected two ($VEGF_{121}$ and $VEGF_{165}$) of the four VEGF isoforms (Hyodo et al. 1998). The standard curve was plotted from the measurements taken with the standard solution (20.6–1000 pg/ml) and the VEGF concentration in the sample was determined. The assay was performed according to the manufacturer's instructions. The limit of the detectable VEGF concentration was 20.6 pg/ml.

The size of the mCNV was measured on fluorescein angiography before treatment. The fluorescein angiography images were digitalized using ImageNet® (Topcon, Tokyo, Japan), and both the mCNV and the disc size were measured using the ImageNet® software. The mCNV area was divided by the disc area and the mCNV size was expressed in disc areas. The axial length was measured using an IOL Master® (Carl Zeiss Meditec, Jena, Germany) in the patients with mCNV. The data were analysed using SIGMASTAT software (version 3.1; Systat Software Inc., Richmond, California, USA) and expressed as the median value. The differences between the VEGF concentrations in the aqueous humour of patients with mCNV and the control patients were compared using the Mann–Whitney rank sum test. The Spearman rank-order correlation coefficient test was used to examine the correlation between the VEGF concentrations in the aqueous humour and the size of the CNV or the axial length. A p-value < 0.05 was considered statistically significant.

This study of the off-label use of bevacizumab was approved by the institutional review board of Shiga University of Medical Science Hospital. All patients provided written informed consent, including those with mCNV and cataract.

Results

The VEGF concentrations in the aqueous humour in eyes with mCNV ranged from < 20.6 to 200 pg/ml (median 35 pg/ml) before intravitreal injection of bevacizumab. VEGF concentrations in the aqueous humour were below 20.6 pg/ml – the lower limit of detection – in six of the 21 eyes with mCNV. The VEGF concentrations in the aqueous humour in the control eyes with cataract ranged from 26 to 218 pg/ml (median 100 pg/ml) (Fig. 1). The median concentration in the aqueous humour was significantly lower in eyes with mCNV than in the control group (Mann–Whitney rank sum test, p < 0.001).

Correlations between VEGF concentration and CNV size or axial length were evaluated. A value of 19 pg/ml was assigned as the VEGF concentration in eyes with VEGF < 20.6 pg/ml and analysed. The CNV sizes ranged from 0.053 to 2.041 disc areas [mean ± standard deviation (SD) 0.664 ± 0.680 disc area] before treatment. No correlation was observed between the VEGF concentrations in the aqueous humour and the CNV size in mCNV (Spearman rank-order correlations coefficients test; $\rho = 0.0946$; $p = 0.678$) (Fig. 2).

In the eyes with mCNV, axial length ranged from 26.90 to 32.55 mm (mean ± SD 29.50 ± 1.47 mm). The VEGF concentrations in the aqueous humour seemed to be correlated with the axial length in the eyes with mCNV (Spearman rank-order correlations coefficients test; $\rho = -0.434$; $p = 0.0488$) (Fig. 3). The axial length in the controls ranged from 20.98 to 31.95 mm (mean ± SD 24.55 ± 2.27 mm).

Discussion

mCNV, a cause of visual loss and legal blindness in young and middle-aged patients, is associated with a poor prognosis (Avia et al. 1984;

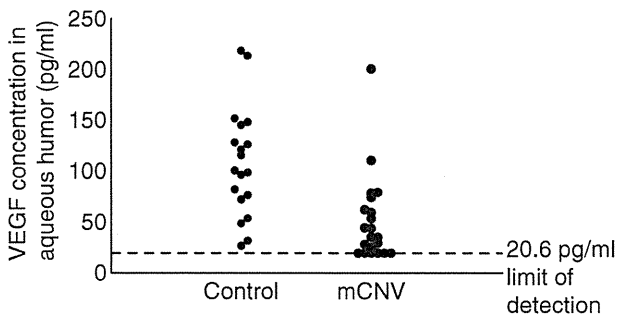

Fig. 1. Vascular endothelial growth factor concentrations in the aqueous humour in eyes with myopic choroidal neovascularization and control eyes.

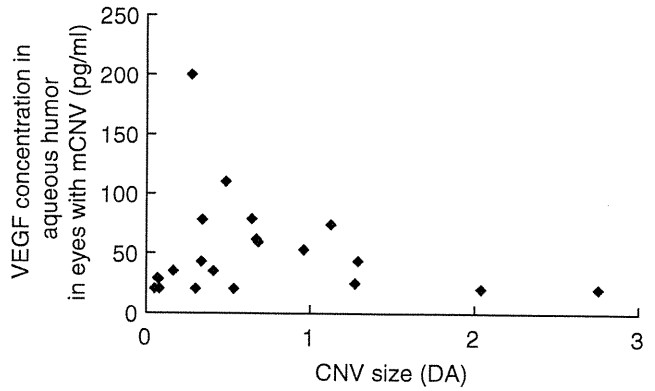

Fig. 2 The correlation between the size of the choroidal neovascularization (CNV) and the aqueous levels of vascular endothelial growth factor (VEGF) in eyes with myopic CNV (mCNV). The aqueous levels of VEGF are not significantly correlated with the size of the CNV (ρ = 0.0946; p = 0.678) (DA, disc area).

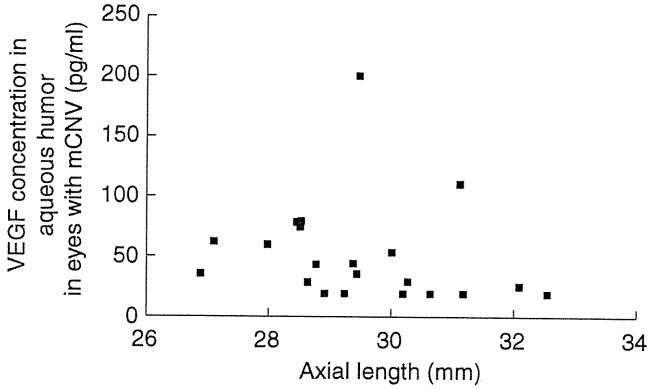

Fig. 3. The correlation between the axial length and aqueous levels of vascular endothelial growth factor (VEGF) in eyes with myopic choroidal neovascularization. The aqueous levels of VEGF are not significantly correlated with the axial length (ρ = −0.434; p = 0.0488).

Yoshida et al. 2003). PDT with verteporfin reduces the risk of visual impairment (Blinder et al. 2003; Ergun et al. 2004; Lam et al. 2004). Currently, PDT or the combination of PDT and intravitreal triamcinolone acetonide is suboptimal for treating mCNV (Degenring & Jonas 2005).

Recent studies have reported that intravitreal injection of an anti-VEGF drug, bevacizumab, seems to be effective for treating mCNV (Chan et al. 2009; Gharbiya et al. 2009; Ikuno et al. 2009). Therefore, VEGF may play a key role in the development of mCNV.

The VEGF concentration in the aqueous humour is higher in patients with diabetic retinopathy and retinal vein occlusion than in healthy individuals (Aiello et al. 1994; Sawada et al. 2007). However, it is controversial whether the VEGF concentration is high in AMD and mCNV. Tong et al. (2006) reported that the VEGF concentrations in the aqueous humour increased markedly in patients with polypoidal choroidal vasculopathy, CNV associated with AMD and CNV associated with myopia compared with control patients. In contrast, Jonas & Neumaier (2007) reported that the VEGF concentrations in the aqueous humour of patients with AMD did not vary significantly compared with controls. The VEGF concentration in eyes with mCNV is also controversial. Chan et al. (2008) reported that the VEGF concentration in the aqueous humour of patients with mCNV was 20.1 ± 28.9 pg/ml, which is similar to the value in the current study, while Tong et al. (2006) reported elevated levels of aqueous VEGF in eyes with mCNV.

In the current study, the VEGF concentrations in the aqueous humour in patients with mCNV were significantly lower than in the controls. In this study, the VEGF concentration in the control eyes (100 pg/ml) was similar to that reported by Noma et al. (2005), who used the same measurement system.

There are several possible explanations for the lower VEGF concentration in the aqueous humour in patients with mCNV compared with controls. VEGF is expressed strongly in subfoveal membranes excised surgically from patients with AMD (Kvanta et al. 1996; Lopez et al. 1996; Hera et al. 2005). However, to the best of our knowledge, the presence of VEGF in the retina and the choroid in mCNV has not been reported. We speculated that VEGF might be localized to a small subfoveal area and might cause mCNV and AMD. If the VEGF is localized to the retina and the choroid and the quantity of VEGF is small, there might not be sufficient VEGF distributed throughout the vitreous cavity and penetrating the anterior chamber. Therefore, it is reasonable that there is no correlation between the VEGF concentration in the aqueous humour and the size of the CNV in mCNV. Another possible explanation is that the VEGF in the anterior chamber and vitreous cavity might be diluted, because the axial length is longer and therefore the intraocular volume is large in patients with high myopia. We observed a negative correlation between the VEGF concentration in the aqueous humour and the axial length in mCNV. However, any VEGF concentration below 20.6 pg/ml was not measured precisely because of the lower limit of the ELISA used in the current study. This correlation might not be definitive. To evaluate this, we compared the adjusted VEGF concentrations in the aqueous humour between the patients with mCNV and the control patients by adjusting for the difference in axial length. The circumferential length of eyes is similar despite differences in the axial length between myopic eyes and non-myopic eyes (Salzmann 1912). Assuming the intraocular volume was linear to the axial length, the adjusted VEGF concentration in the control eyes was 88 pg/ml, which is still higher than in myopic eyes. Therefore, the lower VEGF concentration in mCNV does not seem to be explained solely by the difference in axial length.

Other possible explanations are that VEGF production might decrease because the retina is thin in pathological myopia (Lam et al. 2007) or that retinal thinning might cause relatively increased choroidal perfusion and decreased retinal hypoxia, resulting in