## Biosketch

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CLINICAL INVESTIGATION

# Negative correlation between aqueous vascular endothelial growth factor levels and axial length

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**Abstract**
*Purpose* The aim of this study was to evaluate the relationship between the concentrations 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 cataracts. No patients with diabetes mellitus were 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* The 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 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 cataracts 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 cataracts. 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

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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). The primary antibody against VEGF detected two ($VEGF_{121}$ and $VEGF_{165}$) of the four 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 of 20–1,000 pg/ml for VEGF, and the concentration of VEGF in the sample was determined [28].

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) 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

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

The correlation between the VEGF concentration in the aqueous humor and age or axial length was evaluated. The VEGF concentration in the aqueous humor was negatively correlated with axial length in eyes with cataracts (Pearson product moment correlation test, $\rho = -0.373$; $P = 0.003$) (Fig. 1). The regression line using the VEGF concentration as an outcome variable ($y$) and the axial length as a predictor variable ($x$) was $y = -9.156x + 337.226$. The VEGF concentration in the aqueous humor was not significantly correlated with age (Pearson product moment correlation test, $\rho = 0.173$; $P = 0.185$) (Fig. 2). The VEGF concentrations in the aqueous humor samples from men ranged from 25 to 241 pg/ml (mean ± SD, 108.5 ± 54.4 pg/ml) and in women from 31 to 228 pg/ml (mean ± SD, 120.7 ± 47.2 pg/ml). No significant difference was found between men and women in the VEGF concentrations in the aqueous humor samples (unpaired $t$ test, $P = 0.381$) (Fig. 3), or in the axial lengths (Mann-Whitney test, $P = 0.185$).

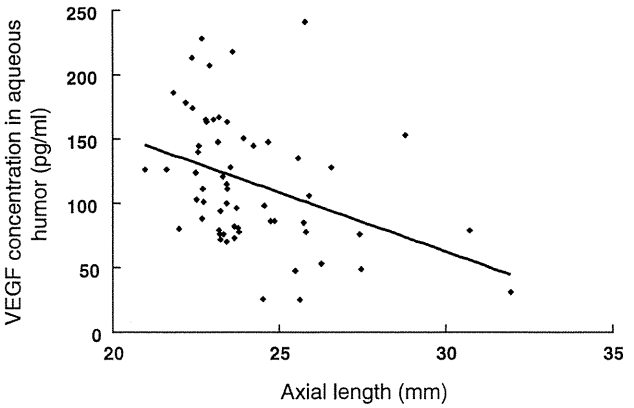


**Fig. 1** Correlation between vascular endothelial growth factor (VEGF) concentrations in the aqueous humor samples and axial length. The VEGF concentration in the aqueous humor samples was negatively correlated with axial length in eyes with cataracts (Pearson product moment correlation test, $\rho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

## Discussion

We measured the VEGF concentrations in the aqueous humor samples from patients without ocular diseases other than cataracts and without diabetes mellitus, and found that the VEGF concentration was negatively correlated with axial length.

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

To evaluate this explanation, regression analysis of the VEGF concentrations in eyes with cataracts in relation to axial length was performed, and we compared the value according to the regression line with the value calculated by the dilution ratio. It might have been better to evaluate the relationship between the VEGF concentration in the aqueous humor and the intraocular volume. But it is

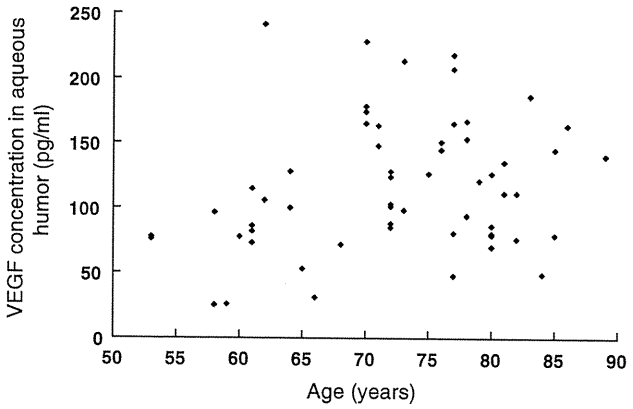


**Fig. 2** Correlation between vascular endothelial growth factor (VEGF) concentrations in the aqueous humor and age. No significant correlation between the VEGF concentrations in the aqueous humor and age was found (Pearson product moment correlation test, $\rho = 0.173$; $P = 0.185$)

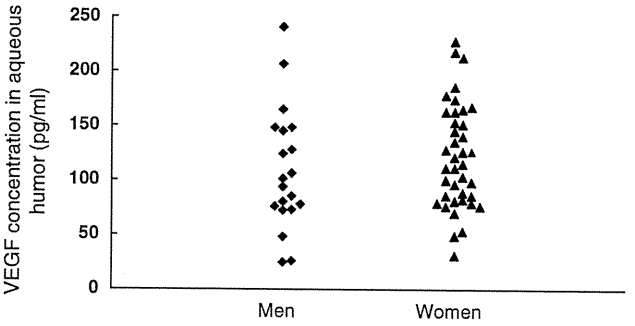


**Fig. 3** Vascular endothelial growth factor (VEGF) concentrations in the aqueous humor from men and women. No significant difference between men and women in the VEGF concentrations in the aqueous humor samples was found ($t$ test, $P = 0.381$)

difficult to measure the intraocular volume of each patient correctly, whereas the methods to measure axial length are well established and widespread. Therefore, we employed the axial length as the index of eyeball size. A significant negative correlation was found between VEGF concentration and axial length. According to the top regression line, ([VEGF concentration] = −9.156 [axial length] + 337.226), the adjusted VEGF concentration was 154.1 pg/ml after substitution of 20 mm for the axial length and 62.5 pg/ml after substitution of 30 mm for the axial length. Because the circumferential length of eyes is similar despite differences in the axial length between myopic eyes and nonmyopic eyes except for the anterior segment, the intraocular volume might be assumed to be linear to the axial length. Assuming the intraocular volume was linear to the axial length, the dilution ratio of the VEGF concentration at 30 mm to that at 20 mm was 20 to 30. The VEGF concentration at 30 mm calculated by the dilution effect was 102.7 pg/ml. This result is still higher than 62.5 pg/ml, the value obtained from the regression line. The lower VEGF level in the aqueous humor samples from eyes with longer axial length is not explained completely by the dilution effect resulting from longer axial length.

Another possible explanation is that VEGF production might decrease because the retina is thinner with axial elongation [29], and the retinal thinning might cause relatively increased choroidal perfusion and decreased retinal hypoxia resulting in decreased VEGF production derived from the retinal pigment epithelium [30].

There was no significant difference between men and women in the VEGF concentrations in the aqueous humor samples and axial lengths in this study.

VEGF plays a key role in the progression of PDR [1]. The current study showed that VEGF concentration was negatively correlated with axial length. The lower VEGF concentration in the aqueous humor samples of eyes with axial elongation might explain why the severity of diabetic retinopathy in patients with myopia is less than that in patients with emmetropia or hypermetropia.

The findings reported herein might contribute to an understanding of the pathogenesis of vitreoretinal disease concerning VEGF.

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CLINICAL INVESTIGATION

# Characteristics of age-related macular degeneration in patients with diabetic retinopathy

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**Abstract**
*Purpose* The purpose of this study was to determine the clinical characteristics of age-related macular degeneration (AMD) in patients with diabetic retinopathy (DR).
*Methods* Retrospective, consecutive case series. Twenty-six eyes of 25 Japanese patients were studied. All patients were diagnosed as having exudative AMD with DR. Patients with no apparent DR, dry AMD, neovascular maculopathy associated with high myopia, and age <50 years were excluded. The clinical characteristics of AMD in patients with DR, e.g., gender, age, stage of DR, and type of AMD were evaluated.
*Results* In the 26 eyes, 2 eyes (7.7%) were classified as mild nonproliferative DR (NPDR), 7 (27.0%) with moderate NPDR, 16 (61.5%) with severe NPDR and 1 eye (3.8%) with PDR. Of the 26 eyes with exudative AMD, 21 eyes (80.8%) were classified as neovascular AMD, 4 (15.4%) as polypoidal choroidal vasculopathy and 1 eye (3.8%) as a retinal angiomatous proliferation. Among the eyes with neovascular AMD, 9 eyes (42.9%) were classified as predominantly classic choroidal neovascularization (CNV).
*Conclusions* There is a predominance of men, neovascular AMD and predominantly classic CNV in Japanese AMD patients with DR. The exudative AMD in patients with DR may have different clinical characteristics from those without DR.

**Keywords** Age-related macular degeneration (AMD) · Diabetic retinopathy · Polypoidal choroidal vasculopathy (PCV) · Choroidal neovascularization (CNV)

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## Introduction

Diabetes mellitus is significantly increasing worldwide, and this is important in part because diabetic retinopathy (DR), a serious complication of diabetic mellitus, is a major cause of adult blindness [1–3]. Age-related macular degeneration (AMD) is another leading cause of legal blindness in elderly patients in developed countries, although the number of patients with AMD in Japan [4, 5] is not as high as in Western countries [6–9]; however, the prevalence of AMD in Japan is also increasing rapidly [10, 11].

Two earlier studies failed to find an association between diabetes and AMD [12, 13], and Klein and Klein [14] reported that diabetic status was not associated with early AMD; however, people >75 years of age with diabetes have been shown to have a higher incidence of exudative AMD (9.4%) than those without diabetes (4.7%) [14].

On the other hand, more recent studies report that the prevalence of AMD is lower in diabetic patients than in the general population [15, 16]. Borrone et al. [15] found that the prevalence of AMD in a sample of diabetic patients aged ≥75 years (2.51%) was significantly lower than in the general population (11%). In addition, Proctor and Ambati

[16] reported that in the USA the prevalence of eyes with both neovascular AMD and DR (0.2%) was significantly lower than in eyes with non-DR (0.8%). These findings support an earlier study that also reported a lower prevalence of exudative AMD in diabetic patients with DR than in diabetics without retinopathy [17]. These observations indicate that the prevalence of AMD appears to be lower in diabetic patients especially in those with DR, than in the general population [15–17].

Aside from the prevalence, a computerized search of Medline did not extract any studies of the clinical features of AMD in patients with DR. Thus, the aim of the present study was to determine the clinical characteristics of AMD in patients with DR.

## Methods

### Subjects

The medical charts of 26 eyes of 25 consecutive diabetic Japanese patients with type 2 diabetes were reviewed. The patients were diagnosed as having exudative AMD and DR at Kansai Medical University, Takii Hospital from 1999 to 2008. Patients with no apparent DR, dry AMD, neovascular maculopathy associated with high myopia, neovascular maculopathy with angioid streaks, and age <50 years were excluded.

### Examination of diabetic retinopathy

All patients underwent a standard ophthalmic examination. The stage of DR was determined by ophthalmoscopy and fluorescein angiography, and the patients were classified according to the severity scale of DR [18] as follows: no apparent DR (NDR), mild nonproliferative DR (mild-NPDR), moderate nonproliferative DR (moderate-NPDR), severe nonproliferative DR (severe-NPDR) and proliferative DR (PDR). Diabetic patients with NDR were excluded from this study.

### Diagnosis of age-related macular degeneration

We diagnosed AMD based on the classification and diagnostic criteria for AMD set out by the Working Group for Establishing Diagnostic Criteria for Age-Related Macular Degeneration [19].

All patients underwent a complete ophthalmologic examination including indirect ophthalmoscopy, slit-lamp biomicroscopy with and without a contact lens, color fundus photography, fluorescein angiography, indocyanine green angiography and optical coherence tomography. Two retinal specialists (NO and TO) evaluated all the data of the patients. Patients were included in this study when the eyes were diagnosed with AMD and DR, and data were analyzed at the point of diagnosis with both AMD and with DR.

We divided the eyes with exudative AMD into 3 subtypes: neovascular AMD, polypoidal choroidal vasculopathy (PCV) [20] and retinal angiomatous proliferation (RAP) [21]. Neovascular AMD was subdivided into predominantly classic choroidal neovascularization (CNV) minimally classic CNV and occult with no classic CNV using the fluorescein angiographic images. A neovascular lesion in which the CNV component was >50% of the total lesion size was defined as predominantly classic CNV; lesions in which the CNV components were <50% of the total lesion size were defined as minimally classic CNV and lesions in which there was no classic CNV component were classified as occult with no classic CNV [22, 23].

### Main outcome measures

The clinical characteristics, e.g., gender distribution, age, stage of DR and type of AMD were evaluated.

## Results

Of the 25 AMD patients with DR, 24 had unilateral AMD (96.2%) and 1 had bilateral AMD (3.8%). There were 23 men (92.0%) and 2 women (8.0%), and the mean age of the patients was 71.1 ± 6.8 years (mean ± SD; 70.4 ± 6.3 men and 78.5 ± 10.6 women) with a range of 60–88 years (Table 1).

Of the 26 eyes, 2 (7.7%) had mild NPDR, 7 (27.0%) had moderate NPDR, 16 (61.5%) had severe NPDR, and 1 eye (3.8%) had PDR. Eleven of these eyes (42.3%) had retinal photocoagulation to treat the DR before they were included in this study, and 15 eyes (57.7%) had not received photocoagulation (Table 1).

None of the eyes had received either grid pattern photocoagulation or photocoagulation for microaneurysmas in the macular region. None of the patients developed AMD from the laser scars or deposits of hard exudates in the macular region. In addition, none of the patients developed AMD from a long-standing cystic macular edema or diabetic retinal pigment epitheliopathy.

Of the 26 eyes with exudative AMD, 21 (80.8%) were classified as neovascular AMD, 4 (15.4%) as PCV and 1 eye (3.8%) was classified as RAP (Tables 1, 2). Nineteen eyes (73.1%) with AMD were located in the subfovea, 4 eyes (15.4%) were located in the juxtafoveal area (within 200 μm of the fovea), and 3 eyes (11.5%) were located in the extrafoveal area (200 μm from the fovea).

Two eyes with mild NPDR had neovascular AMD in 1 eye and PCV in the other eye. Of the seven eyes with moderate NPDR, 5 had neovascular AMD, and 2 had PCV. Of the 16 eyes with severe NPDR, 14 had neovascular AMD, one had PCV, and one had RAP. The one eye with PDR had neovascular AMD (Table 2).

We combined the eyes with mild NPDR and moderate NPDR into a mildly DR group (9 eyes), and the eyes with severe NPDR and PDR into a severely DR group (17 eyes).

**Table 1** Clinical characteristics of AMD patients with diabetic retinopathy

| Characteristics | Number (%) |
|---|---|
| Number of patients | 25 |
| Affected eye of AMD | |
| Bilateral | 1 (3.8) |
| Unilateral | 25 (96.2) |
| Gender | |
| Male | 23 (92.0) |
| Female | 2 (8.0) |
| Mean age (years ± SD) | |
| Total | 71.1 ± 6.8 |
| Male | 70.4 ± 6.3 |
| Female | 78.5 ± 10.6 |
| Stage of DR | |
| Mild-NPDR | 2 (7.7) |
| Moderate-NPDR | 7 (27.0) |
| Severe-NPDR | 16 (61.5) |
| PDR | 1 (3.8) |
| Photocoagulation for DR | |
| Absent | 15 (57.7) |
| Present | 11 (42.3) |
| Type of AMD | |
| Neovascular AMD | 21 (80.8) |
| PCV | 4 (15.4) |
| RAP | 1 (3.8) |

*DR* diabetic retinopathy, *NPDR* nonproliferative diabetic retinopathy, *PDR* proliferative diabetic retinopathy, *AMD* age-related macular degeneration, *PCV* polypoidal choroidal vasculopathy, *RAP* retinal angiomatous proliferation

The mean age of the mildly DR group (72.3 ± 9.6 years) was not significantly different from that of the severely DR group (70.5 ± 5.2 years; $P = 0.53$, unpaired $t$ test). In the mildly DR group, 6 eyes (66.7%) had neovascular AMD, and 3 (33.3%) had PCV. In the 17 eyes in the severely DR group, 15 (88.2%) had neovascular AMD, 1 (5.9%) had PCV and 1 (5.9%) had RAP. The severely DR group had a lower percentage of PCV (5.9%) than the mildly DR group (33.3%) although the difference was not significant ($P = 0.06$, chi-squared test, Fig. 1).

Of the 21 eyes with neovascular AMD, 9 (42.9%) were classified as predominantly classic CNV, 3 (14.2%) were classified as minimally classic CNV and 9 (42.9%) were classified as occult with no classic CNV (Table 3).

## Discussion

The mean age of the AMD patients with DR (71.0 ± 6.8 years) was similar to that reported earlier [24–29]. A recent study reported that typical AMD was unilateral in 94.1% of cases in Japanese patients [25], comparable to the 96.2% unilateral AMD found in our DR group.

In earlier studies, the male/female ratio of non-diabetic patients with AMD was reported to be 0.67–4.3 (Table 4) [24–28]. In our study, we found a higher percentage of male AMD patients with DR. The male/female ratio in our patients was 12.5 (92.0% men) which was a higher predominancee of men compared to earlier reports [24–28].

The Los Angeles Latino Eye Study reported that men had a 50% higher risk of having DR than women [30]. In contrast, three studies in Asia (Beijing Eye Study, the Hisayama Study and the Funagata Study) reported that gender was not associated with DR [31–33]. We do not know why a male predominance was found in the AMD patients who have DR, but the hormonal environment may affect the incidence of AMD in male diabetic patients. Klein et al. [14] reported that diabetes was not related to early age-related maculopathy or geographic atrophy but a relationship between exudative macular degeneration and diabetes was found in older men (age >75 years) but not women.

**Table 2** Types of AMD at different stages of advanced diabetic retinopathy

| | Mild NPDR | Moderate NPDR | Severe NPDR | PDR | Total number (%) |
|---|---|---|---|---|---|
| Neovascular AMD | 1 | 5 | 14 | 1 | 21 (80.8) |
| PCV | 1 | 2 | 1 | 0 | 4 (15.4) |
| RAP | 0 | 0 | 1 | 0 | 1 (3.8) |
| Total number (%) | 2 (7.7) | 7 (27.0) | 16 (61.5) | 1 (3.8) | 26 (100) |

*NPDR* nonproliferative diabetic retinopathy, *PDR* proliferative diabetic retinopathy, *AMD* age-related macular degeneration, *PCV* polypoidal choroidal vasculopathy, *RAP* retinal angiomatous proliferation

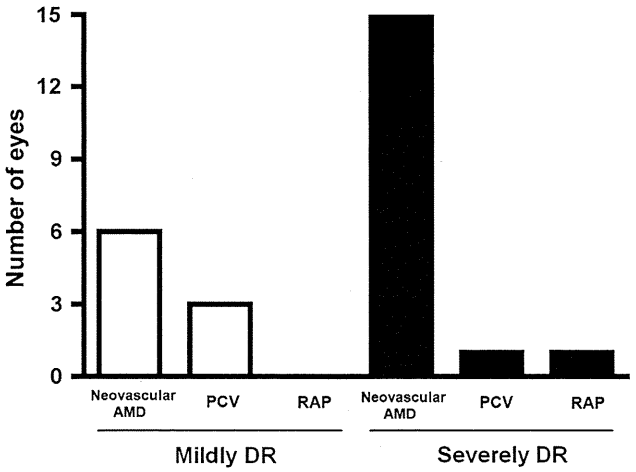


**Fig. 1** Types of AMD relating to the severity of diabetic retinopathy. AMD, age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; RAP, retinal angiomatous proliferation; DR, diabetic retinopathy. We defined the eyes with mild nonproliferative diabetic retinopathy (NPDR) and moderate NPDR as a mildly diabetic retinopathy (9 eyes), and the eyes with severe NPDR and proliferative diabetic retinopathy as severely diabetic retinopathy (17 eyes). The severely DR eyes had a higher percentage of AMD compared to that of mildly diabetic retinopathy eyes

**Table 3** Evaluation of neovascular AMD

| Subgroup[a] of neovascular AMD | Number (%) |
|---|---|
| Predominantly classic CNV | 9 (42.9) |
| Minimally classic CNV | 3 (14.2) |
| Occult with no classic CNV | 9 (42.9) |
| Total | 21 (100) |

*AMD* age-related macular degeneration, *CNV* choroidal neovascularization

[a] A neovascular lesion in which the CNV component was >50% of the total lesion size was defined as predominantly classic CNV; lesions in which the CNV components were <50% of the total lesion size were defined as minimally classic CNV; and lesions in which there was no classic CNV component were classified as occult with no classic CNV

We classified the patients into three subtypes of exudative AMD: neovascular AMD, PCV and RAP. Yannuzi et al. [24] reported that PCV was observed in 8% of eyes with neovascular AMD; however, it was recently reported that PCV occurs more often in pigmented individuals such as Blacks and Asians, and the clinical features differ from those in white patients [25–28, 34, 35]. Sho et al. [29] reported that 100 (24%) of 418 patients with exudative AMD had PCV, and Yoshimura reported that 42% of eyes with exudative AMD had PCV [26]. Maruko et al. [25] reported that 54.7% (158 of 289) of patients with exudative AMD were diagnosed with PCV, 35.3% (102 patients) with neovascular AMD and 4.5% with RAP (13 patients). Thus, there is a predominance of PCV in Japanese patients (Table 4).

In our DR group, we found that 15.4% of patients had PCV and 80.8% of patients were classified as having neovascular AMD. In addition, the severely DR group had a lower percentage of PCV (5.9%) compared to the mildly DR group (33.3%).

In eyes with neovascular AMD, we found a higher percentage (42.9%) of predominantly classic CNV compared to that reported in other studies (21.8% [25], 20.8% [36], 23.0% [37]). These differences may be because the patients in the earlier studies were AMD patients without DR whereas we studied AMD patients with DR.

The effects of hyperglycemia on the development of AMD are not known; however, hyperglycemia may affect the normal structure and functioning of the choroidal circulation, pigment epithelium and Bruch's membrane. Histopathological studies of eyes of individuals with long-standing diabetes have shown a thickening of Bruch's membrane and the basement membrane of the choriocapillaris walls, and a luminal narrowing and drop-out of the choriocapillaris [38–42]. These pathological changes may affect the clinical characteristics of exudative AMD in patients with DR.

It was recently reported that the choroidal blood flow in the foveal region is significantly decreased in patients with diabetes. It is likely that the decreased blood flow in the choriocapillaris may also affect the clinical features of AMD [43].

It has also been reported that exudative AMD was not observed in 431 patients with DR treated with laser photocoagulation (0%), but was diagnosed in 3.3% of 151 non-laser-treated patients [17]. The authors concluded that patients treated for DR by laser photocoagulation are less likely to develop AMD. Alternatively, one Japanese group reported that CNV was present in 5 of 20 eyes with DR after laser photocoagulation for the treatment of DR patients, and they suggested that the risk of CNV in patients with DR might be increased by laser photocoagulation [44]. They also reported that CNV was present in 12 of 20 eyes with DR-associated diabetic maculopathy [44]; however, we observed AMD in 42.3% of patients treated with retinal photocoagulation and 57.7% in non-laser-treated patients. Therefore, we presume that laser photocoagulation itself for DR may not increase the risk of developing AMD. In addition, in the present study, none of the patients developed AMD from long-standing cystic macular edema or diabetic retinal pigment epitheliopathy. Thus, we could not find any correlation between diabetic retinal pigment epitheliopathy and AMD.

The limitation of this study was that it was a clinic-based sample study and not a population-based study. In addition, we did not study diabetic patients without DR. Although a larger number of patients must be examined, the prevalence of PCV in patients with DR in this group

**Table 4** Comparison of proportions of patients with PCV in other AMD studies

| Author(s) | Year | Number of AMD patients | Ethnicity | Mean age | Gender ratio (male/female) | PCV (%) |
|---|---|---|---|---|---|---|
| Yannuzzi et al. [24] | 1999 | 167 | White 20%<br>Black 50%<br>Asian 30% | 74 | 0.67:1 | 8 |
| Ahuja et al. [27] | 2000 | 34 | White 74%<br>Black 20%<br>Asian 6% | 65 | 0.7:1 | 47 |
| Ladas et al. [28] | 2004 | 268 | Greek | 77 | 1.4:1 | 8 |
| Sho et al. [29] | 2003 | 418 | Japanese | – | – | 23 |
| Yoshimura [26] | 2004 | 155 | Japanese | 71 | 4.3:1 | 42 |
| Maruko et al. [25] | 2007 | 289 | Japanese | 73 | 2.7:1 | 54 |
| Current study | 2009 | 25 | Japanese with DR | 71 | 12.5:1 | 15 |

*AMD* age-related macular degeneration, *PCV* polypoidal choroidal vasculopathy

appears to be lower than in the general Japanese population. We still do not know the exact reason but retinal and choroidal blood flow was altered in DR and this alteration would affect the prevalence of PCV.

In conclusion, there is a predominance of men, neovascular AMD, and predominantly classic CNV in patients with DR. Our findings indicate that exudative AMD in patients with DR may have different clinical features from those without DR.

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Acta Ophthalmologica



# 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;

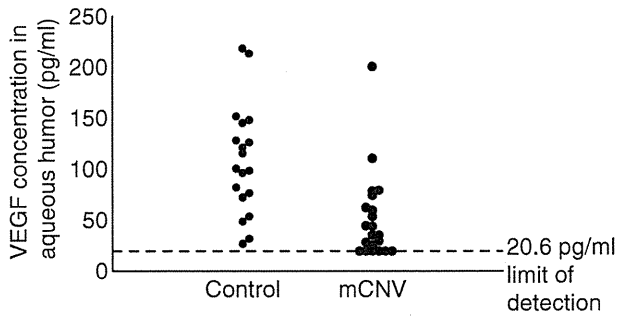


**Fig. 1.** Vascular endothelial growth factor concentrations in the aqueous humour in eyes with myopic choroidal neovascularization and control eyes.

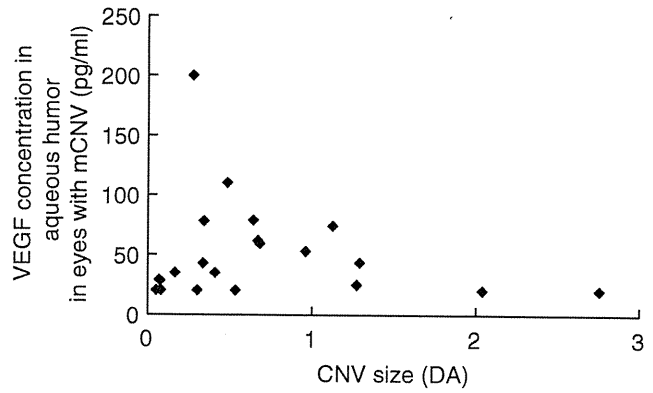


**Fig. 2.** The correlation between the size of the choroidal neovascularization (CNV) and the aqueous levels of vascular endothelial growth factor (VEGF) in eyes with myopic CNV (mCNV). The aqueous levels of VEGF are not significantly correlated with the size of the CNV ($\rho$ = 0.0946; p = 0.678)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

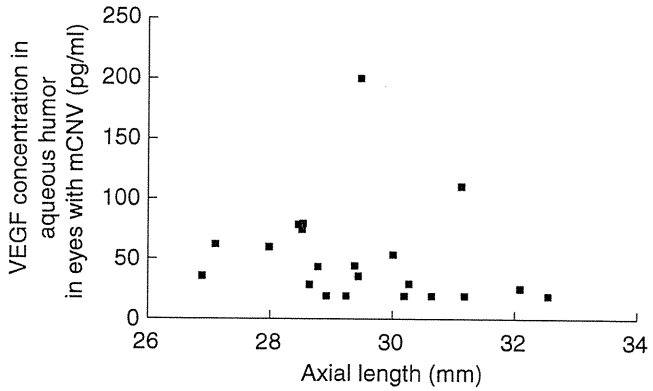


**Fig. 3.** The correlation between the axial length and aqueous levels of vascular endothelial growth factor (VEGF) in eyes with myopic choroidal neovascularization. The aqueous levels of VEGF are not significantly correlated with the axial length ($\rho$ = −0.434; p = 0.0488).

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

decreased VEGF production. In addition, VEGF isoforms other than $VEGF_{121}$ and $VEGF_{165}$ might play a key role in mCNV. The antibody we used can detect free $VEGF_{121}$ and free $VEGF_{165}$. Therefore, we cannot deny the possibility that bound VEGF or other VEGF isoforms might play a key role in mCNV.

In the current study, we found a significantly lower mean VEGF concentration in the aqueous humour in patients with mCNV. To determine the pathogenesis of VEGF in mCNV, further studies are warranted of the local presence and intraretinal expression of VEGF in eyes with mCNV and a comparison of VEGF concentrations in the aqueous humour in patients with high myopia without mCNV.

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MEDICAL OPHTHALMOLOGY

# Effect of 1-year lutein supplementation on macular pigment optical density and visual function

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**Abstract**
*Background* Although it is known that antioxidants including lutein can affect macular pigment optical density (MPOD) and visual function, we still have much to learn about their effect. Our aim was to assess the 1-year changes in MPOD and visual function in response to supplementation containing lutein.
*Methods* We prospectively measured the MPOD level of those who received a supplement containing 6 mg of lutein daily for 1 year. MPOD level was measured every 3 months by using autofluorescence spectrometry with the two-wavelength method. Other examinations, including contrast sensitivity and retinal sensitivity were also measured every 3 or 6 months. Stepwise regression analysis was performed to determine the factors that correlated with the changes observed in those examinations.
*Results* Forty-three eyes of 43 Japanese subjects, including five normal eyes, five fellow eyes with central serous chorioretinopathy (CSC), and 33 fellow eyes with age-related macular degeneration (AMD) were enrolled. The higher baseline MPOD level was correlated with the eye with a clear intraocular lens (IOL). Although no time-dependent changes in the MPOD level were obtained in any area, subjects without cardiovascular diseases showed higher increase in the MPOD level. We observed significant increases in the contrast sensitivity at 1 year ($p$=0.0124) and in the retinal sensitivity at 6 months ($p$<0.0001) and 1 year ($p$<0.0001). Stepwise regression analysis showed that nonsmokers had increased contrast sensitivity ($p$=0.0173), and the fellow eye of those with CSC had less of an increase in retinal sensitivity ($p$=0.0491).
*Conclusions* Daily supplementation with 6 mg of lutein did not affect the MPOD level for 1 year, suggesting that 6 mg of lutein may be insufficient to increase the MPOD level. However, supplementation seems to improve visual functions such as contrast sensitivity and retinal sensitivity.

**Keywords** Macular pigment · Fundus autofluorescence · Lutein · Contrast sensitivity · Microperimetry

## Introduction

Macular pigment, which is comprised of three carotenoids, i.e., lutein, zeaxanthin, and meso-zeaxanthin [1–3], has light-absorbing properties in the 400- to 540-nm range, with maximum absorption at about 460 nm [3–5]. In addition, the macular pigment itself has an antioxidative effect [3, 6–9]. Thus, macular pigment may help retard some destructive processes in the retina and the retinal pigment epithelium, which can cause macular diseases such as age-related maculopathy, age-related macular degeneration (AMD), and possibly central serous chorioretinopathy (CSC) [10–15].

Several studies have investigated the relationship between dietary supplementation with lutein and zeaxanthin and macular pigment optical density (MPOD). It is controversial whether supplementation with these carote-

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noids and/or other antioxidants increases the MPOD level and consequently prevents the development of AMD [16–28]. The role of macular pigment and vitamins in visual function, such as visual acuity (VA) and contrast sensitivity, has also been discussed [28–36]. Some studies have reported that the supplemental antioxidants had a positive effect on visual function, but other studies did not.

In the current study, we investigated the MPOD level in response to supplementation with lutein and other antioxidants and minerals in 43 eyes of 43 Japanese subjects without abnormal fundus autofluorescence (FAF) at the fovea. We also estimated the effect of this supplementation on visual functions, including VA, contrast sensitivity, and retinal sensitivity. To the best of our knowledge, this is the first study to report a relationship between supplementation with carotenoids and time-dependent changes in the MPOD levels and visual function in a Japanese population.

## Materials and methods

### Study population

We conducted a prospective interventional study at Osaka University Hospital from January 2008 to October 2009. The institutional review board approved this study.

We calculated the power (number of eyes) needed for this study, the primary outcome of which was the changes in the MPOD level averaged along the area of an annulus with a retinal eccentricity of 0.5 degree. Considering the normal data that we previously reported, we found that 39 eyes were needed to detect a change in the MPOD optical density of 0.10 density units (DUs) [15]. Because we expected dropout during the follow-up period, we enrolled 43 consecutive Japanese subjects over 40 years old who had never taken supplementation previously. Written informed consent was obtained from all subjects enrolled.

Eyes with less than 0.05 of logMAR score and without apparent retinal disorders and abnormal FAF including the fovea were recruited. All subjects had undergone the detailed fundus examination, optical coherence tomography, and fundus autofluorescence (FAF), and if any macular disorders were suspected, fluorescein and indocyanine green angiography was performed and excluded the subjects with bilateral disease. When one eye had retinal disorders such as CSC and AMD and fellow eyes confirmed to have no apparent abnormalities including FAF examination at the fovea, we selected the fellow eye as the target of our study. Eyes with dry AMD were not included. If both eyes had no ocular disorders, we selected the right eye.

Subjects took daily supplements of 6 mg of lutein and other vitamins and minerals (Ocuvite plus Lutein, Bausch & Lomb Japan, Tokyo, Japan) for 1 year and were examined every 3 months. The ingredients in Ocuvite plus Lutein are shown in Table 1. All participants provided informed consent at the beginning of the supplementation according to the tenets of the Declaration of Helsinki.

### Measurement of the MPOD level

We measured the MPOD level in all eyes using the modified Heidelberg Retina Angiograph (HRA, Heidelberg Engineering, Dossenheim, Germany) every 3 months (0, 3, 6, 9, and 12 months). Autofluorescence spectrometry with the two-wavelength method was the principle measurement of the MPOD [37–39]. Two masked orthoptists who used the same testing device and protocol performed all measurements. Before the study, the reliability of the measurements between the two orthoptists was confirmed as reported previously [15].

Before the measurements, sufficient pupil dilation was obtained with instillation of dilating drops containing 0.5% tropicamide and 2.5% phenylephrine. The subjects sat at a table and fixated on an external light source with the fellow eye. If the fellow eye did not have adequate VA for fixation, the subjects were asked to look straight as much as possible. The modified HRA was aligned with the subject's eye, and movies were taken with the 488- and 514-nm excitation wavelengths (scan size, 30 degrees); computed mean autofluorescence images were obtained at each wavelength, and the two images were subtracted to calculate the MPOD level expressed in DUs. In accordance with other studies using this method, we chose the mean MPOD level averaged along the area of an annulus with a retinal eccentricity of 0.5 degree (MPOD level on 0.5 degree) as a primary outcome. We also calculated the MPOD level averaged within the area of an annulus with a retinal eccentricity of 0.5 degree (MPOD level within 0.5 degree) and 1.0 degree (MPOD level within 1.0 degree). We measured the MPOD two or three times in each eye during

**Table 1** Ingredients in Ocuvite plus Lutein

| | |
|---|---|
| Lutein | 6 mg |
| Beta-carotene (provitamin A) | 1200 μg |
| Vitamin C | 300 mg |
| Vitamin E | 60 mg |
| Vitamin $B_2$ | 3 mg |
| Niacin | 12 mg |
| Zinc | 9 mg |
| Selenium | 45 μg |
| Copper | 0.6 mg |
| Manganese | 1.5 mg |

each visit and then selected the data with the best quality image [37].

### Ophthalmic examinations

To determine the effect of supplementation on visual function, we obtained the best spectacle corrected VA (BCVA) levels using Landolt C charts that then were converted to the logarithm of the minimal angle of resolution (logMAR) score, the low-contrast VA measured using the CSV-1000 LanC10% (Vector Vision Co., Greenville, OH), the contrast sensitivity using the CSV-1000E (Vector Vision Co.), and the mean retinal sensitivity measured by the MP-1 Microperimeter (Nidek Technologies, Padova, Italy).

The BCVA was measured at the beginning and after 1 year of supplementation. Low-contrast VA testing was measured at 0, 3, 6, 9, and 12 months to identify minute changes in visual function [40]. The CSV-1000LanC10% is based on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart (The Lighthouse, New York). This chart displays five letters per line at 10% low contrast. The low-contrast VA was scored by the number of letters identified correctly with full spectacle correction.

Contrast sensitivity usually is measured at several different spatial frequencies. We tested the contrast sensitivity at 3, 6, 12, and 18 cycles per degree (cpd). The contrast level of the last correct response for each cpd was recorded as the contrast threshold in logarithmic value under full spectacle correction. We then calculated the area under the log contrast sensitivity function (AULCSF) as representative of the contrast sensitivity data according to the method of Applegate et al. [41]. The log contrast sensitivity versus log spatial frequency data were fitted to a third-order polynomial curve. The fitted function was integrated between the fixed limits of log spatial frequencies of 0.48 (corresponding to 3 cpd) and 1.26 (18 cpd), and the resulting value was defined as the AULCSF.

Microperimetry was performed at 0, 6, and 12 months [42, 43]. The retinal sensitivity threshold was measured using Goldmann III stimuli (circle with a white background) projected on a white background with background illumination of 1.27 cd/m$^2$ and stimulus presentation time of 200 ms. We tested a radial grid of five stimuli within the area of an annulus with a retinal eccentricity of 0.5 degree and that of 45 stimuli within the area of an annulus with a retinal eccentricity of 6.0 degrees.

### Statistical analysis

The baseline data are expressed as the mean ± standard deviation (SD).

Stepwise regression analysis using the Akaike information criteria was performed to determine the covariates that affected the MPOD level of each area at baseline, i.e., gender, age, smoking, lens status, cardiovascular diseases (stroke, angina, and myocardial infarction), disease in the fellow eye, low-contrast VA, AULCSF, and retinal sensitivity within 0.5 degree [44]. We did not select the logMAR VA as a covariate because low-contrast VA is more sensitive than logMAR VA and they are moderately correlated with each other ($r$=−0.63, $p$<0.0001). Similarly, we did not select retinal sensitivity within 6.0 degrees as a covariate because the MPOD level was measured within 1.0 degree and the retinal sensitivity within 0.5 degree and 6.0 degrees are strongly correlated with each other ($r$=0.86, $p$<0.0001). Stepwise regression analysis was conducted to detect the covariates that correlated with the low-contrast VA, the AULCSF, and the retinal sensitivity within 0.5 and 6.0 degrees at baseline, including gender, age, smoking, lens status, cardiovascular diseases and disease in the fellow eye plus low-contrast VA at baseline when evaluating the AULCSF and the retinal sensitivity.

To estimate the differences compared with baseline at each time point (3, 6, 9, and 12 months) within the variables, we calculated the 95% CI and performed a paired $t$ test and the significance level was adjusted by Bonferroni correction. That is, because we considered $p \leqq 0.05$ significant when comparing just one time point with baseline, $p \leqq 0.025$ and $p \leqq 0.0125$, respectively, were considered significant when comparing two and four time points with baseline. To determine the variables that affected the 1-year change in the MPOD level, stepwise regression analysis was performed, where gender, age, smoking, lens status, cardiovascular diseases, disease in the fellow eye, MPOD on the 0.5 degree, the low-contrast VA, the AULCSF, and the retinal sensitivity within 0.5 degree at baseline were included as covariates. Stepwise regression analysis was also conducted to detect the covariates that correlated with the changes in the low-contrast VA, the AULCSF, and the retinal sensitivity within 0.5 and 6.0 degrees, including gender, age, smoking, lens status, cardiovascular diseases, disease in the fellow eye, MPOD on the 0.5 degree at baseline, and the low-contrast VA at baseline plus the baseline value of the respective factors.

JMP software version 8.0 (SAS Institute Inc., Cary, NC) was used for statistical analysis.

## Results

### Baseline characteristics

A total of 43 eyes of 43 subjects (26 men, 17 women; mean age ± SD, 64.5±9.1 years) were included. Twenty-seven

subjects were smokers and 16 subjects were nonsmokers. Six subjects had had a past history of cardiovascular diseases (stroke, angina, and myocardial infarction). Three eyes had undergone cataract surgery with implantation of clear intraocular lens (IOL) before the study. Five eyes were those of healthy volunteers, five eyes were fellow eyes of those with CSC, and 33 eyes were fellow eyes of those with AMD (18 eyes were polypoidal choroidal vasculopathy, 12 eyes were exudative AMD, two eyes were age-related maculopathy and one eye was retinal angiomatous proliferation). None of the subjects had an unbalanced diet. The subjects had spherical equivalent between −3.88 diopter (D) and 4.63 D (mean ± SD was 0.11±1.88 D) and cylinder magnitude up to 3.50 D. Although 16 eyes had minimal retinal pigment epithelium damage within 6.0 degrees, foveal regions in those eyes were not affected.

Accurate MPOD levels were obtained from all subjects. The mean ± SD MPOD level measured by autofluorescence spectrometry was 0.480±0.136 DU (on 0.5 degree), 0.493±0.138 DU (within 0.5 degree), and 0.474±0.134 DU (within 1.0 degree). The mean±SD logMAR BCVA was −0.078±0.077 (the mean Snellen equivalent; 24/20), and the low-contrast VA was 77.7±6.3 letters. The mean±SD AULCSF was 1.36±0.14, and the retinal sensitivity was 14.8±3.2 dB (within 0.5 degree) and 15.3±2.4 dB (within 6.0 degrees).

Stepwise regression analysis showed that the higher baseline MPOD levels on 0.5 degree and within 0.5 degree were correlated with the eye with clear IOL (T=2.65, $p$=0.0114; and T=2.53, $p$=0.0152, respectively). The low-contrast VA and the retinal sensitivity within 0.5 degree and 6.0 degrees were correlated negatively with age (T=−4.07, $p$=0.0002; T=−2.37, $p$=0.0225; and T=−3.75, $p$=0.0006, respectively). The AULCSF showed no significant correlation with any covariates.

## Time-dependent changes in MPOD

During follow-up, all subjects took daily supplementation containing 6 mg of lutein. No enrolled eyes developed any ocular disorders during the follow-up period.

The time-dependent changes in the MPOD level on 0.5 degree are shown in Fig. 1a. The MPOD levels were 0.480 DU (95% CI, 0.438, 0.521) at baseline, 0.480 DU (95% CI, 0.439, 0.521) at 3 months, 0.485 DU (95% CI, 0.443, 0.527) at 6 months, 0.484 DU (95% CI, 0.442, 0.527) at 9 months, and 0.488 DU (95% CI, 0.445, 0.532) at 12 months. The MPOD differences between the beginning of supplementation (baseline) and each follow-up time point (3, 6, 9, and 12 months) were calculated by paired $t$ test; the respective $p$ values were as $p$=0.9558, $p$=0.4386, $p$=0.5183, and $p$=0.2610. There were no significant changes in the MPOD level at any time point during the follow-up period when compared to a significance level of 0.0125 adjusted by Bonferroni correction. We also measured the mean MPOD level within 0.5 and 1.0 degree (Fig. 1b). There were also no significant time-dependent changes in the MPOD levels in those areas. Stepwise regression analysis showed that the 1-year increase in the MPOD levels on 0.5 degree and within 0.5 degree were correlated with subjects with no cardiovascular diseases (T=2.20, $p$=0.0338; and T=2.42, $p$=0.0201, respectively), and 1 year decrease in the MPOD level within 1.0 degree was correlated with phakic eye (T=−3.96, $p$=0.0003).

## Time-dependent changes in visual function

The mean baseline logMAR was −0.078 (95% CI, -0.102, -0.055) (the Snellen equivalent; 24/20), which did not differ significantly from the mean logMAR at 12 months, -0.096 (95% CI, -0.122, -0.069) (the Snellen equivalent; 25/20) ($p$=0.1658, paired $t$-test).

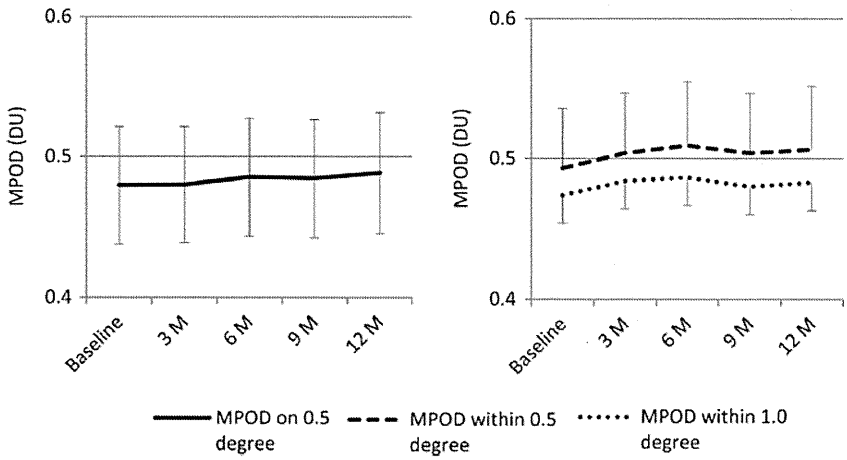


**Fig. 1** Time-dependent changes in the mean MPOD levels on 0.5 degree (**a**) and within 0.5 and 1.0 degree (**b**). There were no significant differences between baseline and 3, 6, 9, and 12 months in those areas. *MPOD* macular pigment optical density; *DU* density unit; *M* months

The low-contrast VA and the AULCSF were measured every 3 months (Fig. 2). There were no significant differences between baseline and 3, 6, 9, and 12 months in the low-contrast VA ($p$=0.9051, $p$=0.6446, $p$=0.5598, and $p$=0.5556, respectively). Although there were also no significant differences between baseline and 3, 6, and 9 months in the AULCSF ($p$=0.3060, $p$=0.8850, and $p$=0.7151, respectively), the AULCSF was significantly ($p$=0.0124) higher at 12 months than at baseline (1.36, 95% CI, 1.32, 1.40 versus 1.41, 95% CI, 1.37, 1.45).

We also analyzed the differences between baseline and 6 and 12 months in the mean retinal sensitivity (Fig. 3). The retinal sensitivities within 0.5 degree were 16.2 decibels (dB) (95% CI, 15.3, 17.2) at 6 months and 17.0 dB at 12 months (95% CI, 16.0, 18.0), which were significantly higher than at baseline, 14.8 dB (95% CI, 13.9, 15.8) ($p$<0.0001, $p$<0.0001, respectively). Similarly, the retinal sensitivity within 6.0 degrees was 16.5 dB (95% CI, 15.8, 17.2) at 6 months and 17.2 dB (95% CI, 16.4, 18.0) at 12 months, and they were significantly higher than at baseline, 15.3 dB (95% CI, 14.6, 16.0) ($p$<0.0001, $p$<0.0001, respectively).

We performed stepwise regression analyses to determine the covariates that correlated with the 1-year changes in factors representative of visual function (Table 2). As a result, the increase in the low-contrast VA correlated with subjects with no cardiovascular diseases (T=2.14, $p$=0.0386) and the lower baseline low-contrast VA (T=−3.18, $p$=0.0029). The increase in the AULCSF correlated with the nonsmoking (T=2.49, $p$=0.0172), the phakic eye (T=2.16, $p$=0.0372) and the lower baseline AULCSF (T=−3.97, $p$=0.0003). The increase in the retinal sensitivity within 0.5 degree correlated with the phakic eye (T=2.42, $p$=0.0202) and the lower baseline retinal sensitivity within 0.5 degree (T=−2.92, $p$=0.0058), and the lower increase of the retinal sensitivity within 6.0 degrees correlated with the fellow eye of the eye with CSC (T=−2.03, $p$=0.0491)

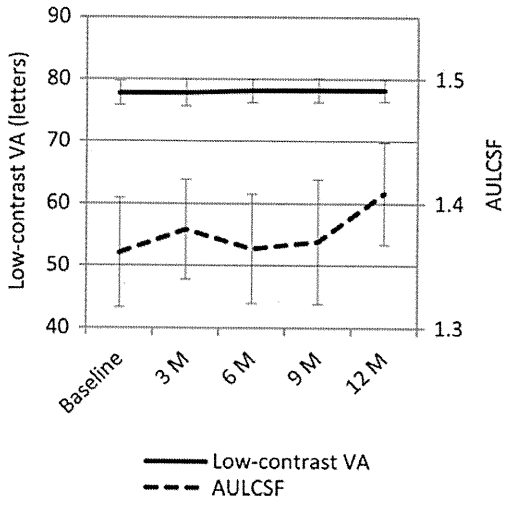


**Fig. 2** Time-dependent changes in the low-contrast VA and the AULCSF. There were no significant differences between baseline and 3, 6, 9, and 12 months in the low-contrast VA. Although there were also no significant differences between baseline and 3, 6, and 9 months in the AULCSF, the AULCSF was significantly higher at 12 months than at baseline ($p$=0.0124). *VA* visual acuity; *AULCSF* area under the log contrast sensitivity function; *M* months

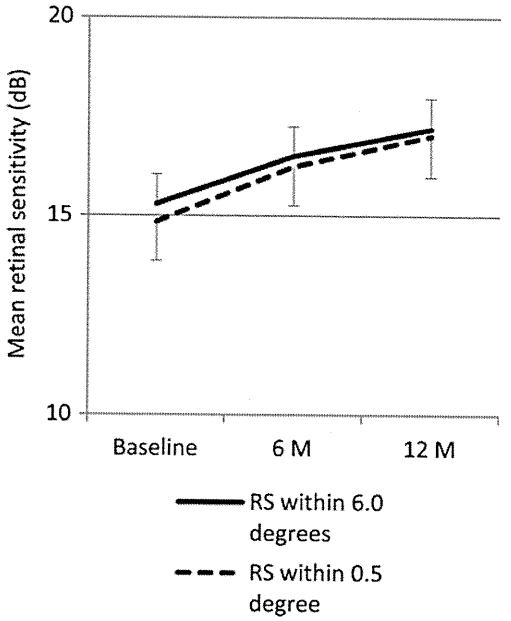


**Fig. 3** Time-dependent changes in the retinal sensitivity. The retinal sensitivities within 0.5 degree at 6 months and 12 months were significantly higher than at baseline ($p$<0.0001, $p$<0.0001, respectively). Similarly, the retinal sensitivity within 6.0 degrees at 6 months and 12 months were significantly higher than at baseline ($p$<0.0001, $p$<0.0001, respectively). *RS* retinal sensitivity; *dB* decibels; *CI* confidence interval

## Discussion

Supplementation with lutein may prevent development and/or progression of AMD because carotenoids help prevent antioxidative stress [3–9]. Whether lutein supplementation increases the MPOD level is now intensely discussed. Most studies have shown that lutein positively affects the MPOD level, but usually the increase in the MPOD level is not high [20–25]. However, a greater increase in the MPOD is likely to be correlated with higher doses of lutein [27, 28]. Previous studies in which a lutein dose under 10 mg was used reported no significant increase in the MPOD level, although the serum level of lutein increased [26].

In the current study, we evaluated the effect of supplementation with 6 mg of lutein, vitamins, and minerals on the MPOD level and the relationship with visual function in Japanese subjects. The 43 subjects who