研 究 等 実 施 計 画 書

1　課題名

　　新生血管黄斑症におけるラビニズマブ（商品名ルセンティス）の投与回数軽減を目的とした
　HLA 拘束性エピトープペプチドによる維持療法第 II 相臨床試験

2　目　的

　　本臨床試験は加齢黄斑変性において標準療法となりつつある抗 VEGF 療法の欠点を補完すること
を目的として Vascular Endothelial Growth Factor Receptor 1 (VEGFR1) および Vascular
Endothelial Growth Factor Receptor 2 (VEGFR2/KDR) 由来の HLA-A*0201 拘束性もしくは
HLA-A*2402拘束性エピトープペプチドを Incomplete Freund's Adjuvant (IFA) とを混和して患者
皮下に投与し、必要とされる抗 VEGF 療法の治療回数を軽減させ、ペプチドワクチンの有用性を検
証する事を主目的とした第 II 相臨床試験である。

　　副次目的としては薬剤の安全性、網膜の解剖的、機能的な側面に対する有効性、および免疫反応
に対する効果を評価することである。

3　対　象

　　対象患者は、血管新生黄斑症で抗VEGF療法（ルセンティス）の硝子体注を選択した患者のうち、
本治療計画の骨子を理解し、同意が得られた患者とする。

3-1. 選択（対象とする疾患等）

　1.　新生血管黄斑症で今後、抗 VEGF 療法の硝子体注が頻回に必要であると考えられる患者。

　2.　同意取得時の年齢が 40 歳以上 85 歳以下。

　3.　新生血管について何らかの治療を受けている場合、もしくは他の疾患により何らかの手術を
　　　受けている場合は、これら処置による影響から回復していること。もしくは、前治療から 4 週間
　　　以上が経過していること。

　4.　骨髄機能（白血球数 2000/mm³ 以上、15000/mm³ 以下、血小板数 7.5/mm³ 以上）、肝機能（GOT
　　　150IU/L 以下, GPT 150IU/L 以下, T-bil 3.0g/dL 以下）、腎機能（Cr 3.0 以下）の主要臓器機能が
　　　保たれていること。

　5.　HLA-A*0201 もしくは HLA-A*2402 を有すること。

　6.　眼底所見にて血圧及び硬化性変化が両眼とも Scheier　H₂S₂ かつ、糖尿病網膜症所見が単純糖尿
　　　病網膜症以下であること。

　7.　治療内容を理解し、患者本人の同意を文書で得られること。

3-2. 除外基準

　1.　妊婦（本臨床研究開始後は妊娠可能な女性は避妊する）。

　2.　授乳中の女性（本臨床研究開始後は授乳を中止する）。

　3.　妊娠の意思のある患者（試験期間中は男女共に適切な避妊をする）。

　4.　制御困難な活動性感染症を持つ患者。

　5.　過去 5 年において脳血管障害、心循環障害（心筋梗塞、狭心症等）を経験した患者。

　6.　治療にもかかわらずコントロール不能の高血圧のある患者。

　7.　試験中に以下の薬剤を投与する必要性がある患者。
　　　　副腎ステロイド剤の全身および眼局所投与又は免疫抑制剤の全身投与
　　　　（非ステロイド性消炎鎮痛剤は使用を認めるが、薬剤名と使用量を記録すること。）

　8.　治癒に至っていない外傷性病変を有する患者。

　9.　腸管麻痺あるいは間質性肺炎が疑われる患者。

　10.　医師、責任医師が不適切と認めた患者。

症例は A*2402 群、A*0201 群、コントロール群それぞれについて共同施設を含めて 40 例ずつで計 120 例とする。各施設の割り当て症例数は決まっておらず、各々の群の目標数のエントリーが済んだ時点で登録完了とする。本施設において 1 群が 40 に到達したのちでも他群が到達するまでエントリーは可能とする。この数字については、期間中抗 VEGF 療法の硝子体投与の回数、1 回分の減少を投与回数の標準偏差 3.0 と予測した場合、検出力 80％、有意水準 0.05 のもとで Wilcoxon 順位検定にて検出できる症例数である。

4　方　法
　文章にて同意取得後、HLA のタイピングにより、患者を A*2402 群、A*0201 群、コントロール群に振り分ける。全患者に対して通常の抗 VEGF 薬ルセンティスの硝子体投与を 1 カ月ごと 3 回行う。A*2402,A*0201 群に対してはこれと同時に、ペプチドそれぞれを 1mg づつをそれぞれ不完全フロイントアジュバント 1mL と混合し、患者腋下またはソケイ部の皮下に投与するペプチド療法を開始する。ペプチドを投与間隔は 2 週間ごとに行う。採血(50ml)やバイタルチェックなどの臨床検査、新生血管の変化を観察するための造影検査や光干渉断層計(OCT)といった眼科的検査は投与直前から 1 か月に一度の感覚で行い、2 年間追跡して最終的な新生血管の退縮度合いから治験薬の効果を判断する。繰り返しとなるが、行われる抗 VEGF 療法は通常の臨床で行われるものと変わりがなく、これにより患者は不利益を得ない設計になっている。

5　実施場所
　関西医科大学附属枚方病院　眼科外来

平成 22 年 6 月 6 日

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III．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tsujikawa M, Nishida K	Regenerative medicine in cornea.	Nihon Rinsho	69	2235-2240	2011
Sasamoto Y, Gomi F, Sawa M, Tsujikawa M, Nishida K	Effect of 1-year lutein supplementation on macular pigment optical density and visual function.	Graefes Arch Clin Exp Ophthalmol	249	1847-1854	2011
Wakabayashi T, Gomi F, Sawa M, Tsujikawa M, Nishida K	Intravitreal bevacizumab for exudative branching vascular networks in polypoidal choroidal vasculopathy.	Br J Ophthalmol	96	394-399	2012
Sawada O, Miyake T, Kakinoki M, Sawada T, Kawamura H, Ohji M	Negative Correlation between Aqueous Vascular Endothelial Growth Factor Levels and Axial Length.	Jpn J Ophthalmol	55	401-404	2011
Sawada O, Kawamura H, Kakinoki M, Sawada T, Ohji M	Vascular Endothelial Growth Factor in the aqueous humor in eyes with myopic choroidal neovascularization.	Acta Ophthalmol	89(5)	459-462	2011
Yoshikawa T, Ogata N, Wada M, Otsuji T, Takahashi K	Characteristics of age-related macular degeneration in patients with diabetic retinopathy.	Jpn J Ophthalmol	55	235-240	2011
Matsuyama K, Ogata N, Matsuoka M, Wada M, Nishimura T, Takahashi K	Effects of intravireally injected bevacizumab on vascular endothelial growth factor in fellow eyes.	J Ocul Pharmacol	55	379-383	2011
Yoshikawa T, Ogata N, Izuta H, Shimazawa M, Hara H, Takahashi K	Increased expression of tight junctions in ARPE-19 cells under endo-plasmic reticulum stress.	Curr Eye Res	36	1153-1163	2011
長央由里子、平本裕盛、髙橋寛二	網膜血管腫状増殖を合併した網膜色素変性の1例	日眼	115	147-150	2011
正健一郎、尾辻剛、津村晶子、津田メイ、髙橋寛二	ラニビズマブ硝子体内注射における反応不良例の検討	眼臨紀	4	782-784	2011
髙橋寛二	加齢黄斑変性の治療指針	Geriat Med	49	379-383	2011
髙橋寛二	網膜血管腫状増殖	臨床眼科	65(増)	274-281	2011
Mai Takagishi, Kazuyuki Hirooka, Tetsuya Baba, Masanori Mizote, and Fumio Shiraga	Comparison of Retinal Nerve Fiber Layer Thickness Measurements Using Time Domain and Spectral Domain Optical Coherence Tomography, and Visual Field Sensitivity.	J Glaucoma	20(6)	383-387	2011
Yasuo Tano and Masahito Ohji on behalf of the EXTEND-I Study Group	Long-term efficacy and safety of ranibizumab administered pro re nata in Japanese patients with neovascular age-related macular degeneration in the EXTEND-I study.	Acta Ophthalmolgica	89(3)	208-217	2011
The Japanese Steroid-Induced Glaucoma Multicenter Study Group	Success Rates of Trabeculotomy for Steroid-Induced Glaucoma: a Comparative, Multicenter, Retrospective, Cohort Study.	Am J Ophthalmol	151(6)	1047-1056	2011
Masanori Mizote, Kazuyuki Hirooka, Kouki Fukuda, Takehiro Nakamura, Toshifumi、Itano, and Fumio Shiraga	D-allose as Ischemic Retina Injury Inhibitor During Rabbit Vitrectomy.	Jpn J Ophthalmol	55(3)	294-300	2011
Chieko Shiragami, Fumio Shiraga, Eri Nitta, Kouki Fukuda, Hidetaka Yamaji	Correlation of Increased Fundus Autofluorescence Signals at Closed Macula with Visual Prognosis after Successful Macular Hole Surgery.	Retina	32(2)	281-288	2012
Shenyang Yang, Kazuyuki Hirooka, Ye Liu, Tomoyoshi Fujita, Kouki Fukuda, Takehiro Nakamutra, Toshifumi Itano, Jiyong Zhang, Masahiro Nishibori, Fumio Shiraga	Deleterious Role of Anti-high Mobility Group Box 1 Monoclonal Antibody in Retinal Ischemia-reperfusion Injury.	Curr Eye Res	36(11)	1037-1046	2011
Kouki Fukuda, Fumio Shiraga, Hidetaka Yamaji, Hiroyuki Nomoto, Chieko Shiragami, Hiroshi Enaida, and Tatsuro Ishibashi, MD	Morphological and functional advantages of macular hole surgery with brilliant blue G-assisted internal limiting membrane peeling.	Retina	31(8)	1720-1725	2011

Shino Sato, Kazuyuki Hirooka, Tetsuya Baba, Masanori Mizote, Takashi Fujimura, Kaori Tenkumo, Hirokazu Ueda, Fumio Shiraga	Efficacy and Safety of Switching from Topical Latanoprost to Bimatoprost in Patients with Normal-Tension Glaucoma.	Journal of Ocular Pharmacology and Therapeutics	27(5)	499-502	2011
Feng Lu, Takehiro Nakamura, Tetsuhiko Toyoshima, Yanan Liu, Kazuyuki Hirooka, Nobuyuki Kawai, Naohiko Okabe, Fumio Shiraga, Takashi Tamiya, Osamu Miyamoto, Richard F Keep, Toshifumi Itano	Edaravone, a free radical scavenger, attenuates behavioral deficits following transient forebrain ischemia by inhibiting oxidative damage in gerbils.	Neuroscience Letters	506(1)	28-32	2012
Ye Liu; Kazuyuki Hirooka; Akira Nishiyama; Bai Lei; Takehiro Nakamura; Toshifumi Itano; Tomoyoshi Fujita; Jinsong Zhang; Fumio Shiraga	Activation of the Aldosterone/Mineralocorticoid Receptor System and Protective Effects of Mineralocorticoid Receptor Antagonism in Retinal Ischemia-Reperfusion Injury.	Experimental Eye Research	96(1)	116-123	2012

Ⅳ．研究成果の刊行物・別刷

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
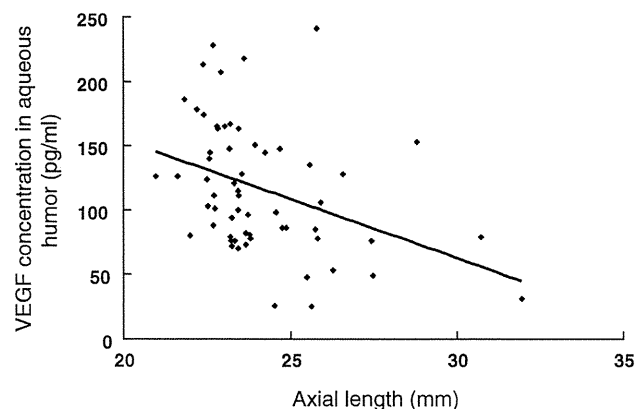

Fig. 1 Correlation between vascular endothelial growth factor (VEGF) concentrations in the aqueous humor samples and axial length. The VEGF concentration in the aqueous humor samples was negatively correlated with axial length in eyes with cataracts (Pearson product moment correlation test, $\rho = -0.373$;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

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, $P = 0.185$).

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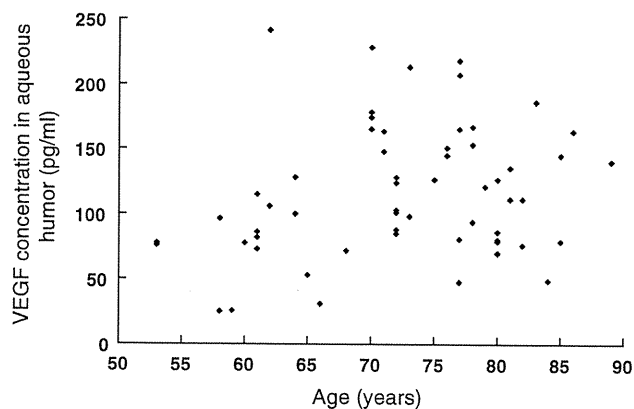
Fig. 2 Correlation between vascular endothelial growth factor (VEGF) concentrations in the aqueous humor and age. No significant correlation between the VEGF concentrations in the aqueous humor and age was found (Pearson product moment correlation test, $\rho = 0.173$; $P = 0.185$)
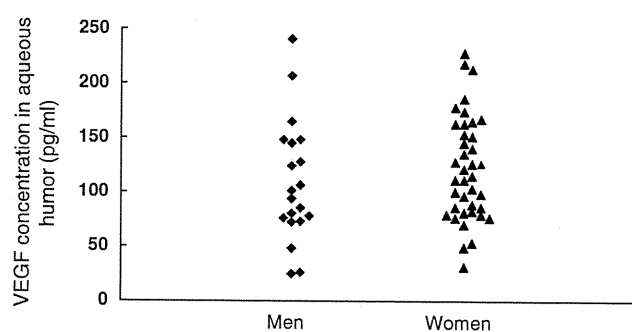

Fig. 3 Vascular endothelial growth factor (VEGF) concentrations in the aqueous humor from men and women. No significant difference between men and women in the VEGF concentrations in the aqueous humor samples was found (t test, $P = 0.381$)

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.

References

1. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med. 1994;331:1480–7.
2. Funatsu H, Yamashita H, Ikeda T, Miura T, Eguchi S, Hori S. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. Ophthalmology. 2003;110:1690–6.
3. Noma H, Funatsu H, Yamasaki M, Tsukamoto H, Mimura T, Sone T, et al. Pathogenesis of macular edema with branch retinal vein occlusion and intraocular levels of vascular endothelial growth factor and interleukin-6. Am J Ophthalmol. 2005;140:256–61.
4. Noma H, Funatsu H, Miura T, Harino S, Hori S. Vitreous levels of interleukin-6 and vascular endothelial growth factor in macular edema with central retinal vein occlusion. Ophthalmology. 2009;116:87–93.
5. Sonmez K, Drenser KA, Capone A Jr, Trese MT. Vitreous levels of stromal cell-derived factor 1 and vascular endothelial growth factor in patients with retinopathy of prematurity. Ophthalmology. 2008;115:1065–70.
6. Nonobe NI, Kachi S, Kondo M, Takai Y, Takemoto K, Nakayama A, et al. Concentration of vascular endothelial growth factor in aqueous humor of eyes with advanced retinopathy of prematurity before and after intravitreal injection of bevacizumab. Retina. 2009;29:579–85.

7. Sato T, Kusaka S, Shimojo H, Fujikado T. Vitreous levels of erythropoietin and vascular endothelial growth factor in eyes with retinopathy of prematurity. Ophthalmology. 2009; Apr 14 (Epub ahead of print).

8. Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR, et al. VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med. 2004;351:2805–16.

9. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006;355:1419–31.

10. Brown DM, Michels M, Kaiser PK, Boyer DS, Kaiser PK, Chung CY, et al. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration. Two-year results of the ANCHOR study. Ophthalmology. 2009;116:57–65.

11. Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology. 2006; 113:363–72.

12. Spaide RF, Laud K, Fine HF, Klancnik JM Jr, Meyerle CB, Yannuzzi LA, et al. Intravitreal bevacizumab treatment of choroidal neovascularization secondary to age-related macular degeneration. Retina. 2006;26:383–90.

13. Avery RL, Pearlman J, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, et al. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. Ophthalmology. 2006;113:1695–705.

14. Oshima Y, Sakaguchi H, Gomi F, Tano Y. Regression of iris neovascularization after intravitreal injection of bevacizumab in patients with proliferative diabetic retinopathy. Am J Ophthalmol. 2006;142:155–8.

15. Spaide RF, Fisher YL. Intravitreal bevacizumab (Avastin) treatment of proliferative diabetic retinopathy complicated by vitreous hemorrhage. Retina. 2006;26:275–8.

16. Iturralde D, Spaide RF, Meyerle CB, Klancnik JM, Yannuzzi LA, Fisher YL, et al. Intravitreal bevacizumab (Avastin) treatment of macular edema in central retinal vein occlusion: a short-term study. Retina. 2006;26:279–84.

17. Spaide RF, Chang LK, Klancnik JM, Yannuzzi LA, Sorenson J, Slakter JS, et al. Prospective study of intravitreal ranibizumab as a treatment for decreased visual acuity secondary to central retinal vein occlusion. Am J Ophthalmol. 2009;147:298–306.

18. Pai SA, Shetty R, Vijayan PB, Venkatasubramaniam G, Yadav NK, Shetty BK, et al. Clinical, anatomic, and electrophysiologic evaluation following intravitreal bevacizumab for macular edema in retinal vein occlusion. Am J Ophthalmol. 2007;143:601–6.

19. Rabena MD, Pieramici DJ, Castellarin AA, Nasir MA, Avery RL. Intravitreal bevacizumab (Avastin) in the treatment of macular edema secondary to branch retinal vein occlusion. Retina. 2007;27:419–25.

20. MIu Sultanov, Gadzhiev RV. The characteristics of the course of diabetic retinopathy in myopia. Vestn oftalmol. 1990;106:49–51.

21. Moss SE, Klein R, Klein BE. Ocular factors in the incidence and progression of diabetic retinopathy. Ophthalmology. 1994; 101:77–83.

22. Dujić M, Misailović K, Nikolić Lj, Ignjacev M. Occurrence of changes in the eye in diabetic retinopathy with significant myopia. Srp Arh Celok Lek. 1998;126:457–60. (in Serbian).

23. Sawada O, Kawamura H, Kakinoki M, Sawada T, Ohji M. Vascular endothelial growth factor in the aqueous humor in eyes with myopic choroidal neovascularization. Acta Ophthalmol. 2010; Jan 22 (Epub ahead of print).

24. Chan WM, Lai TY, Chan KP, Li H, Liu DT, Lam DS, et al. Changes in aqueous vascular endothelial growth factor and pigment epithelial-derived factor levels following intravitreal bevacizumab injections for choroidal neovascularization secondary to age-related macular degeneration or pathologic myopia. Retina. 2008;28:1308–13.

25. Ikuno Y, Sayanagi K, Soga K, Sawa M, Tsujikawa M, Gomi F, et al. Intravitreal bevacizumab for choroidal neovascularization attributable to pathological myopia: one-year results. Am J Ophthalmol. 2009;147:94–100.

26. Gharbiya M, Allievi F, Mazzeo L, Gabrieli CB. Intravitreal bevacizumab treatment for choroidal neovascularization in pathologic myopia: 12-month results. Am J Ophthalmol. 2009; 147:84–93.

27. Chan WM, Lai TY, Liu DT, Lam DS. Intravitreal bevacizumab (Avastin) for myopic choroidal neovascularization: 1-year results of a prospective pilot study. Br J Ophthalmol. 2009;93:150–4.

28. Sawada O, Kawamura H, Kakinoki M, Sawada T, Ohji M. Vascular endothelial growth factor in aqueous humor before and after intravitreal injection of bevacizumab in eyes with diabetic retinopathy. Arch Ophthalmol. 2007;125:1363–6.

29. Lam DS, Leung KS, Mohamed S, Chan WM, Palanivelu MS, Cheung CY, et al. Regional variations in the relationship between macular thickness measurements and myopia. Invest Ophthalmol Vis Sci. 2007;48:376–82.

30. Blaauwgeers HG, Holtkamp GM, Rutten H, Witmer AN, Koolwijk P, Partanen TA, et al. Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localization of vascular endothelial growth factor receptors on the inner choriocapillaris. Evidence for a trophic paracrine relation. Am J Pathol. 1999;155:421–8.

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)

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

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[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
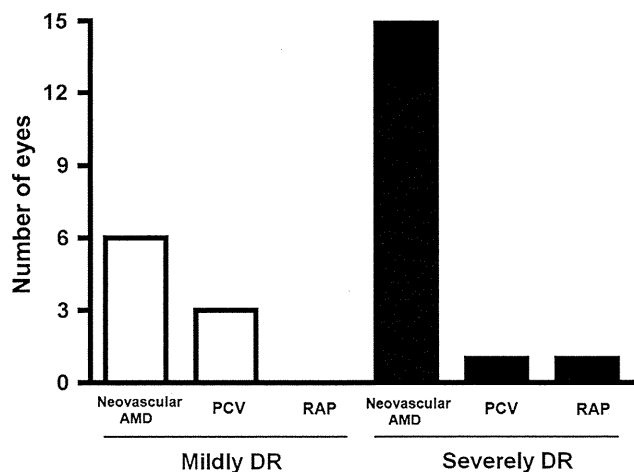
Fig. 1 Types of AMD relating to the severity of diabetic retinopathy. AMD, age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; RAP, retinal angiomatous proliferation; DR, diabetic retinopathy. We defined the eyes with mild nonproliferative diabetic retinopathy (NPDR) and moderate 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% Black 50% Asian 30%	74	0.67:1	8
Ahuja et al. [27]	2000	34	White 74% Black 20% 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.

References

1. King H, Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. Diabetes Care. 1993;16: 157–77.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care. 1998;21:1414–31.
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.
4. Miyazaki M, Nakamura H, Kubo M, Kiyohara Y, Oshima Y, Ishibashi T, et al. Risk factors for age related maculopathy in a Japanese population: the Hisayama study. Br J Ophthalmol. 2003;87:469–72.
5. Oshima Y, Ishibashi T, Murata T, Tahara Y, Kiyohara Y, Kubota T. Prevalence of age related maculopathy in a representative Japanese population: the Hisayama study. Br J Ophthalmol. 2001;85:1153–7.
6. Klein R, Klein BE, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy: the Beaver Dam eye study. Ophthalmology. 2002;109:1767–79.
7. Klein R, Klein BE, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. Ophthalmology. 1997;104:7–21.
8. Mitchell P, Wang JJ, Foran S, Smith W. Five-year incidence of age-related maculopathy lesions: the Blue Mountains Eye Study. Ophthalmology. 2002;109:1092–7.
9. Vingerling JR, Dielemans I, Hofman A, Grobbee DE, Hijmering M, Kramer CF, et al. The prevalence of age-related maculopathy in the Rotterdam Study. Ophthalmology. 1995;102:205–10.
10. Yuzawa M, Tamakoshi A, Kawamura T, Ohno Y, Uyama M, Honda T. Report on the nationwide epidemiological survey of exudative age-related macular degeneration in Japan. Int Ophthalmol. 1997;21:1–3.
11. Kawasaki R, Wang JJ, Ji GJ, Taylor B, Oizumi T, Daimon M, et al. Prevalence and risk factors for age-related macular degeneration in an adult Japanese population: the Funagata study. Ophthalmology. 2008;115:1376–81.
12. Hyman LG, Lilienfeld AM, Ferris FL 3rd, Fine SL. Senile macular degeneration: a case–control study. Am J Epidemiol. 1983;118:213–27.
13. Blumenkranz MS, Russell SR, Robey MG, Kott-Blumenkranz R, Penneys N. Risk factors in age-related maculopathy complicated by choroidal neovascularization. Ophthalmology. 1986;93:552–8.
14. Klein R, Klein BE, Moss SE. Diabetes, hyperglycemia, and age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology. 1992;99:1527–34.
15. Borrone R, Saravia M, Bar D. Age related maculopathy and diabetes. Eur J Ophthalmol. 2008;18:949–54.
16. Proctor B, Ambati J. Age-related macular degeneration and diabetic retinopathy: is diabetic retinopathy protective against ARMD? ARVO. 2007;2149 (Suppl).
17. Zylbermann R, Landau D, Rozenman Y, Abrahami S, Pollack A. Exudative age-related macular degeneration in patients with diabetic retinopathy and its relation to retinal laser photocoagulation. Eye. 1997;11:872–5.
18. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110:1677–82.
19. Takahashi K, Ishibashi T, Ogura Y, Yuzawa M. Classification and diagnostic criteria of age-related macular degeneration. Nippon Ganka Gakkai Zasshi. 2008;112:1076–84. (in Japanese).
20. Yannuzzi LA, Sorenson J, Spaide RF, Lipson B. Idiopathic polypoidal choroidal vasculopathy (IPCV). Retina. 1990;10:1–8.
21. Yannuzzi LA, Negrão S, Iida T, Carvalho C, Rodriguez-Coleman H, et al. Retinal angiomatous proliferation in age-related macular degeneration. Retina. 2001;21:416–34.
22. Barbazetto I, Burdan A, Bressler NM, Bressler SB, Haynes L, Kapetanios AD, et al. Photodynamic therapy of subfoveal choroidal neovascularization with verteporfin: fluorescein angiographic

guidelines for evaluation and treatment-TAP and VIP report No. 2. Arch Ophthalmol. 2003;121:1253–68.

23. Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: one-year results of 2 randomized clinical trials-TAP report. Arch Ophthalmol. 1999;117:1329–45.

24. Yannuzzi LA, Wong DW, Sforzolini BS, Goldbaum M, Tang KC, Spaide RF, et al. Polypoidal choroidal vasculopathy and neovascularized age-related macular degeneration. Arch Ophthalmol. 1999;117:1503–10.

25. Maruko I, Iida T, Saito M, Nagayama D, Saito K. Clinical characteristics of exudative age-related macular degeneration in Japanese patients. Am J Ophthalmol. 2007;144:15–22.

26. Yoshimura N. Polypoidal choroidal vasculopathy. Folia Ophthalmol Jpn. 2004;55:155–72. (in Japanese).

27. Ahuja RM, Stanga PE, Vingerling JR, Reck AC, Bird AC. Polypoidal choroidal vasculopathy in exudative and haemorrhagic pigment epithelial detachments. Br J Ophthalmol. 2000;84:479–84.

28. Ladas ID, Rouvas AA, Moschos MM, Synodinos EE, Karagiannis DA, Koutsandrea CN. Polypoidal choroidal vasculopathy and exudative age-related macular degeneration in Greek population. Eye. 2004;18:455–9.

29. Sho K, Takahashi K, Yamada H, Wada M, Nagai Y, Otsuji T, et al. Polypoidal choroidal vasculopathy: incidence, demographic features, and clinical characteristics. Arch Ophthalmol. 2003;121:1392–6.

30. Varma R, Macias GL, Torres M, Klein R, Pena FY, Azen SP. Biologic risk factors associated with diabetic retinopathy: the Los Angeles Latino Eye Study. Ophthalmology. 2007;114:1332–40.

31. Xie XW, Xu L, Wang YX, Jonas JB. Prevalence and associated factors of diabetic retinopathy. The Beijing Eye Study 2006. Graefes Arch Clin Exp Ophthalmol. 2008;246:1519–6.

32. Kawasaki R, Wang JJ, Rochtchina E, Taylor B, Wong TY, Tominaga M, et al. Cardiovascular risk factors and retinal microvascular signs in an adult Japanese population: the Funagata Study. Ophthalmology. 2006;113:1378–84.

33. Tahara Y, Takada Y, Fujiawa K, Ishibashi T, Inomata H, Kiyohara Y, et al. Prevalence of diabetic retinopathy in one community: the Hisayama study. Rinsho Ganka. 2000;54:573–5. (in Japanese).

34. Yannuzzi LA, Ciardella A, Spaide RF, Rabb M, Freund KB, Orlock DA. The expanding clinical spectrum of idiopathic polypoidal choroidal vasculopathy. Arch Ophthalmol. 1997;115:478–85.

35. Uyama M, Matsubara T, Fukushima I, Matsunaga H, Iwashita K, Nagai Y, et al. Idiopathic polypoidal choroidal vasculopathy in Japanese patients. Arch Ophthalmol. 1999;117:1035–42.

36. Zawinka C, Ergun E, Stur M. Prevalence of patients presenting with neovascular age-related macular degeneration in an urban population. Retina. 2005;25:324–31.

37. Cohen SY, Creuzot-Garcher C, Darmon J, Desmettre T, Korobelnik JF, Levrat F, et al. Types of choroidal neovascularisation in newly diagnosed exudative age-related macular degeneration. Br J Ophthalmol. 2007;91:1173–6.

38. Hidayat AA, Fine BS. Diabetic choroidopathy. Light and electron microscopic observations of seven cases. Ophthalmology. 1985;92:512–22.

39. Vinores SA, Campochiaro PA, May EE, Blaydes SH. Progressive ultrastructural damage and thickening of the basement membrane of the retinal pigment epithelium in spontaneously diabetic BB rats. Exp Eye Res. 1988;46:545–58.

40. Fukushima I, McLeod DS, Lutty GA. Intrachoroidal microvascular abnormality: a previously unrecognized form of choroidal neovascularization. Am J Ophthalmol. 1997;124:473–87.

41. Cao J, McLeod S, Merges CA, Lutty GA. Choriocapillaris degeneration and related pathologic changes in human diabetic eyes. Arch Ophthalmol. 1998;116:589–97.

42. Bhutto IA, McLeod DS, Hasegawa T, Kim SY, Merges C, Tong P, et al. Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. Exp Eye Res. 2006;82:99–110.

43. Nagaoka T, Kitaya N, Sugawara R, Yokota H, Mori F, Hikichi T, et al. Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. Br J Ophthalmol. 2004;88:1060–3.

44. Miyajima H, Takeda M, Imaizumi H, Okushiba U, Furusho F, Miyabe Y, et al. Clinical features of diabetic retinopathy with choroidal neovascularization. Folia Ophthalmol Jpn. 2001;52:498–504. (in Japanese).

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

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 Image-Net® (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 SIGMA-STAT 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; ρ = 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; ρ = −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;
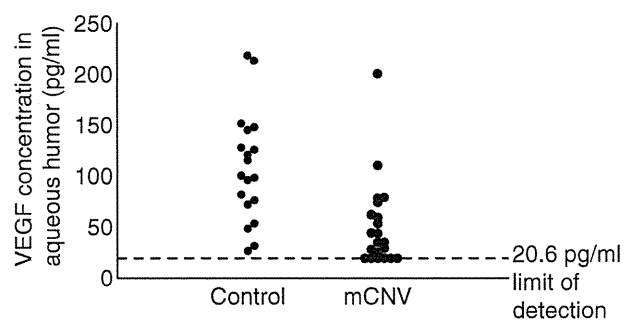

Fig. 1. Vascular endothelial growth factor concentrations in the aqueous humour in eyes with myopic choroidal neovascularization and control eyes.
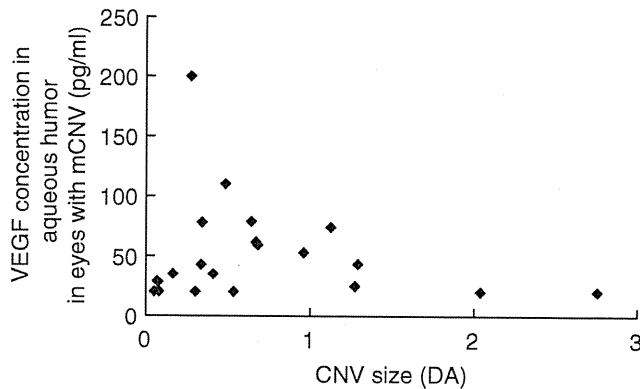
Fig. 2. The correlation between the size of the choroidal neovascularization (CNV) and the aqueous levels of vascular endothelial growth factor (VEGF) in eyes with myopic CNV (mCNV). The aqueous levels of VEGF are not significantly correlated with the size of the CNV (ρ = 0.0946; p = 0.678) (DA, disc area).
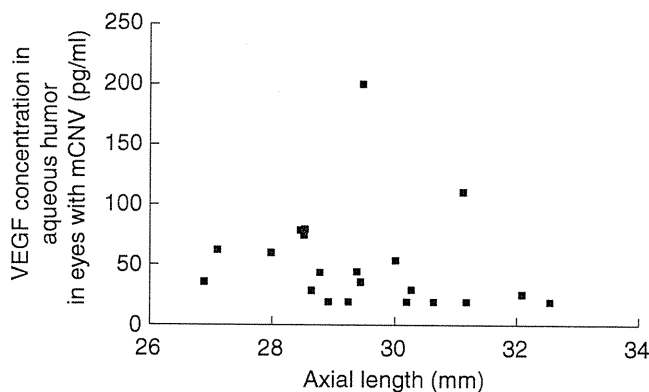

Fig. 3. The correlation between the axial length and aqueous levels of vascular endothelial growth factor (VEGF) in eyes with myopic choroidal neovascularization. The aqueous levels of VEGF are not significantly correlated with the axial length (ρ = -0.434; p = 0.0488).

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

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.

Acknowledgements

References

Aiello LP, Avery RL, Arrigg PG et al. (1994): Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331: 1480–1487.

Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA & Giust MJ (2006): Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology 113: 363–372.

Avia MP, Weiter JJ, Jalkh AE, Trempe CL, Pruett RC & Schepens CL (1984): Natural history of choroidal neovascularization in degenerative myopia. Ophthalmology 91: 1573–1581.

Blinder KJ, Blumenkranz MS, Bressler NM et al. (2003): Verteporfin therapy of subfoveal choroidal neovascularization in pathologic myopia: 2-year results of a randomized clinical trial – VIP report no 3. Ophthalmology 110: 667–673.

Chan WM, Lai TY, Chan KP, Li H, Liu DT, Lam DS & Pang CP (2008): Changes in aqueous vascular endothelial growth factor and pigment epithelial-derived factor levels following intravitreal bevacizumab injections for choroidal neovascularization secondary to age-related macular degeneration or pathologic myopia. Retina 28: 1308–1313.

Chan WM, Lai TY, Liu DT & Lam DS (2009): Intravitreal bevacizumab (Avastin) for myopic choroidal neovascularization: 1-year results of a prospective pilot study. Br J Ophthalmol 93: 150–154.

Degenring RF & Jonas JB (2005): Photodynamic therapy in combination with intravitreal triamcinolone for myopic choroidal neovascularization. Acta Ophthalmol Scand 83: 621.

Ergun E, Heinzl H & Stur M (2004): Prognostic factors influencing visual outcome of photodynamic therapy for subfoveal choroidal neovascularization in pathologic myopia. Am J Ophthalmol 138: 434–438.

Ferrara N (2004): Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 25: 581–611.

Gharbiya M, Allievi F, Mazzeo L & Gabrieli CB (2009): Intravitreal bevacizumab treatment for choroidal neovascularization in pathologic myopia: 12-month results. Am J Ophthalmol 147: 84–93.

Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M & Guyer DR (2004): VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med 351: 2805–2816.

Hera R, Keramidas M, Peoc'h M, Mouillon M, Romanet JP & Feige JJ (2005): Expression of VEGF and angiopoietins in subfoveal membranes from patients with age-related macular degeneration. Am J Ophthalmol 139: 589–596.

Hyodo I, Doi T, Endo H, Hosokawa Y, Nishikawa Y, Tanimizu M, Jinno K & Kotani Y (1998): Clinical significance plasma vascular endothelial growth factor in gastrointestinal cancer. Eur J Cancer 34: 2041–2045.

Ikuno Y, Sayanagi K, Soga K, Sawda M, Tsujikawa M, Gomi F & Tano Y (2009): Intravitreal bevacizumab for choroidal neovascularization attributable to pathological myopia: one-year results. Am J Ophthalmol 147: 94–100.

Ishibashi T, Hata Y, Yoshikawa H, Nakagawa K, Sueishi K & Inomata H (1997): Expression of vascular endothelial growth factor in experimental choroidal neovascularization. Graefes Arch Clin Exp Ophthalmol 235: 159–167.

Jonas JB & Neumaier M (2007): Vascular endothelial growth factor and basic fibroblast growth factor in exudative age-related macular degeneration and diffuse diabetic macular edema. Ophthalmic Res 39: 139–142.

Konstantinidis L, Mantel I, Pournaras JA, Zoqrafos L & Ambresin A (2009): Intravitreal ranibizumab (Lucentis) for the treatment of myopic choroidal neovascularization. Graefes Arch Clin Exp Ophthalmol 247: 311–318.

Kvanta A, Algvere PV, Berglin L & Seregard S (1996): Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. Invest Ophthalmol Vis Sci 37: 1929–1934.

Kwak N, Okamoto N, Wood JM & Campochiaro PA (2000): VEGF is major stimulator in model of choroidal neovascularization. Invest Ophthalmol Vis Sci 41: 3158–3164.

Lam DS, Chan WM, Liu DT, Fan DS, Lai WW & Chong KK (2004): Photodynamic therapy with verteporfin for subfoveal choroidal neovascularisation of pathologic myopia in Chinese eyes: a prospective series of 1 and 2 year follow up. Br J Ophthalmol 88: 1315–1319.

Lam DS, Leung KS, Mohamed S et al. (2007): Regional variations in the relationship between macular thickness measurements and myopia. Invest Ophthalmol Vis Sci 48: 376–382.

Lopez PF, Sippy BD, Lamber HM, Thach AB & Hinton DR (1996): Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. Invest Ophthalmol Vis Sci 37: 855–868.

Noma H, Funatsu H, Yamasaki M et al. (2005): Pathogenesis of macular edema with branch retinal vein occlusion and intraocular levels of vascular endothelial growth factor and interleukin-6. Am J Ophthalmol 140: 256–261.

Rosenfeld PJ, Heier JS, Hantsbager G & Shams N (2006): Tolerability and efficacy of multiple escalating doses of ranibizumab (Lucentis) for neovascular age-related macular degeneration. Ophthalmology 113: 623.

Salzmann M (1912): Anatomy and histology of the human eye ball in the normal state, its development and senescence (translated by E.V.L. Brown). Chicago: University of Chicago Press.

Sawada O, Kawamura H, Kakinoki M, Sawada T & Ohji M (2007): Vascular endothelial growth factor in aqueous humor before and after intravitreal injection of bevacizumab in eyes with diabetic retinopathy. Arch Ophthalmol 125: 1363–1366.

Tong JP, Chan WM, Liu DT, Lai TY, Choy KW, Pang CP & Lam DS (2006): Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. Am J Ophthalmol 141: 456–462.

Yoshida T, Ohno-Matsui K, Yasuzumi K, Kojima A, Shimada N, Futagami S, Tokoro T & Mochizuki M (2003): Myopic choroidal neovascularization: a 10 years follow-up. Ophthalmology 110: 1297–1305.

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