Research Article

CLINICAL INVESTIGATION

Serum levels of Netrin-1 receptor (DCC) and Vascular End othelial Growth Factor Receptor -2 (VEGFR -2) may be biomarkers for wet age-related macular degeneration

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Abstract

Purpose: To evaluate the levels of DCC (deleted in colorectal cancer, well-described Netrin-1 (NTN1) receptor) and vascular endothelial growth factor receptor-2 (VEGFR2) in the serum of patients with dry and wet age-related macular degeneration (d-AMD and w-AMD) and to compare with those of healthy controls.

Methods: Using the enzyme-linked immunosorbent assay (ELISA) method, the serum DCC and VEGFR-2 levels of d-AMD group (n=18), w-AMD group (n=18) were measured and compared with that of controls (n=14)

Results: The statistically significant differences were observed in age and gender distribution among the groups (p>0.05). The DCC levels measured in controls, d-AMD, and w-AMD were 1027.39 ± 547.07/mL; 951.94 ± 452.09 pg/mL, and 606.92 ± 143.65 pg/mL, respectively. The DCC levels in the w-AMD group was significantly lower than in both the control and d-AMG groups (p=0.05).

Conclusion: We observed that serum DCC and VEGFR-2 levels measured significantly lower in the patients with the w-AMD compared to the controls.

Keywords: Age-related macular degeneration • Netrin receptor • DCC • VEGFR-2

Abbreviation

DCC: Deleted in Colorectal Cancer, VEGFR-2: Vascular Endothelial Growth Factor Receptor-2, d-AMD: dry Age-Related Macular Degeneration, w-AMD: wet Age-Related Macular Degeneration, SD: Standard deviation.

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Introduction

Age-related Macular Degeneration (AMD) is an irreversible vision threatening disease in people aged 50 and over in developed countries. The pathogenesis of AMD is not fully understood. In AMD, photoreceptors, Retina Pigment Epithelium (RPE), Bruch membrane, and choriocapillaris in macular region were affected and a neurodegeneration occurs. AMD is divided into two groups as dry (non-neovascular, non-exudative) and wet (neovascular, exudative) AMD [1]. Dry AMD (d-AMD) includes macular RPE changes and drusen in early stages and macular atrophy in late stages. Wet AMD (w-AMD) includes the Choroidal Neovascular Membrane (CNVM) and RPE detachment. The w-AMD is responsible for more than 80% of vision loss due to AMD [1].

The Vascular Endothelial Growth Factor (VEGF) antagonists have been currently used for the w-AMD treatment. VEGF-A is the most major factor in the development of CNVM [2]. It acts *via* tyrosine kinases receptors, VEGF receptor-1 (VEGFR-1) and VEGFR-2. VEGFR-2 is the major angiogenic receptor for the effect of VEGF-A on endothelial

cells [3]. The autoregulatory activities of VEGF-A are mediated by VEGFR-2 [4]. On the other hand, VEGFR1 regulates the activity of VEGF in the vascular endothelium *via* the prevention of VEGF binding to VEGFR-2 [5]. Uehara et al. observed lower serum VEGFR-1 status in patients with w-AMD compared with the patients with d-AMD [6]. Additionally, Sharma et al. reported that there was a strong relation between the serum VEGFR-2 concentration and wet type AMD [7].

The Netrin family is a class of axon guidance proteins. Netrin-1 (NTN1) is a soluble neural projection factor and it has been expressed in the development of the nervous system [8]. It has been reported that NTN1 can induce the pro-angiogenic effect of VEGF, and control the migration and adhesion *via* binding to neogenin [9]. Additionally, Lv et al. observed that NTN1 inhibite the netrin receptor UNC5B in rats resulting inhibition of angiogenesis [10]. Recently, Xu et al. reported that retinal Neovascularization (NV) significantly reduced in the rats with oxygen-induced retinopathy which were treated with specific lentivirusmediated NTN1 shRNA [11]. On the other hand, in a previous study, it has been also demonstrated that NTN1 could inhibit and reverse corneal NV [12]. So, NTN1 can play a significant role in retinal and choroidal NV [13]. DCC (Deleted in Colorectal Carcinoma) proteins are known as axon guidance receptors. They mediate NTN1 responses. In the absence of NTN1, DCC can induce apoptosis. When DCC was bound to NTN1, cellular proliferation and migration signals are transported [8].

To our knowledge, the blood DCC levels in ophthalmic diseases have not previously been evaluated. We hypothesized that DCC may take in part during AMD progression. We aimed to evaluate the serum levels of DCC and VEGFR-2 in patients with AMD.

Methods and Materials

The pilot study was conducted by full accordance to the Declaration of Helsinki and approved by the local ethics committee. All subjects were given informed consents. We included 14 healthy control subjects, 18 patients with dry-age related macular degeneration (d-AMD), and 18 patients with wet-age related macular degeneration (w-AMD).

A full ocular examination including best-corrected visual acuity, slit-lamp biomicroscopy, tonometry, ophthalmoscopy, Fundus Fluorescein Angiography (FFA), and Optic Coherence Tomography (OCT) were carried out. According to ocular examination, patients classified as d-AMD or w-AMD. Patients having macular RPE changes and/or drusen or geographic atrophy without CNVM were classified as d-AMD. Patients with CNVM or disciform scar were classified as w-AMD.

Excluding criteria

The patients with current or past hematological, psychiatric, immune, malignant, vascular inflammatory, connective tissue, cardiac, renal, hepatic or cerebrovascular disease, diabetes mellitus, systemic hypertension, and the patients with ocular or systemic infection and inflammation, morbid obesity, and/or chronic alcohol abuse, retinal vasoocclusive disease, and patients having retinal laser photocoagulation or intravitreal injection were not enrolled.

Biochemical assay

Blood samples were collected from all the study subjects at 9 hours following overnight fasting. DCC measurements were performed with using Human DCC ELISA Kit (Elabscience Biotechnology Co., Ltd., catalog no; E-EL-H2639) according to instructions. VEGFR-2 measurements were performed with using Human VEGFR-2/KDR ELISA Kit (Elabscience Biotechnology Co., Ltd., catalog no; E-EL-H1603) according to the manufacturer's instructions. Absorbances were read spectrophotometrically in EPOCH 2 (BioTek Instrument, Inc, USA) microplate reader at 450 nm.

Statistical analysis

The data obtained from the patients were transferred to The Statistical Package for Social Sciences, version 16.0 (SPSS Inc., Chicago, IL) for statistical analysis. The Kruskal Wallis Test and the correlation analysis with the Spearman-Brown correlation coefficient were used. Mann–Whitney U test with Bonferroni correction was used for among the groups. A "p" value less than 0.05 was considered statistically significant.

Results

Age and gender distributions did not differ between the groups ($p>0.05$). The mean serum DCC levels of controls, d-AMD, and w-AMD were 1027.39 ± 547.07/mL; 951.94 ± 452.09pg/mL, and 606.92 ± 143.65pg/mL, respectively. It was found that the mean DCC level in the w-AMD group was statistically significantly lower than the both control and d-AMG groups ($p=0.05$) (Table 1).

Discussion

The increased levels of various cytokines in ocular fluids and blood of the patients with w-AMD were reported [13]. The main cytokine for CNVM development is the VEGF. The VEGF exists in bound and soluble forms and binds to its specific receptors,

Table 1. Comparison of serum VEFR2 and DCC levels between the groups.

Variable	Control (n=14)	d-AMD (n=18)	w-AMD (n=18)	P value
VEGFR2	726.17 ± 439.21	536.77 ± 238.22	449.97 ± 124.22	0.030
DCC	1027.38 ± 47.07	951.94 ± 452.09	606.92 ± 143.65	0.004

VEGFR-1 and VEGFR2 which are detected in blood circulation to promote angiogenesis and vascular integrity. VEGFR-2 is the main proangiogenic signal transducer for VEGF in angiogenesis [14, 15]. The higher VEGFR-2 has more anti-angiogenic activity [16]. Thus, it may be considered that a reduction in serum VEGFR-2 level enhances angiogenesis in eyes with AMD, especially in eyes with neovascular AMD. As seen in our study, serum VEGFR-2 levels in the patients with d-AMD may be explained by the usage of VEGF and VEGFR-2 in earlier stages of AMD. The reduction of VEGFR-2 level in serum may be a result of allowing VEGF to activate the proangiogenic endothelial cell state and to induce permeability. In a recent study, Tsai et al. observed higher plasma VEGF levels in AMD group compared with controls and moreover, plasma VEGF levels in w-AMD were significantly higher that of d-AMD [17]. The serum VEGFR2 levels also increased like VEGFR-1in patients with w-AMD [6, 7]. Sydorova et al. demonstrated higher VEGF levels in both serum and vitreous in the patients with proliferative diabetic retinopathy and that serum VEGF levels were higher only in proliferative vitreoretinopathy [18]. In our study, we found that the levels of serum VEGFR-2 are lower in patients with w-AMD. The lower serum VEGFR-2 levels in w-AMD may be contributed by the higher binding of the VEGF to VEGFR2 and CNVM development.

Netrins are a family of extracellular proteins that regulate cellular migration, intercellular interactions, and cell-extracellular matrix adhesion during the embryonic development of the nervous and vascular system [19]. NTN1, a well-described member of this family, is an axonal guidance protein and a soluble neural projection factor [20]. NTN1 enhances the proangiogenic function of VEGF and induces vascular cell migration and regulates potent chemoattractant and adhesive properties and VSMCs and endothelial cells by binding to neogenin [9]. It has been well-known that NTN1 mediates attraction or repulsion depends on its receptors. If NTN1 binds to DCC, it induces axonal attraction. On the other hand, if it binds to UNC5, NTN1 causes repulsion. So, NTN1 regulates axon guidance and also cellular migration in the development of vasculature [8-12]. The biological roles of NTN1 are mediated mainly through DCC and UNC5 receptors [21]. The DCC subfamily includes DCC and neogenin [21-23]. In the absence of NTN1, DCC signaling leads to apoptosis. If DCC is absent, downstream signaling is also absent. In a study on rats, it has been shown that NTN1 is vital in fetal survival with its role in angiogenesis in the placenta [24].

To date, there is some evidence on the roles of NTN1 in postnatal angiogenesis and NV in the cardiovascular diseases [25]. Netrin-1 may have important roles in NV. In a study on adult mice brain, it has been reported that NTN1 hyper-stimulation could increase in vivo focal NV [26]. In another study, it has been reported that NTN1 enhances the proliferation and migration of cerebral endothelial and aortic muscle cells [27]. Studies have shown that the bio-molecular mechanism in the induction of endothelial angiogenesis of NTN1 is similar to VEGF signaling, which acts through stimulation of NO production and then caspase inhibition. NTN1 mediated NO stimulation occurs via ERK 1/2 and DCC pathways [28]. NO then mediates endothelial cell growth and increases in migration induced by NTN1 [29]. A recent study has shown that NTN1 reduces apoptosis of endothelial cells and enhances vascular density in ischemia and reperfusion model [30]. It has been reported that NTN1 plays a role in corneal and retinal NV. As it has been shown that NTN1 is widely expressed the retina, this cytokine may also take in part in choroidal NV in AMD [10-12].

The main limitation of our study is lower cohort number. We observed that the levels of serum DCC are lower in AMD patients. To our knowledge, this is the first study evaluating the association between serum DCC levels and AMD. A reason for the lower DCC levels in the blood in patients with w-AMD may be due to the escape of the molecule from the vessel into the choroidal or retinal tissue and the usage or expenditure of DCC. Further studies evaluating the serum levels of DCC and VEGFR-2 in large cohorts are needed to use serum levels of DCC and VEGFR-2 as biomarkers in patients with w-AMD.

Conclusion

We observed that serum DCC and VEGFR-2 levels measured significantly lower in the patients with the w-AMD compared to the controls.

Acknowledgments

Contributions

This study was conducted by BT and NI. Biochemical assays were carried out by NI. Collection and analysis of data, typing and editing of the manuscript were performed by BT, IE, and NI. All authors were reviewed and approved the final manuscript.

Conflict of Interest

The authors declare they have no financial conflict of proprietary interest with this work.

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