

Serum and Aqueous Humor Levels of Brain-Derived Neurotrophic factor in Patients with Primary Open-Angle Glaucoma and Normal Tension Glaucoma

Yu Wan Cha

Chosun University College of Medicine

Seong Taeck Kim (✉ s20age@hanmail.net)

Chosun University College of Medicine <https://orcid.org/0000-0002-8884-1933>

Research Article

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Abstract

Purpose

This study designed to compare the levels of brain-derived neurotrophic factor (BDNF) in the serum and aqueous humor (AH) of patients with primary open-angle glaucoma (POAG) and normal-tension glaucoma (NTG).

Methods

This prospective, observational study consists of 30 patients with POAG, 30 patients with NTG, and 30 healthy controls. The serum and AH BDNF levels were assessed using an enzyme-linked immunosorbent assay.

Results

BDNF levels in serum and AH were markedly lower in the glaucoma groups (POAG and NTG) than in the control group ($p < 0.05$). When comparing the NTG and POAG groups, the average serum BDNF level was significantly lower in the NTG group than in the POAG group ($p < 0.05$). The difference in the mean BDNF levels in AH between the POAG and NTG groups was not statistically significant. ($p = 0.538$).

Conclusion

We confirmed that serum BDNF levels were lower in patients with NTG than in those with POAG. BDNF could be a causative systemic biomarker in NTG.

Introduction

As part of neurodegenerative disease, glaucoma is defined by changes in the optic nerve and visual field defects, which is characterized by retinal ganglion cells (RGCs) loss. In primary open-angle glaucoma (POAG), intraocular pressure (IOP) is the most crucial risk factor, whereas in normal-tension glaucoma (NTG), both intraocular pressure but and hemodynamic factors are considered important [1]. The pathophysiology of neurodegenerative changes in glaucoma is not fully understood.

Dysregulation of neurotrophins is one of the molecular mechanisms leading to neurodegeneration. Neurotrophins are fundamental mediators of neuron vitality [2]. The brain-derived neurotrophic factor (BDNF) is important for RGC survival. It also maintains the function of RGCs and protects them from apoptosis. BDNF passes through the blood-brain barrier, and for this reason, the levels of this factor in the serum can reflect its concentration in the brain [3]. Research on BDNF has been conducted in patients with several systemic diseases like epilepsy, Alzheimer's disease, and Huntington's disease [4, 5]. The eyes are similar to the brain regarding the anatomy and function. The blood-ocular barrier surrounds the eyes and shares features with the blood-brain barrier. The aqueous humor (AH) fills the anterior chamber and contains anti-inflammatory and other factors similar to those in cerebrospinal fluid. Analysis of

neurotrophic factors can help understand the pathogenesis of ocular diseases and determine the effectiveness of treatments that affect the contents of neurotrophic factors.

Several studies have been conducted on BDNF levels in the AH and serum in patients with ocular diseases as glaucoma, age-related macular degeneration (AMD), and diabetic retinopathy. Inanc et al. found that the BDNF level of AH was lower in non-exudative and exudative AMD groups than the controls [6]. Serum and AH concentrations of BDNF in proliferative diabetic retinopathy (PDR) were lower than in patients with non-proliferative diabetic retinopathy (NPDR) and in diabetics without diabetic retinopathy [7]. Several researches have also been studied on the levels of BDNF in glaucoma patients. Shpak et al. reported a decrease in the BDNF concentration in AH, lacrimal fluid, and serum of patients with POAG than controls [8]. Ghaffariyeh et al. observed a decrease in serum BDNF levels in the early and moderate stages of glaucoma [9]. Ghaffariyeh et al. reported that BDNF levels were lower in tears of patients with NTG than normal [10]. When comparing patients with NTG and POAG, the BDNF level in serum was lower in patients with NTG than those with POAG [11].

As far as we know, no study has evaluated the serum and AH BDNF levels in patients with POAG and NTG. Therefore, this research aimed to evaluate the serum and AH BDNF levels in these patients.

Materials And Methods

Patient Selection

This prospective, cross-sectional study included 30 patients with POAG, 30 patients with NTG, and 30 subjects as healthy controls (one eye in each person). The study included POAG patients, NTG patients, and control subjects who needed cataract surgery at Chosun University Hospital between March 2014 and December 2020. The control group consisted of patients who needed uncomplicated age-related cataract surgery without systemic disease.

Inclusion criteria

In the criteria of POAG, POAG was diagnosed when the patient had optic disc change, open-angle on gonioscopy, and visual field defects (Humphrey field analyzer, Carl Zeiss Meditec, Dublin, CA). Patients with POAG but with IOP below 22 mmHg were diagnosed with NTG every time they visited without treatment. The mean deviation (MD) and pattern standard deviation (PSD) were measured using the Humphrey Field Analyzer. Changes in RGC morphology, axons could be assessed using SD-OCT (Cirrus™ OCT, Carl Zeiss Meditec, Dublin, CA, USA). The OCT system measured the average ganglion cell inner plexiform layer (GCIPL) and peripapillary retinal nerve fiber layer (pRNFL) thickness.

Exclusion criteria

Patients with myopia greater than -6 diopters or hyperopia greater than +3 diopters were excluded. We excluded the patients with a previous history of trauma, retinal diseases, any ocular surgery. Systemic

diseases such as Parkinson's disease, dementia and stroke that may affect BDNF level were excluded from the study.

Ethical Approval

The Institutional Review Board Ethics Committee of the Chosun University Hospital approved this study. All study protocols complied with the Declaration of Helsinki. Written informed consent was obtained from patients.

Assessment of Serum and AH BDNF Levels

Blood samples for serum concentrations of BDNF were obtained from a peripheral vein before surgery and centrifuged. AH samples were obtained from the anterior chamber during cataract operation and centrifuged. Samples were stored immediately at -80°C till evaluation. We used a RayBio Human BDNF ELISA kit (Raybiotech Inc., Norcross, GA) based on an ELISA to quantitatively determine the level of BDNF in the samples.

Statistical Analysis

Statistical analysis was performed using SPSS software (SPSS Inc. version 21, Chicago, Illinois, USA). All values are presented as the mean \pm standard error. The distribution of gender in the groups was analyzed using the chi-square test, age, and spherical equivalents using the one-way ANOVA test. Kruskal–Wallis and Mann–Whitney U tests were applied to compare the BDNF levels between groups. Spearman's correlation test used correlation analysis. The p value < 0.05 was considered statistically significant.

Results

We included one eye of each individual (30 POAG patients, 30 NTG patients, and 30 controls) in the study. Table 1 summarizes the basal features of 90 patients. The gender ratio difference between the groups was not significant ($p = 0.175$). The average age of the individuals in POAG, NTG, and control groups was 65.83 ± 6.86 , 67.83 ± 8.66 , and 66.33 ± 7.50 years, respectively. The age of individuals was not significantly different among groups ($p = 0.581$). The mean spherical equivalents were -0.02 ± 1.65 , 0.07 ± 1.51 and -0.28 ± 1.38 in the POAG, NTG, and control groups. The difference among the groups was not significant ($p = 0.653$) (Table 1).

In the POAG, NTG, and control groups, the average serum levels of BDNF were 20.80 ± 7.84 ng/mL, 16.90 ± 6.09 ng/mL, and 25.17 ± 6.08 ng/mL. The mean serum BDNF levels were lower in glaucoma groups (POAG and NTG groups) than in the controls ($p = 0.019$ POAG group, $p < 0.001$ NTG group). Serum BDNF levels were lower in NTG patients than in POAG patients ($p = 0.036$) (Figure 1).

The average AH levels of BDNF in the POAG, NTG, and control groups were 28.13 ± 9.97 pg/mL, 29.67 ± 9.19 pg/mL, and 36.73 ± 9.41 pg/mL, respectively. The mean AH BDNF levels were lower in all glaucoma groups (POAG and NTG) than in the controls ($p = 0.002$ POAG group, $p = 0.005$ NTG group). The

difference in the mean AH level of BDNF between the POAG and NTG patients was not statistically significant ($p = 0.538$) (Figure 2).

Figure 3 shows a simple linear regression between the serum BDNF level and MD (Figure 3A), serum BDNF level and PSD (Figure 3B), serum BDNF level and GCIPL (Figure 3C), and serum BDNF level and pRNFL (Figure 3D) in glaucoma patients.

Serum BDNF levels did not correlate with MD (A), PSD (B), GCIPL (C), and pRNFL (D) ($r=0.037, p= 0.778$), ($r=-0.075, p=0.568$), ($r=0.018, p=0.890$), ($r=-0.028, p= 0.832$).

Figure 4 shows a simple linear regression between the AH BDNF level and MD (Figure 4A), the AH BDNF level and PSD (Figure 4B), the AH BDNF level and GCIPL (Figure 4C), and the AH BDNF level and pRNFL (Figure 4D) in glaucoma patients.

The AH BDNF level was not correlated with MD (A), PSD (B), GCIPL (C), and pRNFL (D) ($r = 0.160, p = 0.223$), ($r = -0.018, p = 0.893$), ($r = 0.160, p = 0.222$), ($r = 0.140, p = 0.287$).

Table 1. Clinical characteristics of eyes with POAG group and NTG group

| | POAG | NTG | Control | <i>p</i> value |
|----------------|--------------|--------------|---------------|----------------|
| Patients (n) | 30 | 30 | 30 | |
| Male / Female | 21 / 9 | 14 / 16 | 16 / 14 | 0.175 |
| Age (years) | 65.83 ± 6.86 | 67.83 ± 8.66 | 66.33 ± 7.50 | 0.581 |
| SE* (diopters) | -0.02 ± 1.65 | 0.07 ± 1.51 | - 0.28 ± 1.38 | 0.653 |

Values are presented as mean ± SD unless otherwise indicated.

*SE : spherical equivalents

Discussion

Diseases like AMD, glaucoma, and diabetic retinopathy are characterized as ocular neurodegenerative diseases [12]. BDNF is one of the key neurotrophic factors. The lack of neurotrophic factors in axon transport disorders contributes to glaucoma. BDNF is necessary for maintaining the vitality of RGCs and protecting them from apoptosis [13] Large differences in BDNF concentration in serum and AH means that this neurotrophin is produced in the eye [14]. In many studies, BDNF expression has been shown in a variety of ocular structures [15].

Several studies have been conducted on serum and AH BDNF levels and ocular disorders like glaucoma, AMD, and diabetic retinopathy. Inanc et al showed that the BDNF level of AH was lower in nonexudative

and exudative AMD patients than the controls [6]. The serum and AH BDNF levels were lower in PDR group than in those with NPDR and diabetes without diabetic retinopathy [7]. The BDNF level in the vitreous samples of PDR group decreased compared to that in controls [16]. Klaassen et al. suggested that BDNF expression in the fibrovascular membrane of PDR was lower than that in the epiretinal membrane [17]. According to large-scale researches, serum BDNF levels are lower in diabetics without diabetic retinopathy than in controls, and low serum BDNF levels are independent risk factors for diabetic retinopathy [18]. Ghaffariyeh et al. indicated decrease in serum BDNF levels and a relative increase in advanced POAG levels. As reduced BDNF levels are closely related to initial glaucoma, BDNF can be used as a biomarker for glaucoma detection. It can be hypothesized that an increase in progressive POAG is due to compensation recovery mechanisms that occur in the progressive phase of neurodegeneration [9]. In this study, we investigated whether there were differences in the BDNF level depending on the glaucoma stage, but no differences were found. Uzel et al. suggested that trabeculectomy affected the serum and AH BDNF levels in POAG. Serum and AH BDNF levels in patients with POAG were lower than controls on the day of surgery. In patients with POAG, serum BDNF levels increased in the 3 months following trabeculectomy, while the controls for cataract surgery had no significant difference [19]. Shpak et al. told a decrease in BDNF concentration in the AH, fluid, and serum of POAG group [8]. Ghaffariyeh et al. reported that BDNF levels were lower in the tears of NTG than normal [10]. When comparing patients with NTG and POAG, serum BDNF concentrations were lower in those with NTG than in those with POAG [11].

Experimental studies reported that high intraocular pressure (IOP) interrupts the anterograde and retrograde axonal transport of BDNF in RGC axons [20]. The deficiency of neurotrophic factors in axonal transport disorders triggers the development of glaucoma. It means that the importance of high IOPs as a major risk factor for POAG, and the therapeutic effect of IOP reduction on glaucoma leading to improvement in BDNF reduction transportation [21]. Because there is no way to improve damaged RGCs and visual loss, it is necessary to manage factors to slow down NTG progression [22]. So, many studies have investigated the pathophysiological mechanisms and risk factors for NTG. It is known that in addition to IOP, various risk factors such as blood flow dysfunction or inflammation of the optic nerve are involved in the pathophysiology of NTG [23]. Vascular spasm, microvascular disease, and autonomic dysfunction are expected to cause perfusion disorders in the optic nerve, retina, and choroid, which will affect the progression of ischemic optic neuropathy and glaucomatous optic neuropathy [24]. BDNF deficiency could be considered as one of the causes of glaucoma progression.

This is the first study to compare the serum and AH BDNF levels in patients with POAG and NTG. Our findings showed that the serum and AH BDNF concentrations were lower in patients with glaucoma than in controls. Serum BDNF levels were also lower in patients with NTG than in those with POAG. No statistically significant relationship has been found between the glaucoma criteria, including OCT and defects of visual field exam, serum and AH BDNF concentrations. High IOP is a major risk factor for glaucoma, but NTG is not fully related to high IOP. Therefore, other factors can play a role in the onset of disease, and BDNF is one of the candidate neurological protection factors.

This study has several limitations. Due to ethical issues, section acquisitions of serum and AH samples allowed BDNF measurements only at a single point in time. Undescribed confusion factors can cause variations in BDNF levels and a complete control over this situation is challenging. Although BDNF measurements in the vitreous sample are considered to be sensitive, obtaining intra-operative vitreous samples is not possible [25]. Further studies on the association between BDNF levels in the AH and vitreous are needed.

In conclusion, we confirmed that the serum BDNF concentration was lower in patients with NTG than in those with POAG. Our data support the theory that BDNF is a potential systemic biomarker in NTG.

Declarations

Acknowledgements

Conflict of interest

Authors have no potential conflict of interest to declare.

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Author contribution

ST Kim designed the study. YW Cha and ST Kim participated in the sample collection and analyzed the obtained data. All Authors read and approved the final manuscript.

Availability of data and material

All relevant data are included in the manuscript.

Compliance with ethical standards

Ethics approval

The Institutional Review Board Ethics Committee of the Chosun University Hospital approved this study.

Informed consent

Written informed consent was obtained from all study participants.

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Figures

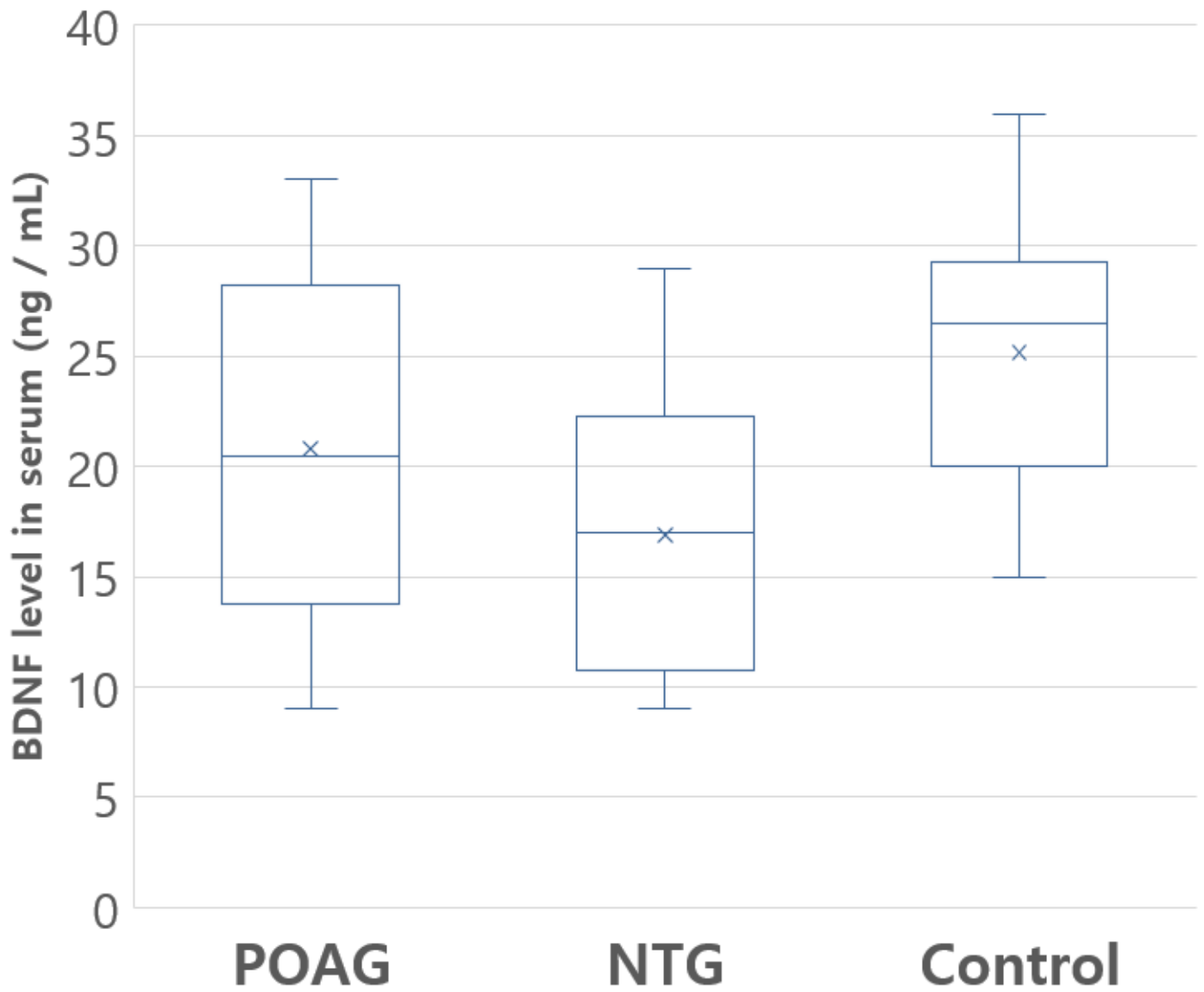


Figure 1

Comparative diagrams between POAG, NTG and controls regarding BDNF in serum

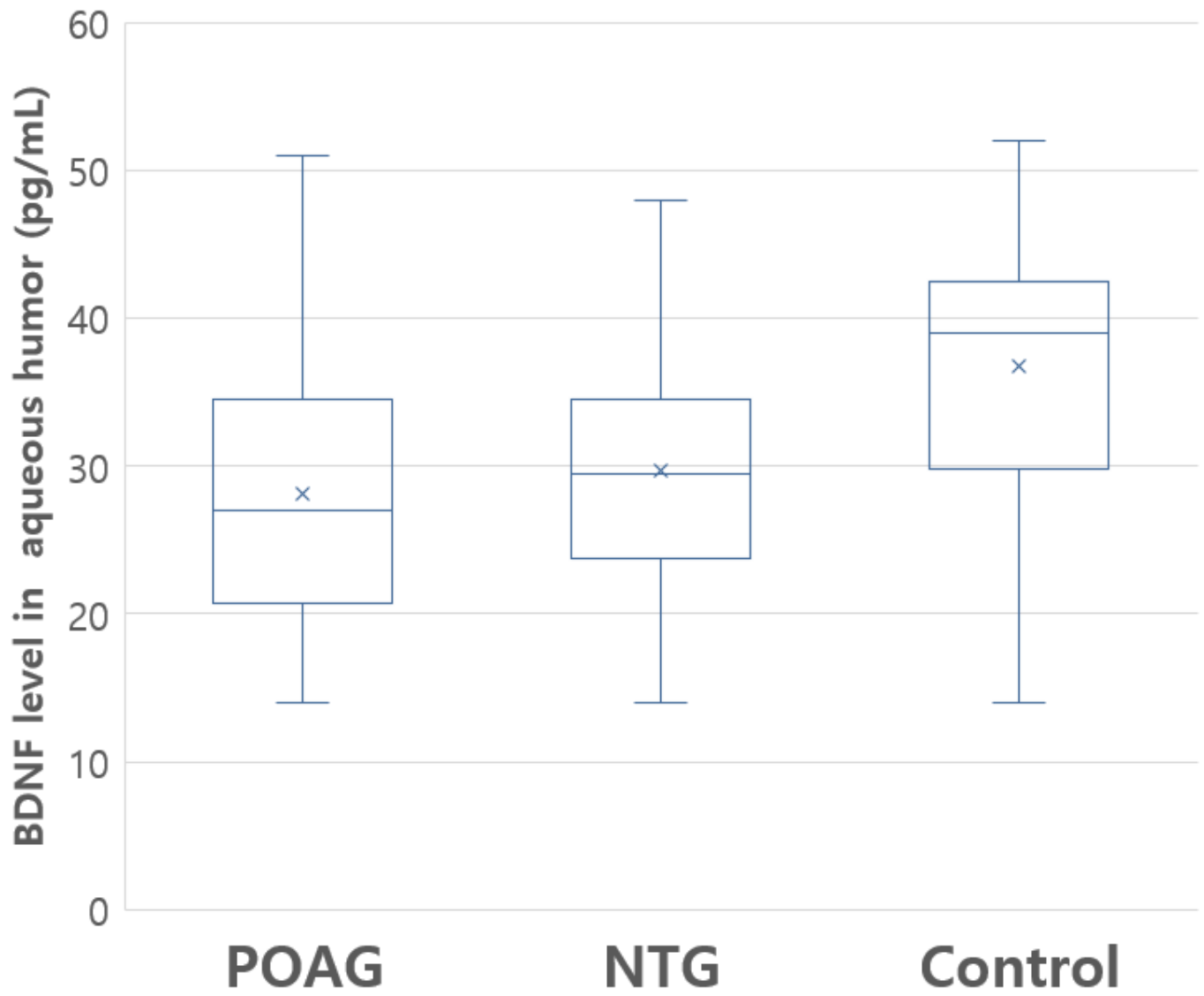


Figure 2

Comparative diagrams between POAG, NTG and controls regarding BDNF in aqueous humor

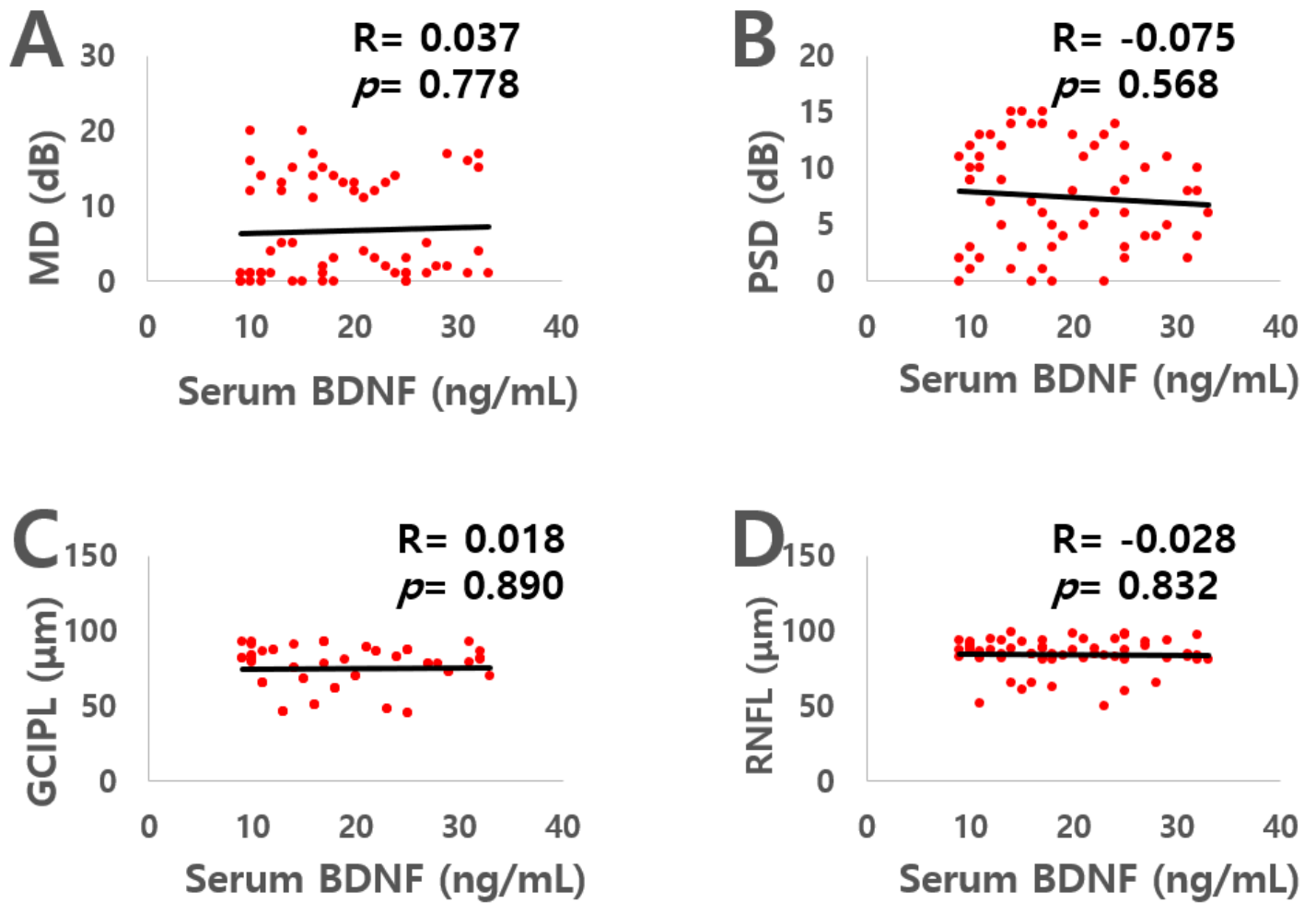


Figure 3

Scatter plot of serum BDNF level versus each parameter in eyes of patients with glaucoma ($n = 60$). No correlation was found between serum BDNF level and MD (A), BDNF and PSD (B), BDNF and GCIPL (C), and between BDNF and pRNFL (D). MD = mean deviation. PSD = pattern standard deviation. GCIPL = ganglion cell inner plexiform layer. pRNFL = peripapillary retinal nerve fiber layer

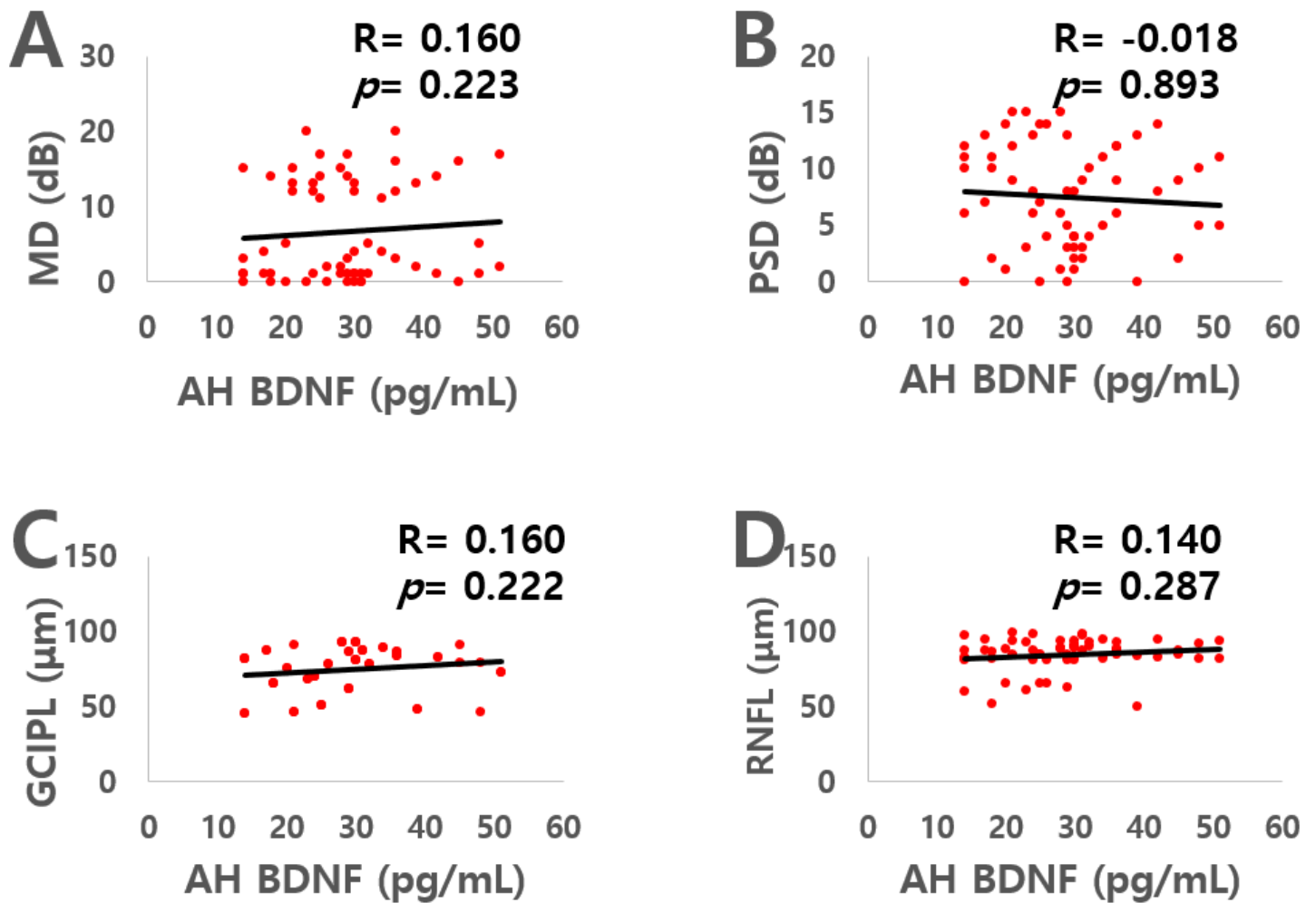


Figure 4

Scatter plot of AH BDNF level versus each parameter in eyes of patients with glaucoma ($n = 60$). There was no correlation between AH BDNF level and MD (A), BDNF and PSD (B), BDNF and GCIPL (C), and between BDNF and pRNFL (D). MD = mean deviation. PSD = pattern standard deviation. GCIPL = ganglion cell inner plexiform layer. pRNFL = peripapillary retinal nerve fiber layer.