—Original—

Serum Brain-Derived Neurotrophic Factor in Glaucoma Patients in Japan: An Observational Study

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Purpose: The aim of this study was to measure serum levels of brain-derived neurotrophic factor (BDNF) in Japanese patients with primary open angle glaucoma (POAG) and normal tension glaucoma (NTG).

Methods: This was a prospective observational study of serum BDNF levels in 78 patients who underwent cataract surgery or trabeculectomy (27 glaucoma patients and 51 non-glaucoma cataract patients as controls). Patient age was 68.8 ± 11.1 years (mean ± standard deviation; range 35-86 years). The numbers of patients with POAG and NTG were 16 and 11, respectively. POAG was diagnosed by intraocular pressure measurement, gonioscopy, optic nerve head change, and presence of a visual field defect.

Results: Serum BDNF concentration was significantly lower in the glaucoma group (including both POAG and NTG) than in the control group (7.2 ± 3.6 ng/mL vs. 12.2 ± 9.3 ng/mL, p=0.004). Serum BDNF concentration was lower in early glaucoma than in moderate glaucoma. There was no correlation between serum BDNF concentration and age. When patients with NTG and POAG were compared, serum BDNF concentration was lower in the former. Serum BDNF concentration was not significantly correlated with glaucoma parameters, including optical coherence tomography and visual field defects.

Conclusion: This is the first study to investigate serum BDNF concentration in glaucoma patients in Japan. Future studies should evaluate the role of BDNF as a potential biomarker of glaucoma.

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Key words: brain-derived neurotrophic factor, serum BDNF, glaucoma, POAG, NTG

Introduction

Glaucoma is a multifactorial neurodegenerative disease characterized by progressive degeneration of retinal ganglion cells (RGCs)[1]. High intraocular pressure (IOP) is the principal risk factor for glaucoma, and standard treatment focuses on reducing IOP by medication or surgery. However, some patients do not respond to these treatments, and some have normal tension glaucoma (NTG), which is not associated with high IOP[2]. Thus, other factors might have a role in glaucoma pathogenesis[3]. One such candidate factor is brain-derived neurotrophic factor (BDNF).

BDNF is a member of the neurotrophin family of growth factors, which are critical for generating and preserving neurons[4]. Some studies suggest involvement of BDNF in the pathology of neurological diseases such as depressive disorder[5–7], epilepsy[8], and Alzheimer[9] and Huntington diseases[10]. In ophthalmology, BDNF expression was investigated in glaucoma models[11–13]. Investigators in Iran reported that the decrease in serum BDNF

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Table 1 Numbers of patients receiving ocular antihypertensive and systemic treatment

	Control	Glaucoma (Total)	POAG	NTG
Carbonic anhydrase inhibitors	0	13	11	2
Prostaglandin analogues	0	21	14	7
β-Adrenergic receptor antagonists	0	15	11	4
α2-Adrenergic receptor agonists	0	7	3	4
Rho-associated coiled-coil forming protein kinase inhibitors	0	7	6	1
Systemic carbonic anhydrase inhibitors	0	1	1	0

POAG: primary open angle glaucoma, NTG: normal tension glaucoma

concentration was greater in patients with primary open angle glaucoma (POAG) and NTG than in healthy persons[14,15]. Similar results were recently reported in Italy[16], Turkey[17], and Russia[18].

BDNF is transported retrogradely from the superior colliculus to RGCs, and this transport is substantially inhibited by acute IOP elevation[1]. Blockade of axonal transport may cause deficits in BDNF and RGC death in glaucoma[1,13]. Moreover, BDNF administration protected RGCs in several experimental glaucoma models[19–23]. Previously, we reported that adeno-associated virus (AAV) vector gene transfer into the inner retina[24] was a new strategy for BDNF replacement. Future studies should investigate BDNF from the viewpoint of neuroprotection, as evidence suggests that BDNF might be a new target for glaucoma treatment.

POAG was the most common type of glaucoma in many population-based prevalence studies[25–27]. However, NTG was found to be prevalent in Northeast Asian countries[28–31]. Elevated IOP is the most important known risk factor for glaucoma, but lowering IOP is not always sufficient to halt progression of optic neuropathy, particularly in NTG. This suggests involvement of neuroprotective factors such an BDNF in the pathology of NTG. Although serum BDNF has been studied in glaucoma patients[14–18], no studies have been performed in Northeast Asia, where NTG is the most common type of glaucoma. This study evaluated serum BDNF levels in Japanese patients with POAG and NTG.

Materials and Methods

Study Population

This prospective observational study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Nippon Medical School Hospital (Approval number: 227026). This study was registered, before patient enrollment, in the Japanese UMIN Clinical Trials Registry (clinical trial identifier: UMIN000021304). Written informed consent was obtained from all participants before any clinical evaluations were performed.

From March 2016 through May 2019, serum BDNF levels were examined in 78 patients who underwent cataract surgery or trabeculectomy (27 glaucoma patients and 51 non-glaucoma cataract patients, as controls) in the Department of Ophthalmology of Nippon Medical School, in Tokyo. Eight patients were excluded in accordance with the exclusion criteria. Mean ± SD patient age was 68.8 ± 11.1 years (range, 35-86 years). The numbers of patients with POAG and NTG were 16 and 11, respectively. POAG was diagnosed by IOP, gonioscopy, optic nerve head change, and presence of a visual field (VF) defect (Humphrey Field Analyzer, Zeiss, Oberkochen, Germany). Patients who received a diagnosis of POAG but had an IOP of 22 mm Hg or lower, without treatment, at all evaluations, were diagnosed as having NTG. Mean deviation (MD) and pattern standard deviation (PSD) were measured with a Humphrey Field Analyzer. Alterations of RGC morphology and axons were evaluated by optical coherence tomography (OCT; RS-3000 Advance2, Nidek, Aichi, Japan). The thickness of the combined inner retinal layers, known as the ganglion cell complex (GCC), includes the retinal nerve fiber layer, ganglion cell layer, and inner plexiform layer[32]. Moreover, circumpapillary retinal nerve fibers (cpRNFLs) were also measured[33]. Glaucomatous VF loss was defined as an MD and PSD with a p value of <0.05, and a cluster in the pattern standard deviation plot of at least 3 points with a p value of <0.05, one of each with a p value of <0.01, not contiguous with the blind spot and not crossing the horizontal midline. A false-positive rate of <15%, fixation losses, and a false-negative rate of <25% were used as reliability indices. Glaucoma stage was classified according to the VF defect as early (MD < −6 dB; 17 eyes), moderate (−6 dB ≤ MD < −12 dB; 15 eyes), and advanced (MD ≥ −12 dB; 22 eyes). Patient treatments are shown in **Table 1**.

The exclusion criteria included severe ophthalmic disease, such as corneal dystrophy, degenerative retinal dis-

Table 2 Demographic characteristics of participants

	Control Mean ± SD	Glaucoma (Total) Mean ± SD	POAG Mean ± SD	NTG Mean ± SD
N	51	27	16	11
Age (years)	71.0 ± 9.59	65.8 ± 12.2	60.3 ± 5.4	74.3 ± 8.3
M/F	25/26	8/19	6/10	2/8
IOP (mm Hg)	14.1 ± 3.1	15.3 ± 4.9	16.4 ± 5.4	13.7 ± 3.5
MD (dB)		–10.4 ± 6.3	–12.0 ± 7.1	–7.9 ± 4.7
PSD (dB)		10.4 ± 4.2	10.7 ± 4.1	9.5 ± 4.7
GCC		66.3 ± 11.8	61.7 ± 10.2	71.9 ± 11.6
RNFL (μm)		65.1 ± 12.8	59.8 ± 9.4	72.7 ± 13.3
BDNF (pg/mL, p value)	12.2 ± 9.3	7.2 ± 3.6 (0.004)	7.9 ± 3.6 (0.038)	6.2 ± 3.4 (0.02)

(p values vs controls)

POAG: primary open angle glaucoma, NTG: normal tension glaucoma, SD: standard deviation, IOP: intraocular pressure, MD: mean deviation, PSD: pattern standard deviation, GCC: ganglion cell complex, cpRNFLs: circumpapillary retinal nerve fibers, BDNF: brain-derived neurotrophic factor

ease, and uveitis, and any ophthalmic surgery within 3 months. Patients with conditions that could affect serum BDNF concentration, such as depression, epilepsy, and Alzheimer disease, were also excluded.

BDNF Concentration

ELISA was used to assess BDNF concentrations in blood. Whole blood collected from patients was kept at room temperature for 15 minutes and centrifuged for 10 minutes at 870 × g. Serum was collected and stored at –80°C until use.

BDNF levels were determined by using a human BDNF Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's protocol.

Statistical Analysis

The mean and SD of measurements were calculated for each group, and comparisons between groups were made by using the Student-Newman-Keuls (SNK) method and unpaired *t*-test (Excel; Microsoft, Tokyo, Japan). The Pearson correlation test was used to analyze correlations between BDNF and clinical parameters. A p value of <0.05 was considered significant.

Results

Study Population

In this study, 27 glaucoma patients and 51 cataract (control) patients were examined. Glaucoma patients were categorized as having POAG (n=16) or NTG (n=11). The demographic and clinical characteristics of the patients are shown in **Table 2**.

Serum BDNF concentration

Serum BDNF level was significantly lower in the glaucoma group (Total) than in the control group (7.2 ± 3.6 ng/mL vs. 12.2 ± 9.3 ng/mL, p=0.004; **Fig. 1**). Subgroup analysis by glaucoma stage showed that, as compared with the control group, BDNF level was significantly lower for early glaucoma (n=17, 5.89±2.54 ng/mL, p=0.004) and advanced glaucoma (n=22, 6.89±3.57 ng/mL, p=0.006) but not for moderate glaucoma (n=15, 9.22±4.29 ng/mL, p=0.11) (**Fig. 2**). There was no significant difference in serum BDNF level between the POAG and NTG groups (7.9 ± 3.6 ng/mL and 6.2 ± 3.4 ng/mL, p=0.11; **Fig. 1**).

Correlations of BDNF with Visual Field and OCT Parameters

Correlations of BDNF with visual field and OCT parameter were investigated. There was no correlation of BDNF with these parameters, including MD (**Fig. 3A**), PSD (**Fig. 3B**), GCC (**Fig. 3C**), and cqRNFL (**Fig. 3D**).

Correlation between BDNF Concentration and Age

There was no correlation between BDNF and age in the control group (**Fig. 4A**) or glaucoma group (**Fig. 4B**). Recently, Neshatdoust et al. reported that serum BDNF level increased linearly with age until age 65 years, after which it decreased markedly[34]. Shimada et al. reported that BDNF level decreased with age after age 65 years[35]. We found no correlation between BDNF concentration and age over 65 years in the control group (**Fig. 4C**) but noted a weak negative correlation in the glaucoma group (**Fig. 4D**).

Discussion

BDNF is anterogradely and retrogradely transported in optic nerve fibers[36–38], and RGCs and optic nerve fibers express the BDNF receptor TrkB in the adult retina[39–41]. Abnormality in BDNF is a putative cause of RGC death

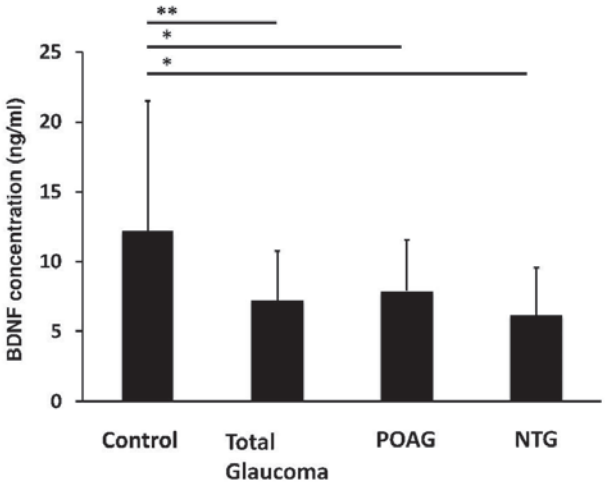

Fig. 1 Serum BDNF concentrations in controls and glaucoma patients.
BDNF concentration was significantly lower in glaucoma (n=27) patients (including Total and POAG; n=16) and NTG (n=11) patients than in controls.
BDNF = brain-derived neurotrophic factor. POAG = primary open angle glaucoma. NTG = normal tension glaucoma. **p<0.01 and *p<0.05, SNK method.

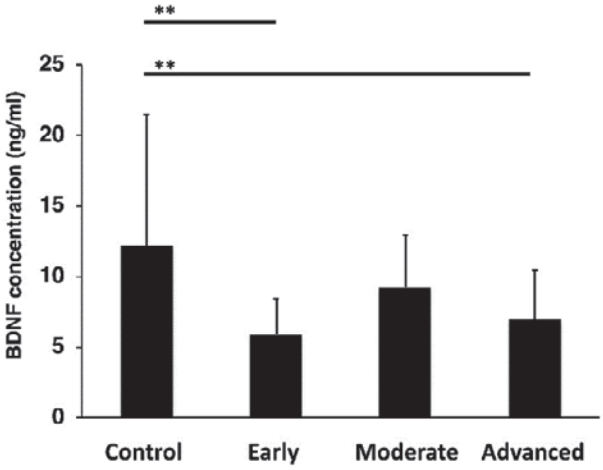

Fig. 2 Serum BDNF concentrations in controls and by glaucoma stage
The BDNF concentration was significantly lower in glaucoma patients (n=54 eyes)—including those with early (n=17 eyes), moderate (n=15 eyes), and advanced (n=22) glaucoma—than in controls.
**p<0.01, SNK method.

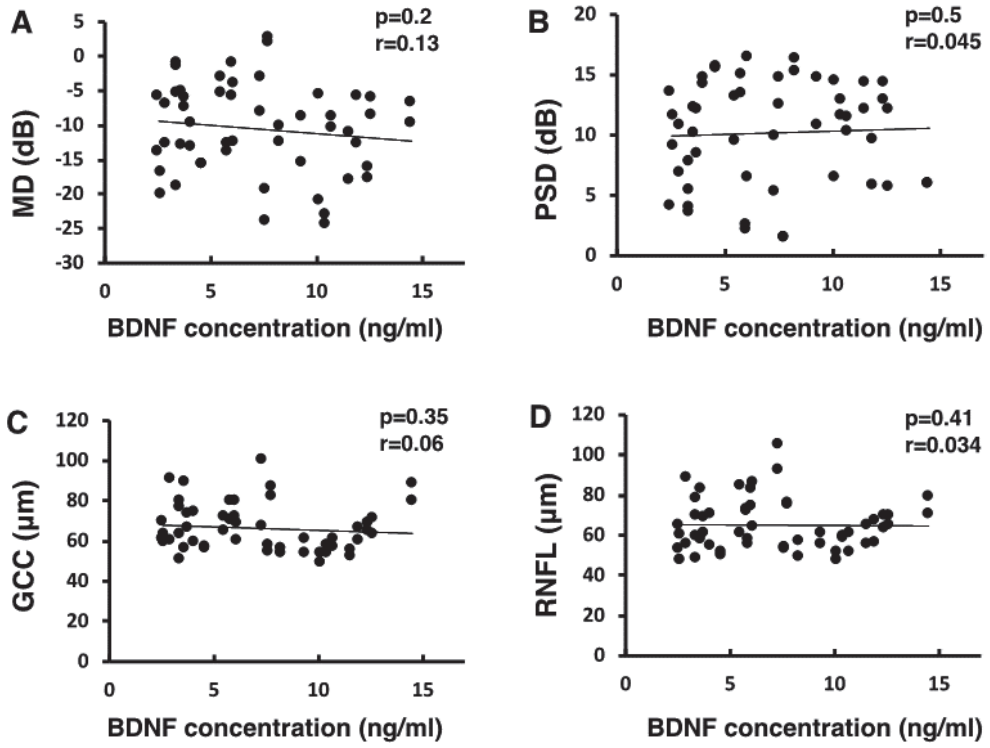

Fig. 3 Correlations of BDNF concentration with glaucoma parameters
There was no correlation between BDNF and MD (A), BDNF and PSD (B), BDNF and GCC (C), or BDNF and cqRNFL (D).
MD = mean deviation. PSD = pattern standard deviation. GCC = ganglion cell complex. cpRNFLs = circumpapillary retinal nerve fibers.

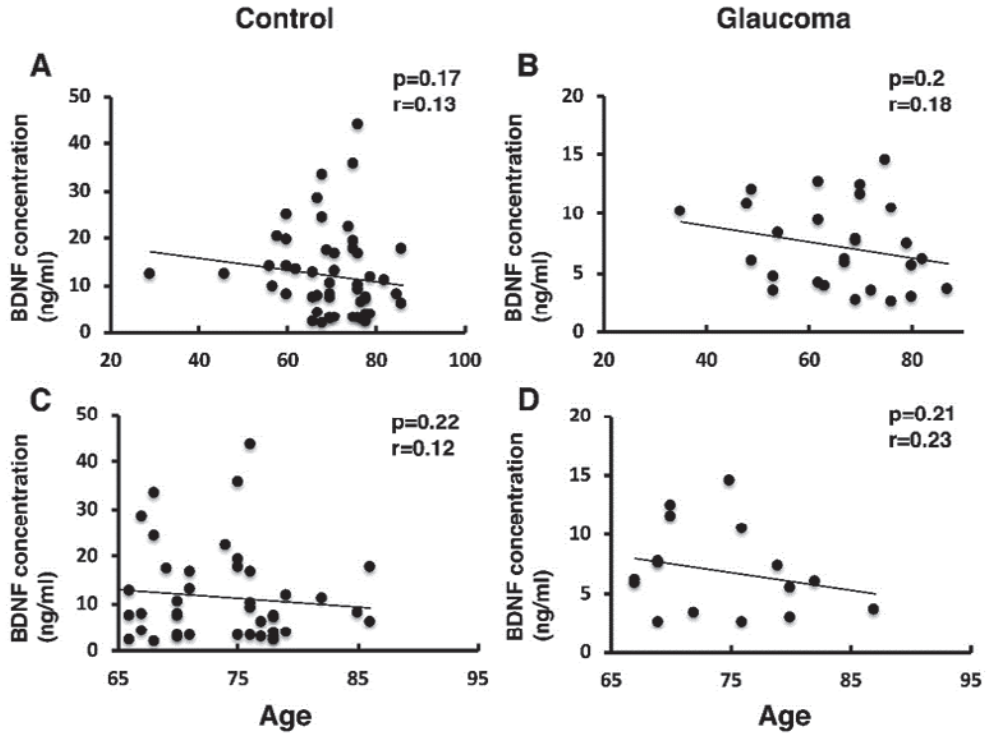

Fig. 4 Correlation between BDNF concentration and age
(A) There was no correlation between BDNF and age in the control group.
(B) There was a weak correlation between BDNF and age in the glaucoma group. (C) There was no correlation between BDNF and age older than 65 years in the control group. (D) There was a weak negative correlation between BDNF and age older than 65 years in the glaucoma group.

in glaucoma. Experimental studies showed that a decreased stream of axonal transport induced by IOP elevation led to BDNF deficits and subsequent RGC death[13,42]. Moreover, BDNF was found to be crucial role for long-term RGC survival in a glaucoma model with induced IOP elevation[43]. Some clinical studies of the relationship between serum BDNF and glaucoma have been conducted. Ghaffariyeh et al. and Shpak et al. reported decreased BDNF levels in patients with early POAG[14,18]. Oddone et al. reported that BDNF levels were lower in patients with early and moderate POAG but not in those with advanced POAG[16].

This study examined serum BDNF concentrations in glaucoma patients who underwent trabeculectomy or cataract surgery and compared those levels with those of non-glaucoma patients who underwent cataract surgery. As was the case in previous studies, serum BDNF concentration in the glaucoma group (including both POAG and NTG) was significantly lower than that in the control group. Serum BDNF concentration was greater than 15 ng/mL in 16 of 51 non-glaucoma patients and in 0 of 27 glaucoma patients. Serum BDNF concentration was lower in early glaucoma than in advanced glaucoma. There was no correlation between serum BDNF concentration and age. When NTG and POAG were compared, serum BDNF concentration was lower in NTG patients than in POAG patients. In the present study, power analysis indicated that the sample size was small for subgroup analyses. Because this was an observational study with descriptive statistics, the low number of cases should be regarded as a limitation.

BDNF has a short half-life and is difficult to measure accurately. To improve accuracy, serum was centrifuged and preserved at the outpatient clinic rather than at a central laboratory. The serum obtained was frozen on dry ice, immediately transferred to a freezer, and stored at −80°C. The cost is about US$100 per sample if a sample is measured every time. In this experiment, we examined BDNF levels in aqueous humor by using the same R&D Human BDNF Immunoassay used by Uzel et al.[17]. However, we were unable to measure BDNF in aqueous humor. Measurement was also performed by using an ultrasensitive human BDNF ELISA kit (Aviscera Bioscience, Inc. Santa Clara, CA, USA) that can measure the lowest concentration of any commercially available product. Although the sensitivity of this kit is high (1-3 pg/mL), most samples were below the measurement limit. In the future, we will measure BDNF in the aqueous humor

with a Simoa HD-1, an ultra-high sensitivity auto-ELISA from Quantaix. This device can measure the low concentration amount 1,000 times as much as before, and we plan to examine BDNF concentration in the anterior chamber.

In the present study, the correlations of glaucoma parameters, including OCT and visual field, with serum BDNF level were examined, but no significant correlations were identified. Although previous studies reported a significant negative correlation of serum or aqueous BDNF level with severity of visual field defects[14,18], BDNF level was lowest in patients with early glaucoma in the present study. The reasons for this finding are unclear, but it may be related to the high proportion of NTG patients.

Studies highlight the importance of BDNF in glaucoma[14–23]. Among neurotrophic factors, BDNF is important because of its potent protective effect on injured RGCs[44]. BDNF is essential for RGC survival during development[21] and in adults[38]. BDNF values could not be measured in this experiment, although it is considered important to be able to quantify BDNF values in the anterior chamber. In the future, patients with low BDNF in the eye may expect replacement therapy with persistent BDNF protein or sustained BDNF derived from an AAV vector. In such cases, preclinical studies using monkeys should be conducted, because of the possible adverse effects of an overdose.

In conclusion, this is the first study to investigate serum BDNF concentrations in glaucoma patients in Japan. Future studies should evaluate the role of BDNF as a potential biomarker of glaucoma.

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Conflict of Interest: The authors declare no conflict of interest.

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