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Glial cell line-derived neurotrophic factor (GDNF) in patients with primary open-angle glaucoma and age-related cataract

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Purpose: To study glial cell line-derived neurotrophic factor (GDNF) concentrations in aqueous humor (AH), lacrimal fluid (LF), and blood serum (BS) in patients with age-related cataract and primary open-angle glaucoma (POAG).
Methods: GDNF was studied in AH, LF, and BS in 47 patients with age-related cataract, and 30 patients with POAG combined with cataract (one eye in each person). AH was sampled during cataract surgery.
Results: GDNF concentration (pg/ml) in patients with POAG and cataract was lower than in cataract-only patients ($p<0.001$), both in AH (46.3±31.1 versus 88.9±46.9) and in LF (222±101 versus 344±134). The difference was not significant for the GDNF concentration in BS (194±56 versus 201±45). In the earlier (early and moderate) stages of POAG, compared to later (advanced and severe) stages, GDNF concentration was significantly lower in LF (176±99 versus 258±91; $p = 0.027$) and in BS (165±42 versus 217±55; $p = 0.017$), while GDNF concentration in AH showed an insignificant difference (40.0±25.7 versus 51.1±34.7). In patients with POAG, GDNF concentration in LF and BS was inversely correlated with the Humphrey visual field index: Pearson's correlation coefficient $r = -0.465$ ($p = 0.01$) for LF and $r = -0.399$ ($p = 0.029$) for BS. When compared to the cataract group, patients in the earlier stages of POAG showed significantly lower GDNF concentrations in all studied biologic fluids.
Conclusions: Compared to patients with cataract only, GDNF levels are lower in the AH and LF of patients with POAG and cataract, especially at earlier stages of the disease (at these stages, the GDNF level in BS is also lower). At earlier stages of POAG, compared to later stages, GDNF content is lower in LF and BS. These data could serve as a reason for the therapeutic use of GDNF in patients with POAG.

and survival of neurons, and their deficiency is believed to be an essential link in neurodegeneration. The powerful neuroprotective effects of neurotrophic factors, including brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), nerve growth factor (NGF), and glial cell-line derived neurotrophic factor (GDNF), suggest that they are good therapeutic candidates for neurodegenerative diseases [1]. The ultimate cause of vision loss in glaucoma, the second leading cause of blindness worldwide and the most common optic neuropathy, is believed to be associated with the deprivation of neurotrophic factors leading to progressive retinal ganglion cell degeneration and death. GDNF, the most studied member of the family of neurotrophic factors that bears its name, has been shown to support the development, survival, and functioning of dopaminergic, sensory, motor, and other neuronal populations [2]. In the human eye, GDNF has been found in corneal stroma [3] and vitreous [4,5]. It has also been detected in lacrimal fluid (LF) [6]. GDNF receptors or their mRNA have been found in aqueous humor (AH) [7], in cultured lamina cribrosa cells and optic nerve head astrocytes [8]. However, quantitative data on GDNF content have been determined only for the vitreous body [4,5].

Disturbances in the GDNF system are associated with glaucoma pathogenesis. Akurathi et al. [9] explored the expression of GDNF and GDNF receptors GFRα1 and GFRα2 in normal and glaucomatous human tissue. The area immunopositive for GFRα2 was significantly decreased in glaucomatous eyes in both the peripapillary region and more peripheral retinal locations. Glaucoma patients, compared to healthy control patients, also showed less GFRα1 expression in the peripapillary retinal ganglion cell complex + retinal nerve fiber layer.

GDNF has been shown to rescue retinal neurons in animal models of retinal and optic nerve diseases, particularly glaucoma [10-13]. Numerous studies have reported that applications of GDNF alone or in combination with other neuroprotective substances improve the survival of photoreceptors,

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retinal pigment epithelial cells, and retinal ganglion cells in both cultures and in vivo models [14-18]. Baranov et al. [19] showed that the small molecule GSK812 was able to induce GDNF in vitro/in vivo and rescue photoreceptors in models of retinal degeneration.

Taking into account the essential involvement of GDNF in the survival of retinal neurons, it is surprising that quantitative data on GDNF concentrations in AH and LF, particularly in patients with glaucoma, have not been established. Blood serum (BS) or plasma GDNF level has been studied in patients with Alzheimer's and Parkinson's diseases [20-22], schizophrenia and mood disorders [23-27], diabetes [28], and other diseases; however, GDNF concentration in the BS of glaucoma patients was not measured in these studies. The purpose of the present work was to examine GDNF concentrations in the AH, LF, and BS of patients with primary open-angle glaucoma (POAG) and age-related cataract.

METHODS

Subjects and clinical assessment: For this study, 77 patients (77 eyes) were examined. The patients were divided into two groups. Thirty patients (30 eyes) with POAG, operated on for age-related cataract, formed the main or "Cataract with POAG" group, and 47 patients (47 eyes) without POAG, operated on for age-related cataract, were included in the comparison or "Cataract" group. All patients underwent uneventful phacoemulsification with intraocular lens implantation by the same surgeon and were selected consecutively in both groups from January 2020 until March 2021.

The inclusion criteria were as follows: age over 50 years, amount of LF sufficient for GDNF measurement, intraocular pressure (IOP) by applanation tonometry less than 21 mmHg, and axial length of the eye less than 26 mm. In addition, patients in the Cataract group needed to have postoperative best corrected visual acuity not less than 20/40, normal Humphrey visual fields, and normal appearance of the optic disc, and a retinal nerve fiber layer. Patients with POAG should have open angles at gonioscopy and reliable repeated Humphrey perimetry.

The exclusion criteria were as follows: any serious ophthalmic disease (uveitis, degenerative diseases of the retina, corneal dystrophies, etc.), any ophthalmic surgery during the last three months, severe somatic pathology (diabetes mellitus, autoimmune or oncological diseases, etc.), and diseases that affect GDNF levels (Alzheimer's and Parkinson's diseases, schizophrenia, depression, etc.). Persons with initial/mild manifestations of somatic diseases, such as essential hypertension, ischemic heart disease, or cardiac arrhythmias, were not excluded.

All patients were under observation at the S. Fyodorov Eye Microsurgery Federal State Institution and were thoroughly examined before and after the operation to confirm the diagnosis and POAG stage. Examinations included automated perimetry (Humphrey Field Analyzer II, Carl Zeiss Meditec Inc., Dublin, CA), using the SITA Standard 24–2 program, and optical coherence tomography without pupil dilation, using the Cirrus HD-OCT 5000 (Carl Zeiss Meditec Inc., Dublin, CA). POAG stage was established according to the Glaucoma Staging System suggested by Mills et al. [29]. This study adhered to the tenets of the Declaration of Helsinki and had local ethics committee approval, with informed consent obtained from all subjects.

Measurement of GDNF concentration: In both groups of patients, AH and blood were collected during phacoemulsification. AH (100–120 µl) was taken immediately after entering the anterior chamber. LF was sampled on the day preceding surgery to evade the influence of drugs instilled into the conjunctival fornix in the evening and morning before surgery.

A pipette was used to sample stimulated LF. (The LF was secreted by the lacrimal gland in response to a very gentle touch of the cornea with the disposable tip of the pipette; this is safe and acceptable for most patients.) A minimal sample of 100 µL was needed to measure the GDNF in LF or AH. To collect serum, blood samples were left at room temperature for 30 min, centrifuged at 1500 × *g* for 15 min, and aliquoted. BS, AH, and LF samples were frozen immediately after being taken, stored at −80 °C in polypropylene tubes (Sarstedt GmbH, Nümbrecht, Germany), and analyzed within three months from sampling. The author performing GDNF analyses (Tatiana A. Druzhkova) was blind to the patients' details.

Upon thawing, samples were centrifuged at 4000 × *g* for 15 min at 4 °C to ensure complete debris removal. Based on previously described methods [30], we used an acid treatment procedure to allow the quantification of total GDNF levels in biologic samples. During the acid treatment procedure, BS, AH, and LF samples were diluted four times, with subsequent multiplication of the results by the dilution factor. The concentration of GDNF was measured in biologic fluids using a Human GDNF ELISA Kit (RayBiotech, Norcross, GA; Code: ELH-GDNF-1), according to the manufacturer's instructions, on a ChemWell 2910 automatic analyzer (Awareness Technology, Inc., Palm City, FL). For each ELISA assay, its own calibration was performed. Recombinant human GDNF samples (RayBiotech, Norcross, GA; Code: 230–00754–10) at concentrations of 10 pg/ml, 100 pg/ml, and 1000 pg/ml were used as controls. Intraassay and interassay coefficients

Table 1. Age and gender characteristics, and axial length of the eye of the patients in the compared groups.

Variable	Cataract with POAG group (n=30)	Cataract group (n=47)
Age, years		
mean±SD	73.9±7.0	74.0±7.4
range	57–89	57–88
median (IQR)	72.5 (69.5–78)	74 (69–80)[a]
Sex,		
women, n (%)	21 (70%)	30 (64%)[b]
Axial length of the eye, mm		
mean±SD	23.05±0.85	23.37±0.76
range	21.43–25.01	21.9–25.1
median (IQR)	23.06 (22.80–23.48)	23.38 (22.96–23.74)[a]

The differences between groups are not significant ([a] Welch's *t* test, [b] Fisher's exact test) POAG – primary open-angle glaucoma; SD – standard deviation; IQR – interquartile range; NS – non-significant

of variation did not exceed 10%, which is consistent with the manufacturer's recommendations. The sensitivity of the assay (minimum quantifiable value) was 4.0 pg/ml. Most measured values were in the validated assay range. Sample permitting, two replicates were used.

Statistical analysis: Statistical analysis was performed using R software package version 3.6.0 (The R Foundation for Statistical Computing, R project, accessed September 12, 2021). Continuous variables are presented as mean (M) ±standard deviation (SD) and median (Me) with interquartile range (IQR). A comparison of continuous variables in the two groups was performed using Welch's *t* test for independent samples or the Mann–Whitney U test as appropriate. A comparison of continuous variables in the three groups was performed using the Kruskal–Wallis test and a post-hoc Dunn test for multiple comparisons. Categorical variables were compared using Fisher's exact test. The associations of variables were analyzed using Pearson's correlation coefficient; $p<0.05$ was considered statistically significant.

The minimum required number of subjects in the main and comparison groups ($n_1 = n_2$) was 35. This number was calculated using the Equation [31] $n_1 = n_2 = (Z_{1-\alpha/2} + Z_{1-\beta})^2 * (\sigma_1^2 + \sigma_2^2) / d^2$, where σ_1 and σ_2 are standard deviations of GDNF levels in AH in these groups (29 and 44 pg/ml in a preliminary study), d is the minimum detectable difference (assumed 25 pg/ml), α was chosen as 5%, and power (1-β) was set at 80%. With an increase in the number of subjects in the comparison group (to 45, for example), the required number of subjects in the main group can be reduced to 29, according to the Equation [32] $n_2 = n*n_1/(2n_1-n)$, where n is the required equal number of subjects in two groups, and n_1 and n_2 are the required unequal numbers of subjects in the first and second groups.

Summary statistics on data sets of GDNF in AH with a portion of observations in the comparison group below the minimum quantifiable value (3 cases, 6.4%) were performed after substitution with half the minimum quantifiable value (2.0 pg/ml; see Limitations). Also, one extreme outlying value in the data set of GDNF in LF in the comparison group was excluded.

RESULTS

The age and gender characteristics as well as the axial lengths of the eyes of subjects in the Cataract and Cataract with POAG groups are presented in Table 1. The groups did not differ according to any of these parameters. The data sets for both groups on the content of GDNF in the studied biologic fluids are presented in Table 2.

GDNF levels varied in a relatively wide range in all biologic fluids studied. In both groups, GDNF concentration was the highest in LF, somewhat lower in BS, and much lower in AH. In BS, GDNF concentration did not differ between groups; however, in LF and AH, significantly lower GDNF levels were revealed in the Cataract with POAG group compared to the Cataract group. The difference in mean GDNF concentration was relatively more pronounced in AH (1.92 times) than in LF (1.55 times).

As seen in Table 1, there were more women than men in both groups. However, this did not have a significant impact on GDNF levels. Thus, the sex-adjusted (mean) concentration of GDNF in the Cataract with POAG group compared to the Cataract group was 44.4 versus 91.0 pg/ml in AH, 217 versus 347 pg/ml in LF, and 195 versus 204 pg/ml in BS.

In the Cataract with POAG group, five patients had early POAG, eight had moderate POAG, five had advanced POAG,

Table 2. GDNF concentrations in the biologic fluids (pg/ml) in cataract patients with and without POAG.

Biologic fluid	Cataract with POAG group (n=30)	Cataract group (n=47[a])	P
Aueous humor			
mean±SD	46.3±31.1	88.9±46.9	<0.001
range	4.4–106.6	2.0–212.5 [b]	
median (IQR)	47.7 (15.4–61.2)	83.0 (58.8–119.4)	
Lacrimal fluid			
mean±SD	222±101	344±134	<0.001
range	61–487	173–683	
median (IQR)	218 (160–293)	312 (244–411)	
Blood serum			
mean±SD	194±56	201±45	NS
range	106–317	103–287	
median (IQR)	180 (158–228)	196 (174–239)	

Statistical analysis was performed by Mann–Whitney U test POAG – primary open-angle glaucoma; SD – standard deviation; IQR – interquartile range; NS – non-significant [a] For lacrimal fluid n=46 (one case with extreme outlying value excluded) [b] In 3 cases, values below 4.0 pg/ml were substituted with half minimum quantifiable value (2.0 pg/ml); without these values range is 32.6–212.5 pg/ml

and 12 had severe POAG. Due to the small number of cases, for further analysis, patients with early and moderate POAG were combined into the "POAG 1–2" subgroup (n = 13), and patients with advanced and severe POAG were combined into the "POAG 3–4" subgroup (n = 17). The detailed characteristics of the patients in these subgroups are presented in Table 3.

Most characteristics were similar in both subgroups. Although patients in the POAG 3–4 subgroup were prescribed β-blockers less often and had surgeries more often than patients in the POAG 1–2 subgroup, these differences did not reach statistical significance.

To analyze the influence of glaucoma progression, GDNF concentrations were compared between POAG subgroups (Table 4). We also studied GDNF changes with a decrease in the Humphrey visual field index (VFI), indicative of glaucoma progression (Figure 1).

Both Table 4 and Figure 1 show significantly less GDNF content in LF and BS at earlier stages of POAG compared to later stages. GDNF concentration in AH showed similar changes, but these were smaller and insignificant.

Comparison of the POAG subgroups with the Cataract group showed that GDNF content in the POAG 1–2 subgroup was significantly lower in all biologic fluids studied: $p = 0.002$ for AH, $p<0.001$ for LF, and $p = 0.027$ for BS (Kruskal–Wallis test with post-hoc Dunn test). GDNF concentration in the POAG 3–4 subgroup was significantly lower in AH ($p = 0.008$) and in LF ($p = 0.043$).

In the patients with POAG, a significant correlation was found between GDNF levels in AH and BS ($r = 0.467$; $p = 0.009$); however, this correlation was absent in the Cataract group. Notably, no correlations were found with GDNF content in LF.

DISCUSSION

GDNF is a strong neuroprotective agent for retinal cells, particularly retinal ganglion cells. Its neuroprotective role has been proven by the increased survival of retinal ganglion cells in animal models of glaucoma [11,15], optic nerve damage [12,33], and retinal ischemia [34]. GDNF also promotes the survival of photoreceptors in animal models of retinal degeneration [17,35,36] and in cell culture models of inflammation and oxidative stress [5].

GDNF is the object of intense research in various neurologic, psychiatric, and other diseases [20-28]. In humans, it is measured mainly in BS [20-28] and rarely in cerebrospinal fluid [20]. Depending on the manufacturer of the ELISA kit, GDNF concentrations in the BS of healthy controls varied widely, from 11.7 pg/ml [28] to 4.1 ng/ml [22]. Among nine studies [20-28], our data fell within the interquartile range of 127 [24] to 690 pg/ml [23]. Obviously, normative data are not interchangeable between studies; therefore, in this study, the data on GDNF content in otherwise healthy cataract patients were regarded as normative data when making comparisons with patients with POAG and cataract (see also Limitations).

Our study was the first to measure GDNF concentration not only in the BS but also in the AH and LF of

Table 3. Age and gender characteristics, intraocular pressure (IOP), medical, laser, and surgical treatment in the POAG subgroups.

Variable	POAG 1–2 subgroup (n=13)	POAG 3–4 subgroup (n=17)	P
Age			
mean±SD	73.5±5.9	74.2±7.9	
Range	62–82	57–89	
median (IQR)	72 (69–78)	73 (71–80)	
Sex			
women, n (%)	10 (77%)	11 (77%)	
IOP, mmHg			
mean±SD	13.3±2.6	13.6±3.1	
range	9–18	9–19	
median (IQR)	13 (12–15)	13 (11–16)	
Medications, n (%)			
β-blockers	10 (77%)	7 (41%)	(0.071) [a]
Prostaglandin analogs	1 (8%)	3 (18%)	
Topical carbonic anhydrase inhibitors	6 (46%)	5 (29%)	
Laser treatment and surgery			
SLT	7 (54%)	9 (53%)	
NPDS	5 (39%)	13 (76%)	(0.061) [a]

The differences between subgroups are not significant; values close to significant are given in parentheses ([a] Fisher's exact test) POAG – primary open-angle glaucoma; SD – standard deviation; IQR – interquartile range; SLT – selective laser trabeculoplasty; NPDS – non-penetrating deep sclerectomy with or without deferred Nd:YAG goniopuncture

cataract patients with and without POAG. Earlier, in a single proteomics study, only GDNF receptor GFRα1 was found in two out of four groups of samples of cataract patients' AH, while GDNF itself was not detected [7]. In another study, GDNF was found in the tears of healthy individuals [6]; however, it was not quantitatively measured.

In three studies, GDNF concentration was measured in the vitreous body of patients with various vitreoretinal

Table 4. GDNF concentration in the studied biologic fluids (pg/ml) in POAG subgroups.

Biologic fluid	POAG 1–2 subgroup (n=13)	POAG 3–4 subgroup (n=17)	P
Aqueous humor			
mean±SD	40.0±25.7	51.1±34.7	NS
range	4.4–77.4	4.4–106.6	
median (IQR)	45.7 (14.4–57.4)	53.2 (32.5–66.0)	
Lacrimal fluid			
mean±SD	176±99	258±91	0.027
range	61–385	141–487	
median (IQR)	176 (96–238)	240 (190–307)	
Blood serum			
mean±SD	165±42	217±55	0.017
range	106–230	150–317	
median (IQR)	163 (128–202)	225 (163–246)	

Statistical analysis was performed by Mann–Whitney U test POAG – primary open-angle glaucoma; SD – standard deviation; IQR – interquartile range; NS – non-significant

pathologies [4,5,37]. In one of these studies, the GDNF level was below the minimum quantifiable value [37]. In the two others, the mean GDNF value was 20.25 pg/ml in controls with retinal detachment [4] and 89.5 pg/ml in controls with various non-diabetic vitreoretinal pathologies [5]. Interestingly, this last value is practically equal to the mean GDNF concentration in the AH of our comparison group. With different manufacturers of ELISA kits, this may be just a coincidence. Alternatively, it may reflect an ease of transition between AH and the vitreous for GDNF and, presumably, for other neurotrophic factors.

The results of the present study showed significantly lower GDNF levels in the AH and LF of patients with POAG and significantly less GDNF content in LF and BS at earlier stages of POAG compared to later stages (GDNF concentration in AH also showed this tendency). Most importantly,

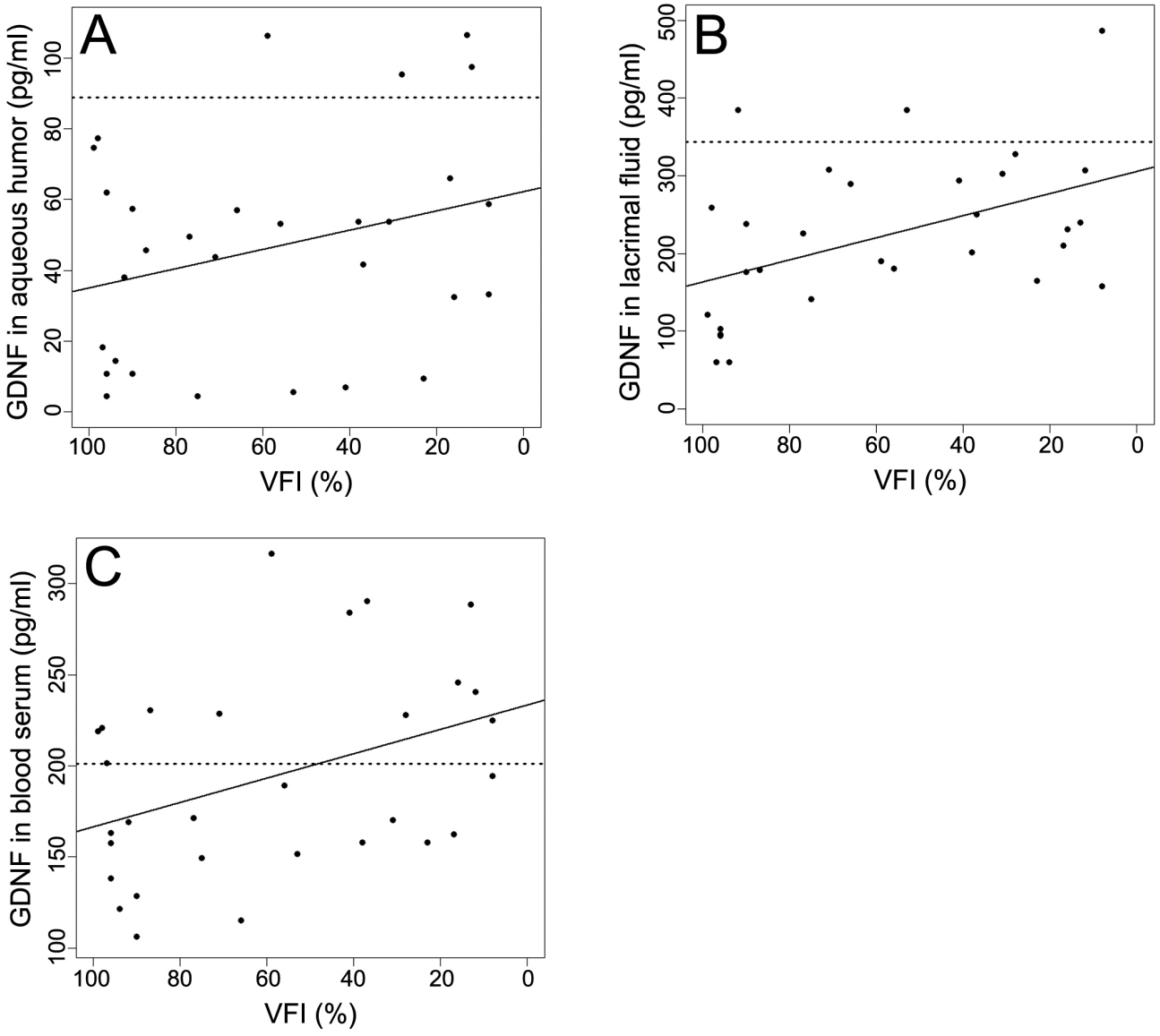

Figure 1. Plots of GDNF content in aqueous humor (**A**), lacrimal fluid (**B**), and blood serum (**C**) versus the Humphrey visual field index (VFI) in the main group. The solid line represents the linear regression fit. Note the reversed x-axis. The dotted line is the average concentration of GDNF in the comparison group. Pearson's correlation coefficients are as follows: −0.289 (p = 0.122) in (**A**), −0.465 (p = 0.01) in (**B**), and −0.399 (p = 0.029) in (**C**).

compared to the Cataract group, GDNF concentration at earlier stages of POAG was significantly lower in all biologic fluids studied.

Previous studies have identified two types of behavior of neurotrophic factors involved in POAG pathogenesis. The first type is a gradual decrease in the concentration of the factor in AH and LF as glaucoma progresses; this has been shown by ciliary neurotrophic factor (CNTF) [38]. Another type is a decrease in the content of a factor in all biologic fluids (AH, LF, BS) in the earlier stages of POAG, with a limited increase in the later stages of the disease, not reaching the level of the comparison group. This type of behavior has been demonstrated previously by brain-derived neurotrophic factor (BDNF) [39] and, in the present study, by GDNF. In a study limited to the BS of patients with POAG, this type of change was shown for BDNF and nerve growth factor (NGF) [40].

These data suggest the hypothesis that a decrease in the concentration of GDNF and other neurotrophic factors in BS, AH, and LF plays an important role in the pathogenesis of earlier stages of POAG and, possibly, in the initiation of the disease. POAG initiation is usually explained by local structural, hydro-, and hemodynamic changes. However, such changes (e.g., ocular hypertension) do not necessarily lead to POAG. Clearly, something else is necessary for POAG initiation. Present and previous studies show that decreased levels of neurotrophic factors (BDNF, GDNF, NGF) may be this supplement, which, when combined with local changes, could lead to glaucoma. Of course, this does not exclude another possibility: the decrease in some neurotrophic factors could be a consequence, rather than a cause, of early POAG. Further studies are needed to investigate these possibilities.

Several limitations may present in this study, including:: 1. We did not compare GDNF contents in the BS and LF of patients with and without cataract. However, in our previous studies, we performed such a comparison for BDNF and CNTF, showing that cataract does not influence the concentration of these neurotrophic factors due to its relatively benign course, which is not accompanied by significant immune or inflammatory changes [38,39]. Based on these results, we accepted the data from patients with cataracts as normative. 2. We collected stimulated LF, which could influence GDNF levels; however, this was the only way to obtain the minimum amount of LF needed for examination, particularly in elderly patients. 3. The LF and AH/BS were not sampled on the same day, so the correlations of GDNF levels in these media could be underestimated or not found. 4. Values below the minimum quantifiable value (non-detects) were substituted with half the minimum quantifiable value. A method of substitution is rightly criticized (Guidelines for computing summary statistics for data-sets containing non-detects); however, other guidelines consider it acceptable if the rate of non-detects is 15% or lower (Guidance for data quality assessment: practical methods for data analysis: EPA QA/G9: QA00). In this study, substitution was used in three cases (6.4%) only, which practically did not affect the results. Thus, with the recommended method of Regression on Order Statistics (Guidelines for computing summary statistics for data-sets containing non-detects) and with substitution, in AH in the Cataract group we obtained the same median (IQR) GDNF concentrations and only slightly different mean±SD: 90.6±44.0 versus 88.9±46.9. At the same time, a significant advantage of substitution is the possibility of using standard statistical calculations.

In conclusion, compared to patients with cataract only, GDNF levels are lower in the AH and LF of patients with POAG and cataract, especially at earlier stages of the disease (at these stages, the GDNF level in BS is also lower). Moreover, at earlier stages of POAG, compared to later stages, GDNF content is lower in LF and BS. These data could serve as a reason for the therapeutic use of GDNF in patients with POAG.

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REFERENCES

1. Kimura A, Namekata K, Guo X, Harada C, Harada T. Neuroprotection, growth factors and BDNF-TrkB signaling in retinal degeneration. Int J Mol Sci 2016; 17:1584-.
2. Cortés D, Carballo-Molina OA, Castellanos-Montiel MJ, Velasco I. The non-survival effects of glial cell line-derived neurotrophic factor on neural cells. Front Mol Neurosci 2017; 10:258-[PMID: 28878618].
3. You L, Kruse FE, Völcker HE. Neurotrophic factors in the human cornea. Invest Ophthalmol Vis Sci 2000; 41:692-702. [PMID: 10711683].
4. Iannetti L, Accorinti M, Malagola R, Bozzoni-Pantaleoni F, Da Dalt S, Nicoletti F, Gradini R, Traficante A, Campanella

M, Pivetti-Pezzi P. Role of the intravitreal growth factors in the pathogenesis of idiopathic epiretinal membrane. Invest Ophthalmol Vis Sci 2011; 52:5786-9. [PMID: 21693611].

5. Boss JD, Singh PK, Pandya HK, Tosi J, Kim C, Tewari A, Juzych MS, Abrams GW, Kumar A. assessment of neurotrophins and inflammatory mediators in vitreous of patients with diabetic retinopathy. Invest Ophthalmol Vis Sci 2017; 58:5594-603. [PMID: 29084332].

6. Sack RA, Conradi L, Krumholz D, Beaton A, Sathe S, Morris C. Membrane array characterization of 80 chemokines, cytokines, and growth factors in open- and closed-eye tears: angiogenin and other defense system constituents. Invest Ophthalmol Vis Sci 2005; 46:1228-38. [PMID: 15790883].

7. Chowdhury UR, Madden BJ, Charlesworth MC, Fautsch MP. Proteome analysis of human aqueous humor. Invest Ophthalmol Vis Sci 2010; 51:4921-31. [PMID: 20463327].

8. Wordinger RJ, Lambert W, Agarwal R, Liu X, Clark AF. Cells of the human optic nerve head express glial cell line-derived neurotrophic factor (GDNF) and the GDNF receptor complex. Mol Vis 2003; 9:249-56. [PMID: 12813408].

9. Akurathi A, Boese EA, Kardon RH, Ledolter J, Kuehn MH, Harper MM. Decreased expression of glial-derived neurotrophic factor receptors in glaucomatous human retinas. Curr Eye Res 2021; Epub ahead of print[PMID: 34738835].

10. Ward MS, Khoobehi A, Lavik EB, Langer R, Young MJ. Neuroprotection of retinal ganglion cells in DBA/2J mice with GDNF-loaded biodegradable microspheres. J Pharm Sci 2007; 96:558-68. [PMID: 17177208].

11. Checa-Casalengua P, Jiang C, Bravo-Osuna I, Tucker BA, Molina-Martínez IT, Young MJ, Herrero-Vanrell R. Retinal ganglion cells survival in a glaucoma model by GDNF/Vit E PLGA microspheres prepared according to a novel microencapsulation procedure J Control Release 2011; 156:92-100. [PMID: 21704662].

12. Flachsbarth K, Jankowiak W, Kruszewski K, Helbing S, Bartsch S, Bartsch U. Pronounced synergistic neuroprotective effect of GDNF and CNTF on axotomized retinal ganglion cells in the adult mouse Exp Eye Res 2018; 176:258-65. [PMID: 30237104].

13. Fudalej E, Justyniarska M, Kasarełło K, Dziedziak J, Szaflik JP. Cudnoch- Jędrzejewska A. Neuroprotective Factors of the Retina and Their Role in Promoting Survival of Retinal Ganglion Cells: A Review. Ophthalmic Res 2021; 64:345-55. [PMID: 33454713].

14. Jiang C, Moore MJ, Zhang X, Klassen H, Langer R, Young M. Intravitreal injections of GDNF-loaded biodegradable microspheres are neuroprotective in a rat model of glaucoma. Mol Vis 2007; 13:1783-92. [PMID: 17960131].

15. Xiao JH, Zhang MN. Neuroprotection of retinal ganglion cells with GDNF-Loaded biodegradable microspheres in experimental glaucoma. Int J Ophthalmol 2010; 3:189-91. [PMID: 22553550].

16. Johnson TV, Bull ND, Martin KR. Neurotrophic factor delivery as a protective treatment for glaucoma. Exp Eye Res 2011; 93:196-203. [PMID: 20685205].

17. García-Caballero C, Lieppman B, Arranz-Romera A, Molina-Martínez IT. Bravo- Osuna I, Young M, Baranov P, Herrero-Vanrell R. Photoreceptor preservation induced by intravitreal controlled delivery of GDNF and GDNF/melatonin in rhodopsin knockout mice. Mol Vis 2018; 24:733-45. [PMID: 30581280].

18. Arranz-Romera A, Hernandez M, Checa-Casalengua P, Garcia-Layana A. Molina- Martinez IT, Recalde S, Young MJ, Tucker BA, Herrero-Vanrell R, Fernandez- Robredo P, Bravo-Osuna I. A safe GDNF and GDNF/BDNF controlled delivery system improves migration in human retinal pigment epithelial cells and survival in retinal ganglion cells: potential usefulness in degenerative retinal pathologies. Pharmaceuticals (Basel) 2021; 14:50-[PMID: 33440745].

19. Baranov P, Lin H, McCabe K, Gale D, Cai S, Lieppman B, Morrow D, Lei P, Liao J, Young M. A Novel Neuroprotective Small Molecule for Glial Cell Derived Neurotrophic Factor Induction and Photoreceptor Rescue. J Ocul Pharmacol Ther 2017; 33:412-22. [PMID: 28441076].

20. Straten G, Eschweiler GW, Maetzler W, Laske C, Leyhe T. Glial cell-line derived neurotrophic factor (GDNF) concentrations in cerebrospinal fluid and serum of patients with early Alzheimer's disease and normal controls. J Alzheimers Dis 2009; 18:331-7. [PMID: 19584438].

21. Liu Y, Tong S, Ding L, Liu N, Gao D. Serum levels of glial cell line-derived neurotrophic factor and multiple neurotransmitters: In relation to cognitive performance in Parkinson's disease with mild cognitive impairment. Int J Geriatr Psychiatry 2020; 35:153-62. [PMID: 31650626].

22. Sharif M, Noroozian M, Hashemian F. Do serum GDNF levels correlate with severity of Alzheimer's disease? Neurol Sci 2021; 42:2865-72. [PMID: 33215334].

23. Pallavi P, Sagar R, Mehta M. Sharma S, Subramanium A, Shamshi F, Sengupta U, Qadri R, Pandey RM, Mukhopadhyay AKSerum neurotrophic factors in adolescent depression: gender difference and correlation with clinical severity. J Affect Disord 2013; 150:415-23. [PMID: 23769609].

24. de Azevedo Cardoso T, Mondin TC, Wiener CD, Marques MB. Fucolo B de Á, Pinheiro RT, de Souza LD, da Silva RA, Jansen K, Oses JP. Neurotrophic factors, clinical features and gender differences in depression. Neurochem Res 2014; 39:1571-8. [PMID: 24899094].

25. Tunca Z, Ozerdem A, Ceylan D, Yalçın Y, Can G, Resmi H, Akan P, Ergör G, Aydemir O, Cengisiz C, Kerim D. Alterations in BDNF (brain derived neurotrophic factor) and GDNF (glial cell line-derived neurotrophic factor) serum levels in bipolar disorder: The role of lithium. J Affect Disord 2014; 166:193-200. [PMID: 25012431].

26. Skibinska M, Kapelski P, Pawlak J, Rajewska-Rager A, Dmitrzak-Weglarz M, Szczepankiewicz A, Czerski P, Twarowska-Hauser J. Glial Cell Line-Derived Neurotrophic Factor (GDNF) serum level in women with schizophrenia and

depression, correlation with clinical and metabolic parameters. Psychiatry Res 2017; 256:396-402. [PMID: 28689143].

27. Tang X, Zhou C, Gao J, Duan W, Yu M, Xiao W, Zhang X, Dong H, Wang X, Zhang X. Serum BDNF and GDNF in Chinese male patients with deficit schizophrenia and their relationships with neurocognitive dysfunction. BMC Psychiatry 2019; 19:254-[PMID: 31420036].
28. Yang Y, Xie B, Ju C, Jin H, Ye X, Yao L, Jia M, Sun Z, Yuan Y. The association of decreased serum GDNF level with hyperglycemia and depression in type 2 diabetes mellitus. Endocr Pract 2019; 25:951-65. [PMID: 31170370].
29. Mills RP, Budenz DL, Lee PP, Noecker RJ, Walt JG, Siegartel LR, Evans SJ, Doyle JJ. Categorizing the stage of glaucoma from pre-diagnosis to end-stage disease. Am J Ophthalmol 2006; 141:24-30. [PMID: 16386972].
30. Okragly AJ, Haak-Frendscho M. An acid-treatment method for the enhanced detection of GDNF in biological samples. Exp Neurol 1997; 145:592-6. [PMID: 9217096].
31. Riffenburgh RH. Statistics in Medicine. 2nd ed. Amsterdam, Netherlands: Elsevier Academic Press; 2005. p.121.
32. Zar JH. Biostatistical analysis. 5th ed. Upper Saddle River (NJ): Pearson Prentice-Hall; 2010. p.146.
33. Hu ZL, Li N, Wei X, Tang L, Wang TH, Chen XM. Neuroprotective effects of BDNF and GDNF in intravitreally transplanted mesenchymal stem cells after optic nerve crush in mice. Int J Ophthalmol 2017; 10:35-42. [PMID: 28149774].
34. Kyhn MV, Klassen H, Johansson UE, Warfvinge K, Lavik E, Kiilgaard JF, Prause JU, Scherfig E, Young M, la Cour M. Delayed administration of glial cell line-derived neurotrophic factor (GDNF) protects retinal ganglion cells in a pig model of acute retinal ischemia Exp Eye Res 2009; 89:1012-20. [PMID: 19735654].
35. Ohnaka M, Miki K, Gong YY, Stevens R, Iwase T, Hackett SF, Campochiaro PA. Long-term expression of glial cell line-derived neurotrophic factor slows, but does not stop retinal degeneration in a model of retinitis pigmentosa. J Neurochem 2012; 122:1047-53. [PMID: 22726126].
36. Wong FS, Wong CC, Chan BP, Lo AC. Sustained Delivery of Bioactive GDNF from Collagen and Alginate-Based Cell-Encapsulating Gel Promoted Photoreceptor Survival in an Inherited Retinal Degeneration Model. PLoS One 2016; 11:e0159342-[PMID: 27441692].
37. Klaassen I, de Vries EW, Vogels IMC, van Kampen AHC, Bosscha MI, Steel DHW, Van Noorden CJF, Lesnik-Oberstein SY, Schlingemann RO. Identification of proteins associated with clinical and pathological features of proliferative diabetic retinopathy in vitreous and fibrovascular membranes. PLoS One 2017; 12:e0187304-[PMID: 29095861].
38. Shpak AA, Guekht AB, Druzhkova TA, Kozlova KI, Gulyaeva NV. Ciliary neurotrophic factor in patients with primary open-angle glaucoma and age-related cataract. Mol Vis 2017; 23:799-809. [PMID: 29225456].
39. Shpak AA, Guekht AB, Druzhkova TA, Kozlova KI, Gulyaeva NV. Brain-derived neurotrophic factor in patients with primary open-angle glaucoma and age-related cataract. Curr Eye Res 2018; 43:224-31. [PMID: 29120253].
40. Oddone F, Roberti G, Micera A, Busanello A, Bonini S, Quaranta L, Agnifili L, Manni G. Exploring serum levels of brain derived neurotrophic factor and nerve growth factor across glaucoma stages. PLoS One 2017; 12:e0168565-[PMID: 28068360].

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