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# Role of Aqueous Humor Light Chain Proteins in Glaucomatous Neurodegeneration

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## Abstract

**Objectives:** This paper aims to evaluate the predictive clinical role of Aqueous Humor Neurofilament light chain (NfL) Proteins in human aqueous humor (AH). **Methods:** In this single-center study, 37 patients were enrolled, including 21 glaucoma patients and 16 non-glaucoma patients. AH samples were collected during planned ophthalmic surgery. AH NfL was quantified using the Single Molecule Array (Simoa)® NF-light assay (Quanterix). Clinical information was obtained by reviewing the medical records. Between-group comparisons were performed using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. **Results:** In a model controlling for age and body mass index (BMI), NfL was significantly elevated in AH from glaucoma patients (519 ± 671 pg/mL) compared to AH from patients without glaucoma (5.6 ± 2.3 pg/ml, $P < 0.001$). **Conclusions:** AH NfL is significantly elevated in glaucoma patients. Our findings suggest that AH NfL may have clinical utility as a marker of glaucomatous neurodegeneration. Larger, multicenter studies are warranted to confirm these findings.

## Keywords

Neurofilament Light Chain, Glaucoma, Aqueous Humor, Neurodegeneration

## 1. Introduction

As a neurodegenerative disease marked by the progressive degeneration of retinal ganglion cells (RGCs), glaucoma constitutes one of the predominant causes of irreversible blindness globally. At present, both medical and surgical therapeutic strategies remain largely predicated on the reduction of intraocular pressure (IOP)—the sole established modifiable risk factor for this condition [1] [2]. Clinicians commonly rely on visual field testing and optical coherence tomography

(OCT) to monitor the health status of retinal ganglion cells (RGCs) and to guide treatment decisions. However, the results of these tests are subject to multiple influencing factors and can be challenging to interpret [3] [4]. Moreover, OCT is of limited utility in very early-stage disease, as it only detects abnormalities after substantial RGC damage has already occurred. Therefore, there is an urgent need for novel biomarkers of glaucomatous neurodegeneration that can monitor early RGC dysfunction.

With advances in medical technology, imaging modalities have emerged as a feasible strategy for monitoring RGC health in glaucoma patients. One such imaging technique is the detection of apoptosing retinal cells (DARC), which employs fluorescently labeled annexin V to identify cells committed to apoptosis-that is, programmed cell death. This monitoring approach has been proven safe and effective in previous studies [5] [6].

Another approach is to identify molecular markers within ocular biofluids that reflect underlying RGC stress or dysfunction in glaucoma. Elevated levels of neurofilament light chain (NfL)—a structural protein of the neuronal cytoskeleton—have been consistently detected in the cerebrospinal fluid and serum of patients afflicted with diverse neurodegenerative disorders, such as Alzheimer's disease, traumatic brain injury, multiple sclerosis, and Parkinson's disease [7]-[9]. Pilot studies have demonstrated elevated AH NfL concentrations in glaucoma patients compared with controls, suggesting NfL as a candidate biomarker for glaucomatous neurodegeneration [10] [11].

It has been demonstrated that the rate of change in serum NfL is associated with disease progression in Alzheimer's disease, particularly in patients at early, presymptomatic stages [12].

In this study, we collected AH samples from glaucomatous and non-glaucomatous patients who underwent ophthalmic surgery as part of routine clinical management, in order to assess the significance of AH NfL in glaucomatous neurodegenerative processes.

## 2. Methods

This single-center study was approved by the Ethics Committee of the First Affiliated Hospital of Yangtze University and was conducted in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent. Glaucoma was diagnosed by a glaucoma specialist according to the presence of characteristic glaucomatous optic neuropathy (increased vertical cup-to-disc ratio, neuroretinal rim thinning, or retinal nerve fiber layer defects) and corresponding visual field defects, irrespective of intraocular pressure. Disease severity was classified as mild, moderate, or severe using the Hodapp-Parrish-Anderson visual field criteria and optic nerve/retinal nerve fiber layer findings.

The case group comprised 21 patients with any type of glaucoma who were managed by a glaucoma specialist and deemed candidates for glaucoma surgery. The control group consisted of 16 patients with age-related cataract who had no

diagnosis of glaucoma, glaucoma suspect, or ocular hypertension, and were scheduled for phacoemulsification with intraocular lens implantation. Exclusion criteria included the presence of active ocular inflammation or optic nerve degeneration unrelated to glaucoma.

AH samples were collected at the initiation of planned ophthalmic surgery. Briefly, a blunt cannula attached to a syringe was introduced into the anterior chamber through the initial peripheral paracentesis site, and 25 - 150 μL of AH was aspirated. The scheduled procedure was then continued. Samples were immediately placed on dry ice and stored at -80 °C until analysis.

NfL levels were measured using the Simoa® NF-light Advantage kit on an automated Simoa® HD-X Analyzer (Quanterix, Billerica, MA) according to the manufacturer's instructions. Demographic and clinical data, including age, sex, type of surgery, type of glaucoma, preoperative IOP, and maximum recorded IOP, were collected.

Statistical analyses were performed using SPSS 21.0. Continuous variables were compared using the Mann-Whitney U test, and categorical variables were compared using Fisher's exact test. A $P$ value < 0.05 was considered statistically significant.

## 3. Results

A total of 37 patients were included (21 glaucoma, 16 controls). Demographic and clinical characteristics are summarized in Table 1. No significant differences were observed between groups in age, sex, BMI, self-reported race, or laterality. Of the patients with glaucoma, 9 (42.9%) were classiffed as having a mild stage, 5 (23.8%) as having a moderate stage, and 7 (33.3%) as having an advanced stage based on ICD-10 criteria. The NfL levels were significantly elevated in AH from patients with glaucoma (519 ± 671 pg/mL) compared with AH from controls (5.6 ± 2.3 pg/ml; $P$ < 0.001).

Table 1. Clinical data of patients.

| Characteristic | Case group (n = 21) | Control group (n = 16) | $P$ value |
|---|---|---|---|
| Age | 70.1 ± 10.3 | 68.5 ± 7.5 | 0.6 |
| Sex | | | |
| Male | 8 (38%) | 5 (31%) | 0.7 |
| Female | 13 (62%) | 11 (69%) | |
| Body Mass Index (BMI) | 26.9 ± 3.2 | 27.4 ± 4.3 | 0.8 |
| Eye (OD/OS) | 12/9 | 8/8 | 0.9 |
| Surgery Type | | | |
| Phaco | 0 (0%) | 16 (100%) | |
| Phaco/MIGS | 9 (42.9) | 0 (0%) | |

**Continued**

| | | | |
|---|---|---|---|
| Incisional | 4 (19.0) | 0 (0%) | |
| Phaco/incisional | 6 (28.6) | 0 (0%) | |
| Other | 2 (9.5) | 0 (0%) | |
| Hypertension (n, %) | 7 (33.3%) | 5 (31.2%) | 1.0 |
| Diabetes (n, %) | 6 (28.6%) | 4 (25.0%) | 1.0 |
| Glaucoma Stage | | | |
| None | 0 | 16 (100%) | |
| Mild | 9 (42.9%) | 0 | |
| Moderate | 5 (23.8%) | 0 | |
| Severe | 7 (33.3%) | 0 | |
| NfL levels (pg/mL) | 519 ± 671 | 5.6 ± 2.3 | <0.001 |

Note: MIGS, minimally invasive glaucoma surgery; NfL, neurofilament light chain.

## 4. Conclusions

By employing the ultrasensitive Simoa immunoassay, we were able to accurately quantify low NfL levels in AH, even at concentrations as low as a few pg/mL. In this study, we found that AH NfL levels were significantly elevated in patients with glaucoma compared with non-glaucomatous controls undergoing cataract surgery. This difference remained statistically significant even after adjustment for age and BMI, both of which have been reported to influence NfL levels in other biofluids [13]. NfL, a neuronally expressed cytoskeletal protein, accumulates in biofluids after neuronal damage. Our results indicate that, akin to neurodegenerative conditions of the brain and spinal cord, glaucomatous injury similarly triggers NfL release into the aqueous humor, providing a quantifiable indicator of retinal ganglion cell (RGC) status. These findings corroborate a previous pilot study that reported higher blood NfL levels in early and advanced glaucoma patients than in controls group [14].

Notably, the elevation in AH NfL in glaucoma patients remained statistically robust after controlling for age and BMI, underscoring that the association is not merely driven by these systemic confounders, which are known to affect NfL in other biofluids. The main difference between our study and previous investigations is that we did not collect serum samples for NfL measurement. Earlier studies have shown that serum NfL levels did not differ significantly between glaucoma patients and healthy controls, suggesting that larger-scale studies are warranted to evaluate serum NfL in this population.

Our findings may have clinical implications. For example, patients with elevated AH NfL levels may have ongoing RGC neurodegeneration despite apparently stable visual field and OCT findings, potentially requiring closer monitoring and/or intensified therapy. Moreover, high NfL levels may indicate rapid progres-

sion or more severe RGC injury, supporting earlier intervention in this subgroup.

This study has several limitations. First, the modest sample size limited our ability to control for all potential covariates affecting AH NfL, and the single-center design restricts generalizability. Second, although we adjusted for age and BMI, other demographic or clinical factors may confound the observed associations. Third, our reports no longitudinal outcomes or association with visual field or OCT measures. Fourth, surgical procedures differed between groups (all controls underwent phacoemulsification, whereas glaucoma patients underwent various surgeries), which may influence AH NfL levels. Future larger, multicenter studies are needed to determine whether serum NfL is also significantly elevated in glaucoma patients and to assess the correlation between serum and AH NfL.

In summary, AH NfL is significantly elevated in patients with glaucoma and may serve as a promising biomarker of glaucomatous neurodegeneration. These findings support the need for further large-scale, multicenter studies to validate AH NfL as a clinical tool for monitoring RGC health and guiding treatment decisions.

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## Author Contributions

Conceptualization, T.T.; methodology, Q.W.; formal analysis, T.T.; investigation, G.J.Z.; resources, Q.W.; writing—original draft preparation, T.T.; writing-review and editing, T.T., Y.N.Z.; funding acquisition, T.T. All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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