Ghrelin Levels in Patients With Primary Open-Angle Glaucoma

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**Purpose:** This study was aimed to assess the levels of ghrelin in aqueous humor and plasma of human eyes with primary open-angle glaucoma (POAG) and to correlate their concentrations with the severity of glaucoma.

**Design:** This was a case-control, prospective study.

**Methods:** Fifty patients with POAG and 35 patients with senile nonpathologic cataract (control group) were enrolled in the study prospectively. Aqueous humor samples were obtained by paracentesis from patients with glaucoma and cataract who underwent elective surgery. Aqueous humor and corresponding plasma samples were analyzed for ghrelin concentrations by radioimmunoassay diagnostic kits assay.

**Results:** Ghrelin levels were significantly lower in aqueous humor of patients with POAG with respect to the comparative group of patients with cataract (P < 0.001). There was no significant difference in the levels of ghrelin in the plasma of patients with POAG and that of patients with cataract. A positive correlation was found between plasma/aqueous humor ghrelin concentration in patients with POAG versus the control group (P < 0.001). No significant correlation was found between the levels of ghrelin and the severity of visual field loss.

**Conclusions:** Lower levels of aqueous humor ghrelin may be associated with POAG and may be a consequence of glaucomatous damage.

**Key Words:** ghrelin, aqueous humor, POAG, cataract


**Laboratory Science**

Glaucoma, which is characterized by retinal ganglion cell apoptosis, is a major cause of blindness. Retinal ganglion cell apoptosis may be the result of increased intraocular pressure (IOP), neurotoxicity and apoptosis, extracellular matrix changes, oxidative stress, and hypoxia due to ocular and systemic vascular dysregulation.

Ghrelin is the endogenous ligand for the human G protein-coupled growth hormone secretagogue receptor type 1a (GHS-R1a). It is a 28-amino-acid peptide hormone described in the rat’s stomach oxyntic mucosa in 1999 by Kojima and Hosoda. The acylated form of ghrelin is a relatively unstable molecule and exerts mostly neuroendocrine effects after binding to the GHS-R1a. The des-acylated form (des-acyl-ghrelin) of the peptide, which constitutes more than 90% of the total circulating ghrelin, does not bind to the GHS-R1a, and exhibits important peripheral metabolic and cardiovascular effects.

Ghrelin is a peptide that exerts both endocrine and paracrine effects because it is involved in the regulation of metabolic balance and energy homeostasis, as well as cardiovascular function. Ghrelin expression in vascular endothelial cells and its effects on vascular smooth muscle cells have been recently described, including an endothelium-independent vasodilatory effect of similar potency and efficacy between both the acylated and des-acylated forms of ghrelin.

In the present study, we measured the aqueous humor and plasma levels of ghrelin in patients with primary open-angle glaucoma (POAG) and senile nonpathologic cataract. In addition, we assessed their relation to the severity of POAG.

**MATERIALS AND METHODS**

**Study Design**

This was a case-control, prospective study. After explaining to the patients the details of the study, we obtained written informed consent from all patients before enrollment. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

**Patients**

Fifty patients with POAG aged from 54 to 73 years were scheduled for glaucoma filtering surgery. The cataract group consisted of 35 patients aged from 53 to 74 years who were scheduled for phacoemulsification.

Full ophthalmic examination for POAG patients and the subjects in the control group was performed. This included assessment of visual acuity, slit-lamp anterior and posterior segment biomicroscopy, and IOP measurement by Goldmann applanation tonometry. In addition, patients with glaucoma were subjected to gonioscopy using Goldmann 3-mirror contact lens, 24-2 program Humphrey visual field analyzer (Humphrey Instruments, San Leandro, Calif), and estimation of cup/disc ratio. A detailed medical history including age, sex, glaucoma medications, systemic hypertension, systemic medications, and previous ocular surgery or laser treatment was recorded.

Inclusion criteria were as follows: (1) patients with POAG with medically uncontrolled IOP, correlated visual field loss, and glaucomatous optic nerve head changes (scheduled for trabeculectomy); (2) patients with senile cataract who had normal IOP and did not receive any topical medications (scheduled for phacoemulsification); and (3) systolic blood pressure of less than or equal to 140 mm Hg and diastolic blood pressure of less than or equal to 90 mm Hg.

The IOP measurements were measured at least 5 times on different times of the day from 8 A.M. to 5 P.M. The highest and the lowest IOP values were used to determine the diurnal IOP range. The visual field categories were as follows: (1) normal; (2) mild, an arcuate defect; (3) moderate, abnormal in 1 hemifield.
TABLE 1. Demographic and Clinical Features in the Study and Control Groups

<table>
<thead>
<tr>
<th>Group/Parameters</th>
<th>POAG Group</th>
<th>Control Group</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>50</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61.53 ± 4.62</td>
<td>63.24 ± 3.41</td>
<td>F = 5.54, P = 0.533</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23 (46)</td>
<td>17 (48.5)</td>
<td>x² = 0.035</td>
</tr>
<tr>
<td>Female</td>
<td>27 (54)</td>
<td>18 (51.5)</td>
<td>P = 0.421</td>
</tr>
<tr>
<td>Cup/disc ratio</td>
<td>0.73 ± 0.12</td>
<td>0.39 ± 0.14</td>
<td>F = 5.33, P = 0.012*</td>
</tr>
<tr>
<td>IOP at time of examination</td>
<td>22.4 ± 3.7</td>
<td>18.5 ± 4.6</td>
<td>F = 5.45, P = 0.0684</td>
</tr>
<tr>
<td>Diurnal IOP range</td>
<td>11.15 ± 1.6</td>
<td>5.37 ± 2.4</td>
<td>F = 6.54, P = 0.016*</td>
</tr>
<tr>
<td>Mean deviation</td>
<td>−7.42 ± 2.13</td>
<td>−2.32 ± 0.41</td>
<td>F = 4.63, P = 0.014*</td>
</tr>
</tbody>
</table>

*Significant at P < 0.05.
F indicates 1-way ANOVA test.

and not within 5 degrees of fixation; and (4) severe, abnormal in both hemifields or within 5 degrees of fixation. Classification of visual field loss was done based on the most recent reliable results of Humphrey visual field tests before the elective ocular surgery.

Exclusion criteria were as follows: (1) patients with any type of glaucoma other than POAG such as ocular hypertensive, angle-closure glaucoma, secondary glaucoma, pigment dispersion, or exfoliation glaucoma; (2) patients with previous intraocular surgery and laser surgery; (3) diseases that could influence the ghrelin levels such as diabetes mellitus, ocular trauma, inflammation that affected the blood-aqueous barrier; and (4) patients with a family history of glaucoma.

Patients with POAG were on topical antiglaucomatous medications: 36% (18/50 patients) were on b-blocker, 38% (19/50 patients) on prostaglandin analog, 32% (16/50 patients) on carbonic anhydrase inhibitor, and 24% (12/50 patients) on a-agonist.

Sample Collection
Aqueous Humor Sampling
Aqueous humor samples were obtained from each patient requiring either elective glaucoma surgery or cataract surgery. Aqueous humor concentration of 100 to 200 μL was collected at the beginning of the surgery through a clear corneal paracentesis of the anterior chamber with a 27-gauge needle attached to a tuberculin microsyringe before any tissue manipulation. Blood contamination was meticulously avoided. Aqueous humor samples were immediately centrifuged and stored at −20°C before the subsequent biochemical tests. All samples were protected from light.

Blood Sampling
Five-milliliter venous blood samples were drawn from an antecubital vein without any anticoagulant from participants who had fasted for at least 12 hours just before the operation. Each blood sample was stored in chilled tubes containing ethylenediaminetetraacetic acid (1 mg/mL of blood) and citrate (3.2% sodium citrate). The samples were centrifuged at 3000 rpm for 5 minutes at 4°C, and the separated plasma was rapidly frozen for storage until the time of assay.

Assay
The total ghrelin (active + des-acyl) concentrations in aqueous humor and plasma were measured using radioimmunoassay diagnostic kits, including the active ghrelin ELISA kit and the des-acyl-ghrelin ELISA kit (Sceti, Tokyo, Japan). The kits have an analytical sensitivity of 50 pg/mL and calibrating range of 0 to 4500 pg/mL with intraassay and interassay variations less than 5% and 7.5%, respectively. Ghrelin radioimmunoassay kits are calibrated against the internal test of Hannover Medical School. All sample assays were performed, duplicated, and included in the same run. The reference range for assay was 150 to 1250 pg/mL, and the sensitivity was 25 to 50 pg/mL.

Statistical Analysis
All data were analyzed with SPSS version 16 (SPSS Inc, Chicago, Ill). Data were presented as mean ± SD. Results were compared by using parametric unpaired t test and x² to detect any difference in either demographic or clinical characteristics. Analysis of variance (1-way ANOVA) was performed to evaluate the differences between the groups and also to adjust the variables. Bonferroni correction was used as multiple correlations were investigated. Pearson correlation coefficient was used to examine the association between variables. A P value of less than 0.05 was considered to be statistically significant.

RESULTS
Eighty-five eyes of 85 patients were enrolled in the present study according to the above-mentioned inclusion/exclusion criteria. There were 50 POAG eyes (POAG group) and 35 senile nonpathologic cataract eyes (control group).

Table 1 summarizes the demographic and the clinical features of the studied groups. There was no statistically significant difference between the POAG group and the control group in regard to age, sex, and IOP (P = 0.533, P = 0.421, and P = 0.684, respectively). As expected, cup/disc ratio, diurnal IOP range, and mean deviation were statistically significant (P = 0.012, P = 0.016, and P = 0.014, respectively).

Table 2 shows the ophthalmic characteristics of patients with glaucoma, such as visual acuity and cup/disc ratio. The table also includes the number of glaucoma eye drops used by patients, and the severity of visual field loss.

Aqueous humor concentration of ghrelin was significantly lower in the POAG group when compared to that of the

TABLE 2. Ophthalmologic Characteristics of the Patients With POAG

<table>
<thead>
<tr>
<th>Visual Field Loss</th>
<th>No. Eye Drops</th>
<th>Cup/Disc Ratio</th>
<th>VA (log MAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>1</td>
</tr>
<tr>
<td>POAG group (N = 50)</td>
<td>30%</td>
<td>48%</td>
<td>22%</td>
</tr>
</tbody>
</table>

logMAR indicates logarithm of the minimum angle of resolution; VA, visual acuity.

Note: This text is a summary of the methodology and results of a research paper focusing on ghrelin levels in patients with POAG. The data presented here are part of a larger study that examines the association between ghrelin levels and glaucoma, specifically POAG.
control group (95.13 ± 12.59 pg/mL vs 164.65 ± 24.31 pg/mL; P = 0.003). Ghrelin concentration in plasma of the POAG group was lower but not statistically significant when compared to that of the control group (586.51 ± 134.45 pg/mL vs 621.36 ± 123.61 pg/mL; P = 0.456; Fig. 1 and Table 3). A positive correlation was found between the plasma/aqueous humor ghrelin concentration in the POAG group versus the control group (P < 0.001; Table 4).

Table 5 shows the correlation between ghrelin and the severity of visual field loss in the POAG group. No significant correlation was found between ghrelin levels and the severity of visual field loss.

Topical therapy (β-blocker, prostaglandin analog, carbonic anhydrase inhibitor, and α-agonist) was not associated with ghrelin levels in the aqueous humor (t-test; P > 0.4 in all types of topical medication). Furthermore, no association could be found between the ghrelin levels in the aqueous humor and the number of topical drugs used for the treatment of glaucoma (r = 0.455; F = 0.361; r = 0.542; F = 0.542, respectively, 1-way ANOVA).

**DISCUSSION**

The pathogenesis underlying the development and/or progression of POAG remains unknown; there are many data that state that POAG is a disease involving retinal ganglion cell apoptosis and abnormal extracellular matrix production.2

In the present study, we compared the aqueous humor and the plasma levels of ghrelin in patients with POAG undergoing cataract extraction versus nonglaucomatous persons undergoing cataract extraction (control). In addition, we correlated their concentrations with the severity of glaucoma.

Ghrelin has emerged as the first identified circulating hormone. It is produced mainly in the small and the large intestines but can also be secreted by the lungs, pancreatic islets, gonads, adrenal cortex, placenta, kidney, and brain. Ghrelin plays a significant role in neurotroph, particularly in the hippocampus, and is essential for cognitive adaptation to changing environments and the process of learning.15,16 Ghrelin has been shown to activate the endothelial isoform of nitric oxide synthase in a pathway that depends on various kinases. In addition, ghrelin is produced in small amounts locally in areas of the central nervous system.15

A statistically significant decrease in ghrelin in the aqueous humor in patients with POAG as compared to the control group is in agreement with a study conducted by Katsanos et al.14 Our study found that plasma ghrelin levels in patients with POAG were lower (586.51 ± 134.45 pg/mL) than in those in the control group (621.36 ± 123.61 pg/mL) although this was not statistically significant when compared to the controls (P = 0.456). These findings are in agreement with a study conducted by Rocha-Sousa et al.15 However, Katsanos et al.14 found that the plasma levels of ghrelin were higher (490.5 ± 156.0 pg/mL) in the POAG group than in the control group (482.2 ± 125.4 pg/mL) with no statistical significance (Mann-Whitney U test, P = 0.897).

Studies have shown that circulating ghrelin can pass the blood-brain barrier in a complex and highly regulated process. In the eye, the role of the blood-aqueous barrier and the extent to which circulating ghrelin can affect its aqueous levels are not known. However, there is evidence that ghrelin is indeed locally produced in the anterior segment because ghrelin transcripts have been identified in the iris posterior epithelium, the iris stroma, and the nonpigmented ciliary epithelium in rats.7

Concerning its functional significance, there is initial evidence for a role of ghrelin in iris muscle dynamics.7 Its effects seem to be mediated by the GHS-R1a for the dilator and a different receptor for the sphincter muscle in animal studies.

**TABLE 3. Ghrelin Levels in Patients**

| Group/Parameter, pg/mL | POAG Group (n = 50) | Control Group (n = 35) | *P*
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Aqueous humor ghrelin</td>
<td>95.13 ± 12.59</td>
<td>164.65 ± 24.31</td>
<td>0.003*</td>
</tr>
<tr>
<td>Plasma ghrelin</td>
<td>586.51 ± 134.45</td>
<td>621.36 ± 123.61</td>
<td>0.456</td>
</tr>
</tbody>
</table>

*Significant at *P* < 0.05.

**TABLE 4. Correlation Between Aqueous Humor and Plasma Ghrelin Levels Among the Studied Groups**

<table>
<thead>
<tr>
<th>Correlation Between Studied Variables</th>
<th>P</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin (aqueous)-ghrelin (plasma)</td>
<td>&lt;0.001</td>
<td>0.338</td>
</tr>
<tr>
<td>Control</td>
<td>0.434</td>
<td>0.423</td>
</tr>
</tbody>
</table>
Ghrelin promotes the decrease of the muscle tension, either actively developed after carbachol precontraction or the basal tone. This relaxing effect is not species dependent, being independent from GHS-R1a.7

Ghrelin’s messenger RNA that is observed in the iris posterior segment and in the nonpigmented segment has been reported in glaucoma, which may imply these peptides in the pathophysiology of the disease.17

The present study revealed differences in aqueous levels of total ghrelin in patients with POAG compared to patients in the control group. These findings may indicate a role of ghrelin on the tissues that are anatomically and functionally related to the aqueous production and/or circulation. All patients with glaucoma enrolled in this study were under topical treatment without glaucoma therapy drugs. Another potential explanation of our findings is that glaucoma medication lowers the concentration of ghrelin. A third potential explanation of our findings may be related to the elevated IOP that patients with glaucoma generally have.

The present study was carried on white Arab populations. Others studies14,15 conducted on other ethnic groups had the same results. This revealed that there is no potential genetic difference between our study and other studies.

The effect of ghrelin on iris sphincter is mediated by prostaglandins.7 Most of the patients with glaucoma in this study (36/50) were receiving prostaglandin analogs. As the pathways involved in the ghrelin cycle in the eye are barely understood, the possibility that prostaglandin analogs may have influenced ghrelin aqueous levels cannot be excluded. In the present study, however, there was no significant difference in aqueous or plasma levels of ghrelin between patients on prostaglandins and patients on other glaucoma medications.

The significantly lower levels of ghrelin in aqueous humor associated with nonsignificant serum changes could be attributed to enhanced intraocular synthesis. The increased ghrelin levels may reflect an increased protein turnover, which might play a role in the pathophysiology of POAG.

The insignificant correlation between either ghrelin levels with visual field loss in POAG patients at any stage may indicate absence of potential secondary consequences such as ischemia, hypoxia, or reactive oxygen species caused by glaucomatous damage.

In the current study, there was a significant correlation between the aqueous humor and plasma levels of ghrelin, which suggested that ghrelin levels in aqueous humor were related to the breakdown of the blood-retinal barrier. This correlation supported that ghrelin may be consequential to glaucomatous damage.

Lower aqueous levels of ghrelin was found in eyes with POAG compared to eyes in the control group. Also, the present study revealed that intraocular expression of ghrelin may be associated with optic nerve damage. Although a causal relationship cannot be established by this study, our data may indicate a link between ghrelin expression and the glaucomatous process, or an association between glaucoma medications and decreased aqueous levels of these peptides. Further research will be needed to elucidate the potential role of ghrelin in glaucoma and other ocular diseases.

### TABLE 5. Correlation Coefficient Values Between Severity of Glaucoma Stage and Ghrelin Levels in Patients With Glaucoma

<table>
<thead>
<tr>
<th>Parameters, pg/mL</th>
<th>Mild VF Loss</th>
<th>Moderate VF Loss</th>
<th>Severe VF Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous humor ghrelin</td>
<td>r</td>
<td>−0.512</td>
<td>−0.321</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.432</td>
<td>0.252</td>
</tr>
<tr>
<td>Plasma ghrelin</td>
<td>r</td>
<td>−0.426</td>
<td>−0.346</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.279</td>
<td>0.233</td>
</tr>
</tbody>
</table>

VF indicates visual field.

### REFERENCES