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CLINICAL INVESTIGATION

Aqueous humour levels of ghrelin in exfoliation syndrome and exfoliation glaucoma patients

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Abstract
Purpose To investigate levels of ghrelin in the aqueous humour (AqH) of patients with exfoliation syndrome and exfoliation glaucoma and compare them to levels of ghrelin in control subjects.
Methods This cross-sectional study involved 15 patients with exfoliation syndrome, 8 with exfoliation glaucoma and 12 control subjects for whom cataract surgery was indicated. The AqH was aspirated from the anterior chamber with a 27-G needle under sterile conditions prior to tissue manipulation. Ghrelin levels were quantified using radioimmunassay kits.
Results Levels of ghrelin in the AqH were 187.87 ± 80.1 pg/mL in the eyes exhibiting exfoliation syndrome, 98.53 ± 50.9 pg/mL in the eyes exhibiting exfoliation glaucoma and 111.40 ± 77.5 pg/mL in the controls. Ghrelin level of patients with exfoliation syndrome were significantly higher than those of patients with exfoliation glaucoma and the controls ($P < 0.05$). Ghrelin levels of patients with exfoliation glaucoma were lower than those of the controls but were not reach statistically significant ($P > 0.05$). Age, gender and IOP did not have a significant effect on ghrelin levels in patients with exfoliation syndrome and exfoliation glaucoma.
Conclusion This study is the first to report elevated levels of ghrelin in the AqH in eyes exhibiting exfoliation syndrome. Findings suggest ghrelin might play role in the etiopathogenesis of exfoliation syndrome to exfoliation glaucoma.

Keywords Ghrelin · Aquous humor · Exfoliation syndrome · Exfoliation glaucoma

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Introduction

Exfoliation syndrome, first described by Lindberg in 1917, is an age-related disorder characterized by abnormal synthesis and deposition of fibrillar extracellular material (ECM) in ocular and systemic tissue [1]. The pathogenetic mechanism is thought to be multifactored, including aging, genetics, oxidative stress and ischemia, changes in levels of growth factor activity, erythropoietin, connective tissue growth factor (CTGF) and asymmetric dimethyloarginin (ADMA) [2–6]. The amount of ECM in the juxtacanalicular tissue and the outflow filtration area are correlated with intraocular pressure (IOP) and optic nerve damage [7]. Patients with exfoliation syndrome have a 5.3 % chance of developing glaucoma within 5 years, increasing to 15.4 % within 10 years [8]. Exfoliation glaucoma is a relatively severe and progressive type of glaucoma, more resistant to medical therapy than primary open angle glaucoma (POAG) and results in more extensive complications following surgery [9].

Ghrelin is a 28 amino acid acylated peptide hormone that was first discovered in rat stomachs in 1999 by Kojima et al. [10, 11], the actions of which are mediated by the growth hormone secretagogues receptor type 1a (GHSR-1a). Ghrelin manifests a widespread tissue distribution including endocrine, cardiovascular, musculoskeletal and immune systems, and the eye [12]. The presence of ghrelin

m-RNA has been identified in the anterior segment of the rat eye, specifically, the posterior epithelium of the iris and non-pigmented ciliary epithelium in 2006 [13]. Ghrelin has been shown to have a relaxing effect on iris sphincters (through a GHSR-1a-independent mechanism) and dilator muscles (through a GHSR-1a-dependent mechanism) [13].

Two clinical research protocols assessed the potential relationship between ghrelin and glaucoma. Rocha Sousa et al. [14] and Katsanos et al. [15] found significantly lower AqH levels of ghrelin in glaucoma patients when compared to controls. However, it is not clear if lower levels of ghrelin are a cause or a consequence of the glaucoma process. We believe the answer to this question can be found by examing ghrelin levels in patients with exfoliation syndrome. Therefore, the purpose of this study was to investigate the differences in concentrations of ghrelin in the AqH of exfoliation syndrome patients, exfoliation glaucoma patients and control subjects.

Materials and methods

The Human Research Ethics Committee of the University of Cumhuriyet approved this study protocol and this study adheres to principles outlined in the Declaration of Helsinki. Written informed consent was obtained from each participant.

All participants had cataracts and were planning to undergo cataract surgery. One eye per patient was included in the study. The 35 participants formed 3 groups, 15 patients with exfoliation syndrome, 8 patients with exfoliation glaucoma and 12 controls.

Each patient underwent a detailed examination including slit-lamp biomicroscopy, Goldmann tonometry and dilated funduscopy. The diagnosis of exfoliation syndrome without glaucoma was based on the presence of typical pseudoexfoliation material on the anterior lens capsule in one or both eyes with a normal optic disc appearance, and IOP level of 21 mmHg or less (measured by Goldmann applanation tonometry, documented on at least 2 consecutive visits more than 1 week apart). Patients included in the exfoliation syndrome group were operated on for the first time, and did not receive any topical medication. The inclusion criterion for the exfoliation glaucoma group was the patient having a clinically significant senile cataract in the presence of exfoliation glaucoma (medically controlled IOP). The inclusion criterion for the control group was a clinically significant senile cataract. All glaucoma patients were using topical antiglaucoma medication.

Exclusion criteria were previous ocular surgery including laser trabeculoplasty and history or signs of conditions that may affect the blood–aqueous barrier such as ocular trauma, inflammation and diabetes mellitus.

All participants fasted overnight (for at least 12 h). The protocol for preoperative mydriasis was the same in all participants undergoing cataract surgery and included tropicamide 1 %, cyclopentolate 1 %, phenylephrine 5 % and diclofenac drops administered four times within an hour before surgery. AqH samples (0.1–0.2 mL) were collected before and at the beginning of phacoemulsification surgery through clear corneal paracentesis. The AqH was aspirated from the central pupillary area using a 27-gauge needle on a tuberculin syringe, with special care taken to avoid vascular contact or damage to the iris and other intraocular structures. Care was taken to avoid blood contamination of the aqueous samples. AqH samples were stored at −20 °C.

AqH ghrelin levels were measured using radioimmunoassay (RIA) diagnostic kits (KIPMR90, Diasource Europe SA, Belgium). The ghrelin kit is suitable for measuring total human ghrelin, with no cross-reactivity found with other proteins. The kit has an analytical sensitivity of 40 pg/mL and a calibratorion range of 0–6400 pg/mL, and the intra- and inter-assay variation is <5 and 7.6 %, respectively. The radiotracer used in all kits was 125 iodine (I-125, half-life $t_{1/2}$ 60 days, 35.5 keV gamma radiation, 27–32 keV X-rays, no beta radiation). All sample assays were performed in duplicate and included in the same run. If the difference between duplicate results of a sample was more than 5 %, the sample assay was repeated. An automatic gamma counter (Cobra II/5010, Packard Instrument Company, Meriden, CT, USA) was used to count the radioactivity and calculate the results.

All statistical analyses were performed using the Statistical Product and Services Solutions (SPSS) statistical software (SPSS, version 11.0, SPSS Inc, Chicago, IL, USA). The Kruskal–Wallis ANOVA test with a post hoc Tukey test was used for comparison of the cup:disc ratio, IOP and ghrelin levels. Multiple regression analysis was used to evaluate the contribution of age, gender and IOP level on the ghrelin levels. A P value of <0.05 was considered statistically significant.

Results

Descriptive patient characteristics are presented in Table 1. There was no difference in age, sex and preoperative IOP between the groups ($P > 0.05$). The cup:disc ratio of patients with exfoliation glaucoma was significantly higher than those of patients with exfoliation syndrome and the controls ($P < 0.05$) (Table 1). The patients' preoperative antiglaucoma medication is presented in Table 2.

All ghrelin measurements were completed successfully without any failure due to insufficient sampling or other complications during the analyses. One AqH sample from each of the 15 exfoliation syndrome eyes, the 8 exfoliation

Table 1 Patient characteristics

	Exfoliation syndrome $n = 15$	Exfoliation glaucoma $n = 8$	Controls
Sex (male/female)	12/3	7/1	10/2
Age (mean ± SD, years)	75.0 ± 8.4	76.7 ± 6.8	75.17 ± 6.1
Cup:disc ratio (mean ± SD)	0.26 ± 0.08	0.67 ± 0.26*	0.24 ± 0.06
IOP (mean ± SD, mmHg)	17.2 ± 1.3	17.5 ± 1.5	17.0 ± 1.1
Humphrey MD (mean ± SD, dB)	N/A	8.90 ± 4.84	N/A
Humprey PSD (mean ± SD, dB)		7.52 ± 2.18	
Number of antiglaucoma drugs (mean ± SD)	N/A	2.14 ± 0.19	N/A

IOP intraocular pressure, *MD* mean deviation, *PSD* pattern standard deviation, *dB* decibel, *N/A* not applicable

* $P < 0.05$ vs. exfoliation syndrome and controls

Table 2 Exfoliation glaucoma patients' topical medication

Class of topical medication	Exfoliation glaucoma patient ($n = 8$)
PG	1
PG + α-agonist	3
PG + CAI + α-agonist	2
PG + β-blocker + CAI + α-agonist	2

CAI carbonic anhydrase inhibitor, *PG* prostoglandin/prostannoid

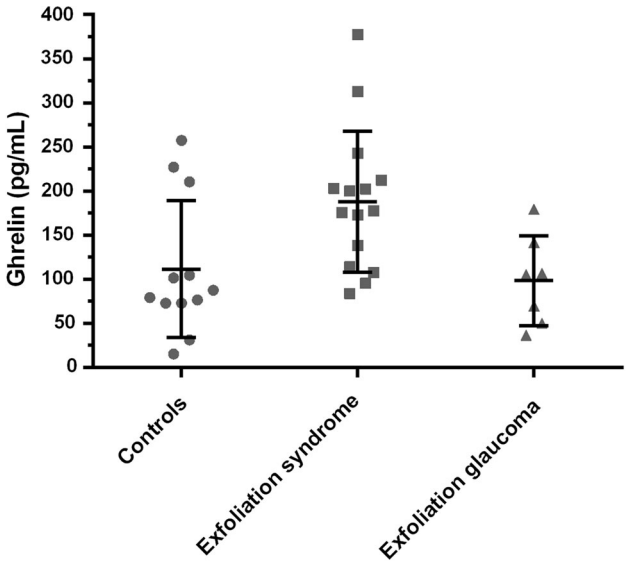

Fig. 1 Aqueous humour levels of ghrelin in patients with exfoliation syndrome or exfoliation glaucoma and in control subjects. *Asterisk* data are presented as mean ± SD. $P < 0.05$ vs. exfoliation glaucoma and controls

glaucoma eyes and the 12 control participants was analysed. AqH levels of ghrelin were 187.87 ± 80.1 pg/mL (max 377.56; min 83.86 pg/mL) in the eyes with exfoliation syndrome, 98.53 ± 50.9 pg/mL (max 179.51; min 36.64 pg/mL) in the eyes with exfoliation glaucoma, and 111.40 ± 77.5 pg/mL (max 257.64; min 31.19 pg/mL) in the controls (Fig. 1). AqH ghrelin levels of patients with exfoliation syndrome were significantly greater than those of patients with exfoliation glaucoma and the controls ($P < 0.05$). AqH ghrelin levels of patients with exfoliation glaucoma were lower than that of the controls but were not statistically significant ($P > 0.05$). Age, gender and IOP did not have a significant effect on ghrelin levels in patients with exfoliation syndrome and exfoliation glaucoma.

Discussion

We compared the AqH ghrelin levels of patients with exfoliation syndrome or exfoliation glaucoma to those of control subjects. This is the first study evaluating ghrelin levels in the AqH of eyes exhibiting exfoliation syndrome. This study indicates elevated levels of ghrelin in the AqH of patients with exfoliation syndrome as compared to those of patients with exfoliation glaucoma and controls.

Previous studies indicate that protein concentrations and growth factors are increased in the anterior chamber in cases of exfoliation syndrome [3, 16, 17]. They described how growth factors induce cellular proliferation and that they may play a role in the pathogenesis of exfoliation syndrome through the increase of ECM. On the other hand, the AqH of patients with exfoliation syndrome and exfoliation glaucoma was found to have elevated levels of CTGF. Some researchers suggest that CTFG, which is thought to take place in fibrotic pathology, is a pathophysiological marker for exfoliation syndrome and exfoliation glaucoma [18, 19]. In our study, the greatest and most significant ghrelin levels were observed in patients with exfoliation syndrome. Our hypothesis, similar to the above studies, is that a possible cause for the increase in ECM in patients with exfoliation syndrome may be increased ghrelin levels, because ghrelin is a strong endogenous growth hormone releaser. It has been found through experimental studies that GHSR1a is produced in the anterior segment and this area has been shown to comply with the localization of the trabecular meshwork [12].

On the other hand, it has been proven that ghrelin has a potent hypotensive effect on ocular tissue. In 2005 Shinde et al. [19] first noted that ghrelin had an ocular hypotensive effect by increasing the levels of nitric oxide.

Subsequently, Rocha-Sousa et al. [13] proved that ghrelin caused a decrease in muscle tone via prostaglandins in both iris sphincter and iris dilator muscles. In 2012 Rocha-Sousa et al. [20, 21] found that intraocular pressure decreased after the application of ghrelin in experimental animals that established experimental glaucoma. In clinical studies, Rocha-Sousa et al. and Katsanos et al. [14, 15], glaucomatous patients of either type demonstrated a significant reduction of AqH levels of ghrelin when compared to control subjects. Katsanos et al. [15] contained two additional findings; first, there was no difference in AqH levels of ghrelin between POAG and exfoliation glaucoma patients; and, second, the antiglaucomatous therapy administrated to patients did not affect AqH ghrelin levels. Unlike these previous studies, our glaucoma group consisted of only exfoliation glaucoma eyes, in which ghrelin levels were lower (98.53 ± 50.9 pg/mL). Ghrelin levels were compared statistically and found to be similar to those of exfoliation syndrome (187.87 ± 80.1 pg/mL and $P < 0.05$) and the control group (111.40 ± 77.5 pg/mL, $P > 0.05$). Ghrelin levels were lower in exfoliation glaucoma eyes than in the control group, but there was no statistically significant difference.This can be explained by fewer eyes in the study; it is one of the weaknesses of our study. However, the results are consistent with the literature in terms of achieving levels at least similar to those of the group with glaucoma.

Our hypothesis is that while on one hand ghrelin leads to the accumulation of ECM (high ghrelin associated with exfoliation syndrome in our study) on the other hand it leads to ocular hypotension, resulting in individuals with exfoliation syndrome exhibiting an intraocular pressure within normal limits. When ghrelin levels decrease [14, 15, and our study] causing ocular hypotension to subside, the transition from exfoliation syndrome to exfoliation glaucoma with elevated intraocular pressure occurs and the accumulation of ECM prevents the drainage of the AqH. In another study of individuals with exfoliation syndrome and exfoliation glaucoma, AqH ADMA levels were found to be lower in exfoliation syndrome and higher in exfoliation glaucoma. A direct correlation was detected between the increase in the levels of ADMA and the development of glaucoma [22]. There is an inverse relation in ghrelin; when it decreases, glaucoma occurs. One theory about the decrease of ghrelin in patients with glaucoma is the use of a prostaglandin derivative antiglaucoma. The effect of ghrelin on the iris sphincter is mediated by prostaglandins [13]. However, in Katsanos et al. [15] there was no significant difference in AqH or plasma levels of ghrelin between patients on prostaglandins and patients receiving other glaucoma medications. In our study the limited numbers of patients with exfoliation glaucoma and the presence of prostaglandins in the anti-glaucomatous combination of all individuals prevented us from reporting results. Further investigations with a large series will be required to reveal if there is an association between the ghrelin regulation pathway and the glaucoma processes in exfoliation syndrome and exfoliation glaucoma patients.

Studies found that ghrelin may pass the blood–brain barrier, albeit, at lower levels, but the real effect occurs through the vagus nerve indirectly [23, 24]. However, the passage of ghrelin through the blood–aqueous barrier has not been verified. In Rocha-Sousa et al. a relationship between aqueous humour and plasma ghrelin levels was not demonstrated, but in Katsanos et al. [14, 15] it is reported that there was a correlation, even if it was marginal. Results are contradictory and further studies are needed. Although it may seem like a weakness of this study, the levels of plasma ghrelin have not been evaluated due to the fact that ghrelin is locally produced in the anterior segment of the eye [13], and this was not the aim of this study.

In conclusion, this is the first study to assess ghrelin levels in patients with exfoliation syndrome and to support the idea that a reduction of ghrelin may be a contributing factor during the development of glaucoma. Further studies are required to investigate the factors playing a role in the decrease of ghrelin during the course of exfoliation syndrome and how ghrelin exerts its effect as a preventative factor for the development of exfoliation glaucoma.

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