• Clinical Research •

Comparison of total/active ghrelin levels in primary open angle glaucoma, pseudoexfoliation glaucoma and pseudoexfoliation syndrome

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Abstract

• AIM: To investigate the levels of ghrelin (Gh), acylated ghrelin (AGh) and AGh/Gh ratio in the humor aqueous (HA) of cases with pseudoexfoliation syndrome (PXS), pseudoexfoliation glaucoma (PXG), primary open angle glaucoma (POAG) and to compare these with control subjects.

• METHODS: A prospective examination was made of the total Gh, and AGh levels in HA of 67 patients undergoing cataract surgery. Patients were divided into 4 groups. HA samples were aspirated at the beginning of the surgery, stored at -70°C. Gh and AGh quantification was performed with ELISA kits and the AGh/total-Gh ratios were calculated. ANOVA, Kruskal-Wallis, Chi-square and post-hoc tests were used for statistical analysis.

• RESULTS: Total Gh levels in HA were 189.2±45.6 pg/mL in the control group, 199.2±32.9 pg/mL in PXS, 180.6±20.9 pg/mL in PXG and 176.8±21.4 pg/mL in POAG groups (*P*>0.05). AGh levels in HA were 23.09±5.01 pg/mL in the control group, 24.13±5.22 pg/mL in PXS, 22.29±1.55 pg/mL in PXG and 19.69±2.93 pg/mL in POAG groups (*P*>0.05). The ratio of AGh/Gh was 10.3%±2.34% in the control group, 13.03%±2.58% in PXS, 12.3%±1.54% in PXG and 11.79%±1.41% in POAG groups (*P*=0.044). The difference between the PXS and control groups was significant (*P*=0.03).

• CONCLUSION: In spite of statistically insignificant results, the HA total Gh levels were lower than those of the control subjects but not parallel with the AGh levels in glaucoma patients. The relative increase in the AGh/Gh ratio in glaucoma cases supports the view that proportional increases of AGh might play a role in the pathogenesis of glaucoma.

• KEYWORDS: ghrelin; acylated ghrelin; humour aqueous; pseudoexfoliation syndrome; pseudoexfoliation glaucoma; primary open angle glaucoma

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INTRODUCTION

Glaucoma is still one of the major causes of blindness throughout the world and is characterized by progressive retinal ganglion cell (RGC) death and axonal loss[1-4]. High intraocular pressure (IOP) is the most important risk factor of the disease and the therapies for IOP reduction remain the main treatment modalities[5-6]. However, glaucoma progresses in many people despite low IOP levels and this might be related to pathogenetic factors for the disease[1-4]. Primary open angle glaucoma (POAG) and pseudoexfoliation glaucoma (PXG) are the most frequent types of open angle glaucoma[7-9] and both may cause visual loss especially in the conditions of late diagnosis and improper treatment.

The inadequacy of IOP lowering treatment in many cases has encouraged investigators to study the pathogenesis of glaucoma. Ghrelin (Gh) is a polypeptide hormone and is synthesized in the digestive and endocrine systems in particular. Gh receptor growth hormone secretagogue receptor type 1α (GHSR-1α), belongs to G-protein coupled receptors superfamily, used phospholipase C, inositol triphosphates and intracelluler calcium pathways. Nascent Gh peptides are subjected to posttranslational modification by acylation of hydroxyl group of serin amino acid. Acylation is essential for its binding to GHSR-1α[10-11]. It promotes the release of growth hormone from the pituitary gland. It also has an antioxidant effect and because of this property it could be neuroprotective in glaucoma cases[12]. Three clinical research protocols defined the correlation between Gh and glaucoma. Rocha-Sousa *et al*[13], Katsanos *et al*[14] and Ozec *et al*[15] detected significantly lower humor aqueous (HA) levels Gh in glaucoma patients compare with the controls. Gh has been shown to related with pupil movements by decreasing iris dilatator and sphincter muscle tension[16-17]. Gh mRNA is expressed in the anterior and posterior segment of rat and rabbit eyes[18]. The aim

of this study was to investigate the levels of Gh, acylated ghrelin (AGh) and AGh/Gh ratio in the HA of cases with pseudoexfoliation syndrome (PXS), PXG and POAG and compare them to the control subjects.

SUBJECTS AND METHODS

A prospective evaluation was made of 67 eyes of 67 patients (34 females and 33 males) who underwent phacoemulsification surgery for senile cataract at Ulucanlar Eye Research Hospital between October 2014 and March 2015. All the study procedures were conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all participants. Approval for the study was granted by the Ethics Committee of Numune Training and Research Hospital. All the patients were Turkish Caucasians.

The patients were divided into 4 groups as control, PXS, PXG and POAG groups. The control group consisted of 22 eyes of 22 cases with no ocular disease other than senile cataract, the PXS group consisted of 20 eyes of 20 cases with PXS and cataract, the PXG group consisted of 12 eyes of 12 cases who had PXG and senile cataract and the POAG group consisted of 13 eyes of 13 cases with POAG and senile cataract.

A detailed ophthalmology examination was made including best corrected visual acuities (BCVA) with Snellen charts, anterior and posterior segment examinations, IOP measurements with Goldmann applanation tonometer, central corneal thickness (CCT) measurements with ultrasonic pachymeter, visual field examinations with Humphrey automated perimeter and retinal nerve fiber layer investigation using spectral-domain optic coherence tomography (OCT). The inclusion criteria for all the groups were age >40y and BCVA between hand motion and 20/40. The control group had no ocular disease other than cataract and refractive error. POAG was determined by the presence of glaucomatous visual field defects such as nasal step, seidel or arcuate scotome with an IOP≥22 mm Hg, grade 3-4 open angle according to Shaffer angle grading system and optic nerve head changes such as cup to disc ratio ≥0.3, localized neuro-retinal rim defects, peripapillary choroidal atrophy or splitter hemorrhage. The presence of pseudoexfoliation material (PXM) on the surface of the lens and/or pupillary margin without glaucomatous findings revealed PXS and the presence of PXM with glaucomatous findings revealed PXG. Cases were excluded from the study if aged younger than 40y, if they had types of glaucoma other than POAG and PXG, a history of any ocular trauma, surgery, uveitis, retinal or corneal pathologies which might cause vision loss, systemic diseases such as diabetes mellitus or rheumatological disorders. All the glaucoma cases in this study had mild to moderate glaucoma with controlled IOP and other glaucomatous findings treated with a maximum of 2 medical anti-glaucoma drops and which did not require surgery.

Sample Collection After pupil dilatation with topical 1% tropicamide and 1% cyclopentolate drops, topical anesthesia was applied with proparacaine hydrochloride 0.5%, preoperative antisepsis of the eyelids and conjunctiva with 5% povidone-iodine and after covering with ophthalmic drape, a sideport incision paracentesis was performed with a 23-gauge MVR knife. Under sterile conditions, a HA sample (0.1-0.2 mL) was aspirated from the anterior chamber with a 27-gauge insulin syringe and a cannula without any contact. After this procedure, phacoemulsification and posterior chamber intraocular lens (IOL) implantation surgery was performed. The samples were placed in special tubes and transferred immediately to -70℃ deep-freeze, where they were stored until the sample examination.

Sample Examination All the HA samples were transferred with a cold chain to Ankara Training and Research Hospital. Total Gh and AGh levels were measured with human Gh enzyme-linked immunoassay (ELISA) and human AGh ELISA kits (Hangzhou Eastbiopharn Corporation, Hangzhou, China). The human Gh ELISA kit has analytical sensitivity of 10 pg/mL and a calibration range of 50-10 000 pg/mL. The human AGh ELISA kit has analytical sensitivity of 2.6 pg/mL and a calibration range of 15-1000 pg/mL. As the HA sample was not sufficient in some cases, the total Gh and AGh levels could not be measured in all cases. The HA total Gh levels were obtained from a total of 50 cases, comprising 15 control, 15 PXS, 9 PXG and 11 POAG. The AGh levels were obtained from a total of 42 cases, comprising 11 control, 11 PXS, 9 PXG and 11 POAG. The AGh/total Gh ratios were calculated in 38 cases, comprising 9 control, 10 PXS, 9 PXG and 10 POAG.

Statistical Analysis SPSS for Windows version 18.0 software was used for statistical analysis. One-way ANOVA, Kruskal-Wallis, Levene, post-hoc Games-Howell and Pearson's Chi square tests were used for statistical analysis. Conformity to normal distribution was tested with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables with normal distribution were evaluated with one-way ANOVA, and those not with normal distribution, with the Levene test. If the variances were not homogeneous, the post-hoc Games-Howell test was used, and for the analysis of variance of nominal data, the Pearson's Chi-square test.

RESULTS

Total Gh in HA levels were measured in 50 cases, consisting of 23 females (46%) and 27 males (54%) with a mean age of 68.6±9.2y. The cases in the PXS and PXG groups were significantly older than those in the POAG and control groups (P<0.05; Table 1). AGh levels were measured in 42 cases consisting of 24 females (57.1%) and 18 males (42.9%) with a mean age of 68.8±7.4y. No significant differences of age or gender were found among the groups (P>0.05; Table 1).

Table 1 The demographic characteristics of the cases mean±SD

Parameters	Control	PXS	PXG	POAG	*P*
Total Gh					
Sex (F/M)	4/11	5/10	5/4	9/2	0.026
Age (y)	64.3±10.5	73.5±6.6	71.2±8.5	65.5±7.8	0.020
AGh					
Sex (F/M)	5/6	5/6	5/4	9/2	0.265
Age (y)	68.7±6.4	73.1±6.9	68.6±6.5	64.8±7.8	0.144
AGh/total Gh					
Sex (F/M)	2/7	4/6	5/4	8/2	0.076
Age (y)	63.3±12.4	72.9±7.3	68.6±6.5	67.9±9.1	0.135

PXG: Pseudoexfoliative glaucoma; POAG: Primary open angle glaucoma; PXS: Pseudoexfoliation syndrome; Gh: Ghrelin; AGh: Acylated ghrelin.

Table 2 The levels of total Gh, AGh and AGh/total Gh ratio in the groups mean±SD

Parameters	Control	PXS	PXG	POAG
No. of eyes	15	15	9	11
Total Gh level (pg/mL)	189.2±45.6	199.2±32.9	180.6±20.9	176.8±21.4
No. of eyes	11	11	9	11
AGh level (pg/mL)	23.09±5.01	24.13±5.22	22.29±1.55	19.69±2.93
No. of eyes	9	10	9	10
AGh/total Gh ratio (%)	10.3±2.34	13.03±2.58	12.3±1.54	11.79±1.41

PXG: Pseudoexfoliative glaucoma; POAG: Primary open angle glaucoma; PXS: Pseudoexfoliation syndrome; Gh: Ghrelin; AGh: acylated ghrelin; AGh/total Gh ratio: Acylated ghrelin to total ghrelin ratio.

Thirty-eight cases in whom HA total Gh and AGh levels were measured and the AGh/Gh ratio was calculated, consisting of 19 females (50%) and 19 males (50%) with a mean age of 67.9±9.1y. No significant differences of age or gender were found among the groups (P>0.05; Table 1).

Total Gh levels of HA were determined to be 189.2±45.6 pg/mL in the control group, 199.2±32.9 pg/mL in the PXS, 180.6±20.9 pg/mL in the PXG and 176.8±21.4 pg/mL in the POAG groups and there were no statistically significant differences among all groups (P>0.05; Table 2). AGh levels of HA were determined to be 23.09±5.01 pg/mL in the control group, 24.13±5.22 pg/mL in the PXS, 22.29±1.55 pg/mL in the PXG and 19.69±2.93 pg/mL in the POAG groups and no statistically significant differences were detected between the groups (P>0.05; Table 2). The ratio of AGh/Gh was calculated to be 10.3%±2.34% in the control group, 13.03%±2.58% in the PXS, 12.3%±1.54% in the PXG and 11.79%±1.41% in the POAG groups (Table 2). The difference between the PXS and control groups was significant (P=0.03) but there were no statistically significant differences between the control and PXG or control and POAG groups (P>0.05).

DISCUSSION

Although high IOP is thought to be the most important risk factor, glaucoma pathogenesis is associated with many other factors[1-4]. Low levels of total Gh in HA is one of the recent hypotheses for glaucoma pathogenesis, especially in PXG and POAG cases[13-15]. Rocha-Sousa *et al*[13] investigated the total Gh levels in HA of glaucoma cases and found statistically significantly low levels compared with control subjects. In a similar study, Katsanos *et al*[14] found significantly low levels of total Gh in HA of POAG and PXG cases compared with the normal subjects[14]. In both studies no significant differences were found in the plasma levels of total Gh. Ozec *et al*[15] compared HA total Gh levels of cases with PXS and PXG and found significantly lower levels in PXG than PXS. It was thought that the low level of Gh in HA could be associated with the progression of PXS to PXG. In the current study, the levels of total Gh, AGh and AGh to total Gh ratio were investigated in PXS, PXG, POAG and normal subjects and it was attempted to determine the associations of Gh levels in HA with glaucoma pathogenesis.

Gh is known to be found in some ocular tissues in the anterior and posterior segment and to have some important roles in cellular mechanisms. It has been shown that Gh is expressed in iris and ciliary body epithelial cells and retinal pigment epithelium[16-17]. It has also been shown that Gh has a relaxation effect on the iris muscles. The ciliary body produces many neuroendocrine peptides which might have major roles in the production and outflow of HA, the vascularization and immune mechanisms of the anterior segment and the circadian rhythm of IOP[17]. Gh is thought to have a messenger-type role in the anterior segment and may affect IOP as a result of its effects on the production and outflow of HA[16-17].

The correlation between Gh and glaucoma pathogenesis has been investigated in some experimental studies. Rocha-Sousa *et al*[18] induced raised IOP in rats and rabbits with intravitreal injection of NaCl 20% in 2 animal models. Gh and des-acyl Gh were injected subconjunctivally and Gh was observed to significantly reduce IOP. This effect was considered to be related with the induced production of prostaglandines and/or nitric oxide. However, no similar effect was determined with des-acyl Gh and the AGh/Gh ratio was not measured as it was in the current study[18]. It was considered in the current study that AGh was the main form of Gh that might be related with glaucoma pathogenesis because it was the only form that could bind to GHSR-1α receptor. More than 90% of Gh in circulation is in the form of inactivated des-acyl Gh because a large amount of the produced Gh is rapidly inactivated by deacetylation[19-20]. For this reason we should focus on not only total Gh levels but also AGh levels and AGh/Gh ratio because of des-acyl Gh not able to bind Gh receptors.

In this study, the levels of total Gh and AGh in HA were compared in cases with PXS, PXG, POAG and normal subjects. Although the results were not statistically significant, lower levels of total Gh were determined in POAG and PXG cases than in the control group but higher levels were found in PXS cases than in normal subjects. AGh levels were also compared and the results were found to be similar to those of total Gh in spite of insignificant *P* values. To the best of our knowledge, this study is the first to have compared AGh levels of HA in glaucoma patients and parallel results were found for AGh and Gh levels of HA. In addition to these results, the AGh to Gh ratio was calculated and these ratios from the highest to the lowest were determined in the PXS, PXG, POAG and control groups. The difference between the PXS cases and normal subjects was significant in respect of this ratio.

In spite of many common characteristics, POAG and PXG are known to have different etiological, pathogenetic and clinical properties[7-9]. In the current study, the levels of both Gh and AGh in HA were compared in POAG and PXG cases. All the cases had controlled glaucoma under topical anti-glaucoma medications and used different kinds of anti- drops. No significant differences were found and because of these insignificant differences, it can be considered that the anti-glaucoma medications had no effect on Gh and AGh levels.

The capability of Gh to permeate the blood-aqueous barrier is unclear. Rocha-Sousa *et al*[13] found no correlations between the HA and plasma levels of Gh. Katsanos *et al*[14] showed some correlations between HA and plasma levels but the results of that study were not supported by other studies. On the basis of these findings it is still unclear whether locally produced or systemic Gh might have effects on glaucoma pathogenesis.

In the current study, very small different levels of total Gh were found to be correlated with previous results, which may have been due to the study methodology. Unlike in previous studies, instead of radioimmunassay, ELISA was used in the current study, which was 4 times more sensitive. The ELISA kit could measure Gh and AGh in all body fluids. Furthermore, the samples were stored at -70℃ instead of -20℃. No comparison could be made of the current study AGh levels with previously reported levels because to the best of our knowledge, this study was the first to examine AGh levels of HA.

There were some limitations to this study. Pipetting was performed manually because very small amounts (40 μL) were necessary for sampling. During pipetting, some HA was lost and therefore, all total Gh, AGh and the ratio could not be measured in all cases.

In conclusion, although the level of active and inactive Gh in glaucoma patients decreases with respect to control, the ratio of active Gh to inactive Gh is actually increased. This proportional increase might affect receptor-acyl Gh interaction and might trigger glaucoma. That would be more accurate to concentrate on AGh levels and AGh/Gh ratio in order to clarify the pathogenesis of glaucoma. Further researches with a great number of cases and with different types of glaucoma will be required to lighten the actual role of Gh in glaucoma.

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REFERENCES

1 Doucette LP, Rasnitsyn A, Seifi M, Walter MA. The interactions of genes, age, and environment in glaucoma pathogenesis. *Surv Ophthalmol* 2015;60(4):310-326.

2 Wang JW, Chen SD, Zhang XL, Jonas JB. Retinal microglia in glaucoma. *J Glaucoma* 2016;25(5):459-465.

3 Yue B. Biology of the extracellular matrix: an overview. *J Glaucoma* 2014;23(8 Suppl 1):S20-S23.

4 Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA* 2014;311(18):1901-1911.

5 Miglior S, Bertuzzi F. Relationship between intraocular pressure and glaucoma onset and progression. *Curr Opin Pharmacol* 2013;13(1):32-35.

6 Clement CI, Bhartiya S, Shaarawy T. New perspectives on target intraocular pressure. *Surv Ophthalmol* 2014;59(6):615-626.

7 Janssen SF, Gorgels TG, Ramdas WD, Klaver CC, van Dujin CM, Jansonius NM, Bergen AA. The vast complexity of primary open angle glaucoma: disease genes, risks, molecular mechanisms and pathobiology. *Prog Retin Eye Res* 2013;37:31-67.

8 Anastasopoulos E, Founti P, Topouzis F. Update on pseudoexfoliation syndrome pathogenesis and associations with intraocular pressure, glaucoma and systemic diseases. *Curr Opin Ophthalmol* 2015;26(2):82-89.

9 Dewundara S, Pasquale LR. Exfoliation syndrome: a disease with an environmental component. *Curr Opin Ophthalmol* 2015;26(2):78-81.

10 Kojima M, Kangawa K. Ghrelin: structure and function. *Physiol Rev* 2005;85(2):495-522.

11 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999;402(6762):656-660.

12 Erşahin M, Toklu HZ, Erzik C, Cetinel S, Akakin D, Velioglu-Ogunc A, Tetik S, Ozdemir ZN, Sener G, Yegen BC. The anti-inflammatory and neuroprotective effects of ghrelin in subarachnoid hemorrhage-induced oxidative brain damage in rats. *J Neurotrauma* 2010;27(6): 1143-1155.

13 Rocha-Sousa A, Alves-Faria P, Falcao-Pires I, Falcao-Reis F, Leite-Moreira AF. Analyses of aqueous humour ghrelin levels of eyes with and without glaucoma. *Br J Ophthalmol* 2009;93(1):131-132.

14 Katsanos A, Dastiridou A, Georgoulias P, Cholevas P, Kotoula M, Tsironi EE. Plasma and aqueous humour levels of ghrelin in open-angle glaucoma patients. *Clin Exp Ophthalmol* 2011;39(4):324-329.

15 Ozec AV, Dursun A, Toker MI, Demirci Y, Topalkara A, Erdogan H, Arici MK, Ersalcan T. Aqueous humour levels of ghrelin in exfoliation syndrome and exfoliation glaucoma patients. *Jpn J Opthalmol* 2014; 58(4):348-352.

16 Rocha-Sousa A, Saraiva J, Henriques-Coelho T, Falcao-Reis F, Correia-Pinto J, Leite-Moreira AF. Ghrelin as a novel locally produced relaxing peptide of the iris sphincter and dilator muscles. *Exp Eye Res* 2006;83(5):1179-1187.

17 Coca-Prados M, Escribano J. New perspectives in aqueous humor secretion and in glaucoma: the ciliary body as a multifunctional neuroendocrine gland. *Prog Retin Eye Res* 2007;26(3):239-262.

18 Rocha-Sousa A, Pereira-Silva P, Tavares-Silva M, Azavedo-Pinto S, Rodrigues-Araujo J, Pinho S, Avelino A, Flcao-Reis F, Leite-Moreira A. Identification of the ghrelin-GHSR 1 system and its influence in the modulation of induced ocular hypertension in rabbit and rat eyes. *Peptides* 2014;57:59-66.

19 Kojima M, Hosoda H, Kangawa K. Purification and distribution of ghrelin: the natural endogenous ligand for the growth hormone secretagogue receptor. *Horm Res* 2001;56(suppl 1):93-97.

20 Patterson M, Murphy KG, le Roux CW, Ghatei MA, Bloom SR. Characterization of ghrelin-like immunoreactivity in human plasma. *J Clin Endocrinol Metab* 2005;90(4):2205-2211.