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ASSOCIATION BETWEEN GLAUCOMA DAMAGE AND PLASMA CONCENTRATION OF HEAT SHOCK PROTEIN 70

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ABSTRACT

Heat shock proteins (HSP) or stress proteins are induced in cells. They protect the cell and increase cell survival.

The aim of our research was to determine the plasma concentration of HSP 70 in patients with primary open-angle glaucoma with elevated intraocular pressure (POAG-HTG) and patients with pseudoexfoliative open-angle glaucoma (XFG), and to investigate the relationship between this biomarker and the structural and functional characteristics of glaucoma.

We included 90 participants in this study, divided into three groups: 37 patients with primary open-angle glaucoma with increased intraocular pressure (hypertensive glaucoma, POAG-HTG), 24 patients with pseudoexfoliative open-angle glaucoma (XFG), and 29 participants without systemic diseases and without glaucoma, matched by sex and age (control group of subjects, CONT). The concentration of circulating HSP 70 was measured in participants' plasma using the sandwich enzyme linked immunosorbent assay (ELISA).

Plasma levels of HSP 70 were very similar in all three groups of participants, without any significant differences among the examined patients. A significant negative correlation of the plasma concentration of HSP 70 and RNFL Savg ($p<0.05$) was found in POAG-HTG patients, whereas the negative correlations of HSP 70 with MD ($p= 0.0538$) and with RNFL Iavg ($p= 0.0584$) were very close to statistical significance.

There was no increase in the plasma concentration of HSP 70 in POAG-HTG and XFG patients. There was an interdependence between the plasma level of HSP 70 and the examined clinical parameters of POAG-HTG patients (MD, RNFL Avg, RNFL Savg and RNFL Iavg). HSP 70 can be a significant biomarker for glaucoma.

Keywords: heat shock protein 70, glaucoma, open-angle, hypertension glaucoma, pseudoexfoliation, plasma

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POVEZANOST OŠTEĆENJA IZAZVANIH GLAUKOMOM I KONCENTRACIJE PROTEINA TOPLOTNOG ŠOKA 70 U PLAZMI

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Proteini toplotnog šoka (HSP) ili proteini stresa se indukuju u ćelijama. Oni štite ćeliju i povećavaju opstanak ćelije.

Cilj našeg istraživanja bio je da se utvrdi koncentracija HSP 70 u plazmi pacijenata obolelih od primarnog glaukoma otvorenog ugla sa povišenim intraokularnim pritiskom (POAG-HTG) i pacijenata obolelih od pseudoeksfolijativnog glaukoma otvorenog ugla (PEXG) i da se ispita veza između ovog biomarker i strukturnih i funkcionalnih karakteristike glaukoma.

U ovu studiju smo uključili 90 ispitanika, podeljenih u tri grupe: 37 pacijenata obolelih od primarnog glaukoma otvorenog ugla sa povišenim intraokularnim pritiskom (hipertenzivni glaukom, POAG-HTG), 24 pacijenta obolela od pseudoeksfolijativnog glaukoma otvorenog ugla (PEXG), 29 ispitanika bez sistemskih bolesti i bez glaukoma, sparenih po polu i starosti (kontrolna grupa ispitanika, CONT). Koncentracija cirkulišućeg HSP 70 je merena metodom sendvič enzimskog imunosorbentnog testa (ELISA) u plazmi ispitanika.

Nivoi HSP 70 u plazmi bili su veoma slični u sve tri grupe ispitanika, bez značajne razlike između ispitivanih pacijenata. POAG-HTG pacijenti su imali značajnu negativnu korelaciju koncentracije HSP 70 i RNFL Savg u plazmi ($p<0,05$), a veoma blizu statističke značajnosti su negativne korelacije HSP 70 sa MD ($p= 0,0538$) i sa RNFL Iavg ($p = 0,0584$).

Nema povećanja koncentracije HSP 70 u plazmi pacijenata sa POAG-HTG i PEXG. Postoji međuzavisnost između nivoa HSP 70 u plazmi i ispitivanih kliničkih parametara POAG-HTG (MD, RNFL Avg, RNFL Savg i RNFL Iavg). HSP 70 može biti značajan biomarker glaukoma.

Ključne reči: protein toplotnog šoka 70, glaukom, otvoreni ugao, hipertenzivni glaukom, pseudoeksfolijacija, plazma

INTRODUCTION

Heat shock proteins (HSPs) are stress proteins and their production in cells protect the cell and increase cell survival. Their concentration increases in physiologically or environmentally stressful conditions. HSPs function as chaperone molecules that prevent aggregation and facilitate remodeling of deactivated proteins in the cell (1, 2).

HSPs act by the most diverse mechanisms including direct interaction with components of cellular signaling pathways, downstream or upstream regulation of caspase-dependent programmed cell death, or at the mitochondrial level. HSPs can also affect caspase-independent apoptosis, by interacting with apoptogenic factors such as Apoptosis-inducing factor (AIF) or acting at the level of lysosomes. HSP 70 is a guardian of the integrity of the lysosomal membrane. Due to oxidative stress, HSP carboxylation occurs, subsequently causing lysosome rupture. In addition, HSP 70 dysfunction activates nuclear factor-κB (NF-κB) signaling that may also promote neurodegeneration (1-6).

The heat shock protein 70 family (HSP 70 family) includes several members such as the constitutive form HSP 70, inducible HSP 72, mitochondrial GRP 75, and endoplasmic reticulum GRP 78. These proteins exhibit distinctive neuroprotective effects and function in normal, developmental, and stressful conditions. Although the constitutive form exists under normal conditions, it is more pronounced under stressful conditions (7-13).

HSP 70 is the most structurally and functionally conserved protein in the HSP family. HSP 70 is a ubiquitous class of ATP-dependent chaperone proteins that exert cytoprotective effects. It plays a central role in the cellular control of protein quality. HSP 70 binds to the protein substrate facilitating its unfolding, degradation, transport, regulation, and preventing aggregation (1).

Elevated IOP is one type of mechanical stress that activates HSPs. Several studies have shown that HSPs and anti-HSP antibodies play an important role in glaucoma pathogenesis. Cellular stress and cell death are interrelated events, and HSPs, induced in response to stress, play a role in controlling apoptosis (11). HSPs include anti-apoptotic and proapoptotic proteins that interact with various cellular proteins involved in apoptosis. Their expression level can determine cell fate in response to a stimulus (14).

Increased immunohistochemical staining of HSPs in the glaucomatous retina reflects the function of these proteins in terms of cellular defense and stress response in glaucoma (15, 16).

Optic neuropathy and progression of glaucoma occurs due to overexpression of HSP and activation of the auto-stimulatory response (17). Therefore, in patients with glaucoma, an elevated titer of anti-HSP antibodies occurs compared to healthy subjects, which reduces the protective ability of HSP (16). These data indicate that HSPs are essential for RGC survival as molecular chaperones and have pathogenic significance in glaucoma patients (18).

HSPs are involved in multiple stages of apoptosis and their function is to inhibit apoptosis (19). Overexpression of HSP 70 protects mitochondria from the harmful effects of reactive oxygen species (20). HSP 70 inhibits apoptosis by reducing the release of cytochrome-c and increasing the activity of caspase-3 (21). HSP 70 has also been described to inhibit protein kinase/c-Jun N-terminal kinase (SAPK/JNK) (22).

Kim et al. (8) demonstrated that HSP 70 is transferred from cell to cell. However, RGCs are not the sole target of HSP 70-induced neuroprotection. Glial cells also participate in HSP 70 transfer and contribute to neuroprotection.

The aim of our research was to determine the plasma concentration of HSP 70 in patients with primary open-angle glaucoma with elevated intraocular pressure (POAG-HTG) and patients with pseudoexfoliative open-angle glaucoma (XFG), and to investigate the relationship between this biomarker and the structural and functional characteristics of glaucoma disease.

MATERIAL AND METHODS

The research was carried out at the Clinic for Ophthalmology, University Clinical Center in Niš, Department of Biochemistry and Laboratory for Functional Genomics and Proteomics, and Scientific Research Center for Biomedicine, University of Niš. All participants were informed about the objectives of the research and signed an informed consent to participate, according to the Declaration of Helsinki. The study was approved by the Ethical Committee of the Faculty of Medicine in Niš (decision number 01-2625-18) and the Ethical Committee of the University Clinical Center in Niš (decision number 338/43).

We included 90 participants in this study, divided into three groups: 37 patients with primary open-angle glaucoma with increased intraocular pressure (hypertensive glaucoma, POAG-HTG), 24 patients with pseudoexfoliative open-angle glaucoma XFG, and 29 participants patients without systemic diseases and without glaucoma, matched by sex and age (control

group, CONT).

We performed a complete ophthalmic examinations, including a review of the medical history, determination of visual acuity with refractions (Snellen tables), slit-lamp biomicroscopy of the anterior and the posterior segment, gonioscopy with three mirrors Goldman gonioscope, Goldmann applanation tonometry (GAT), indirect ophthalmoscopy and determination of the cup size of the optic nerve head (C/D ratio), using a 90D lens, standard automatic perimetry (Humphrey Visual Field Analyzer, HFA, USA; Carl Zeiss Meditec, Inc., Threshold Test 24-2), and glaucoma OCT scan protocols (OCT, Stratus, Carl Zeiss Meditec, Inc., Dublin, CA) and determination of retinal peripapillary nerve fiber thickness (RNFL). We measured RNFL average (RNFL Avg), in the superior quadrant (RNFL Savg) and in the inferior quadrant (RNFL Iavg).

Inclusion criteria for patients with POAG-HTG were as follows: elevated IOP, characteristic Bjerum's arcuate scotoma, and/or paracentral scotoma, and/or nasal, Rönne's step in Humphrey's computerized visual field, or other relevant defects in the visual field, optic disc cupping, and/or thinning of the nerve fiber layer on OCT, the finding of an open angle, and the absence of a secondary cause of glaucomatous optic neuropathy, such as previous trauma, previous corticosteroid administration, inflammation, or uveitis. Patients with POAG-HTG had intraocular pressure values greater than 21 mmHg upon daily measurement before diagnosis.

Inclusion criteria for patients with XFG were: elevated IOP, changes in the visual field, thinning of the RNFL on OCT, as for POAG, with the presence of pseudoexfoliations on the anterior capsule of the lens and/or along the pupillary margin.

Patients with systemic arterial hypertension, diabetes mellitus, systemic vasculopathies, retinal disease, eye surgery, trauma and inflammation of the eye were excluded from the study. All control subjects were without glaucoma as confirmed by applying the same diagnostic criteria used for the diagnosis and with no family history of the condition, matched by sex and age. Patients with history of congenital glaucoma or suspected normotensive (NTG) or hypertensive glaucoma (HTG) were also excluded from further examination.

Collected whole blood samples obtained from participants using EDTA as an anticoagulant, were centrifuged for 10 minutes at 3500 spins, at the temperature of +4°C. The plasma was separated and frozen at a temperature of -80°C afterward.

The concentration of circulating HSP 70 was measured by the sandwich enzyme linked immunosorbent assay (ELISA) method in participants' plasma, according to the manufacturer's instructions (Cusabio, CSB-E13463h, P.R. China). The concentration was determined using a standard curve and expressed in ng/ml. Minimum detectable dose (MDD) was 78 pg/ml. In

accordance with the manufacturer's instructions, there was no significant cross-reactivity or interference with other proteins.

STATISTICAL ANALYSIS

We used the methods of descriptive (absolute numbers, relative numbers, arithmetic mean, standard deviation, median, interval of variation (minimum and maximum values)) and analytical statistics (Mann-Whitney U test, Student's t-test of independent samples, Kruskal-Wallis test, ANOVA, χ^2 test or Fisher's test, Pearson's simple linear correlation coefficient, Spearman's rank correlation coefficient, univariate linear regression analysis). Statistical processing of the results was done with the SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA). A value of $p<0.05$ was used as the threshold for statistical significance.

RESEARCH RESULTS

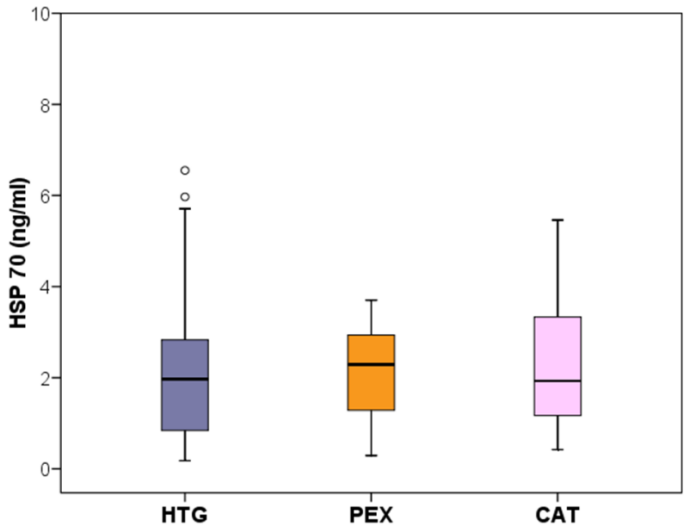
Table 1 shows demographic characteristics of 90 participants (37 POAG-HTG + 24 XFG + 29 CONTROL), basic clinical parameters of glaucoma (IOP, C/D ratio, MD, RNFL Avg, RNFL Savg, and RNFL Iavg), and plasma levels of HSP 70 (graphic 1).

Table 1. Demographic and clinical characteristics, and plasma levels of HSP 70 of glaucoma patients and the control group of participants without glaucoma.

	POAG-HTG (n=37)	**XFG** (n=24)	**CONTROL** (n=29)	
Age (year) X±SD (Me) Min-Max	70.95±8.01 70.00 58 - 87	73.41± 6.25 76.00 59 - 84	71.77±9.38 74.0 51 - 88	Kruskal-Wallis test Mann-Whitney test
Gender (M/F)	20 (54.05%) /17 (45.94%)	14 (58.33%) /10 (41.67%)	14 (48.27%) /15 (51.73%)	
IOP (mmHg) X±SD (Me) Min-Max	**21.86±7.43**^{c***} (20.00) 10-48	**23.58±11.31**^{c***} (20.50) 10-56	14.76±2.39 (14.00) 8-20	c- vs. CONT, ***-p<0.001 Kruskal-Wallis and Mann-Whitney test

C/D ratio X±SD (Me) Min- Max	0.64±0.16 (0.60) 0.4-1	0.63±0.18 (0.55) 0.4-1		Mann-Whitney test
MD (dB) X±SD (Me) Min- Max	-11.73±9.35 (-8.46) -0.38 - -31.27	-12.72±11.21 (-8.62) -0.07 - -29.69		Mann-Whitney test
RNFL Avg (µm) X±SD (Me) Min- Max	78.09±24.48 (80.98) 24.46-143.71	78.62±21.54 (81.63) 45.77-103.69		Mann-Whitney test
RNFL Savg (µm) X±SD (Me) Min- Max	92.38±34.87 (96.00) 26-180	100.64±37.66 (104.00) 44-161		Mann-Whitney test
RNFL Iavg (µm) X±SD (Me) Min- Max	94.41±37.95 (101.00) 29-157	96.10±31.11 (91.00) 57-144		Mann-Whitney test
HSP 70 (ng/ml) X ± SD (Me), Min-Max	2.27±1,70 (1.97) 0.18 – 6.55	2.14±0.96 (2.29) 0.29 – 3.70	2.20±1.32 (1.93) 0.42 - .,46	Kruskal-Wallis and Mann-Whitney test, Student´s t-test

Legend: n- number of participants/eyes and examined plasma samples, POAG-HTG- primary open-angle glaucoma with elevated IOP, hypertensive glaucoma, XFG- pseudoexfoliative glaucoma, CONTROL- control group without glaucoma, IOP- intraocular pressure, C/D- cup/disk ratio, MD- mean deviation, RNFL Avg- average peripapillar retinal nerve fibre leyer thicknes, RNFL Savg- peripapillar retinal nerve fibre leyer thicknes in the superior quadrant, RNFL Iavg- peripapillar retinal nerve fibre leyer thicknes in the inferior quadrant, HSP 70- heat shock protein 70.



Graph 1. Median, minimum, maximum and 25th and 75th percentile values for HSP 70 in glaucoma and control group.

We found no difference in age and sex between the examined groups (Kruskal-Wallis test and Mann-Whitney test). POAG-HTG and XFG were more prevalent in men (54.05%, i.e., 58.33%), whereas, in the control group, women were more prevalent (51.73%), without any significant difference between the groups. XFG patients had the highest IOP, and IOP values in both glaucoma groups were significantly higher compared to the control group ($p<0.001$). There was no significant difference in the values of the C/D ratio between POAG-HTG and XFG. Although the absolute value of MD was higher in eyes with XFG, it was not significantly different from the value of this parameter in eyes with POAG-HTG. All POAG-HTG and XFG patients were in the second and the third group according to the Hadopp classification, without significant differences in distribution. Average RNFL thickness and RNFL thickness in the superior and inferior quadrants were higher in XFG patients, however, not significantly compared to POAG-HTG patients.

Plasma levels of HSP 70 were very similar in all three groups of subjects. Neither Kruskal-Wallis, nor Mann-Whitney, nor Student's t-test of independent samples, revealed any significant differences between the examined patients.

Spearman's rank correlation coefficient and Pearson's linear correlation coefficient were used to examine the connection between plasma concentration of HSP 70 and the intraocular pressure (IOP), C/D ratio, MD, RNFL Avg, RNFL Savg and RNFL Iavg of the same patient (table

2). A significant negative correlation of the plasma concentration of HSP 70 and RNFL Savg ($p<0.05$) was found in POAG-HTG patients, whereas the negative correlations of HSP 70 with MD ($p= 0.0538$) and with RNFL Iavg ($p= 0.0584$) were very close to statistical significance.

Table 2. Spearman's and Pearson's correlation coefficients of HSP 70 and the examined parameters in glaucoma patients.

ρ (*r*) HSP 70 (ng/ml) and	IOP	C/D ratio	MD	RNFL Avg	RNFL Savg	RNFL Iavg
POAG-HTG	0.12	–0.12	–0.27	–0.26	*–**0.33**	–0.30
XFG	0.07	0.12	–0.46	*0.14*	*–0.05*	*–0.171*

* - p<0,05, ρ - Spearman rank correlation coefficient

r - Pearson coefficient of linear correlation (values in italics)

Univariate linear regression analysis did not find any effects of HSP 70 on the values of IOP, C/D ratio for POAG-HTG patients or XFG patients (table 3). This analysis confirmed a significant effect of HSP 70 concentration on MD and RNFL Avg, RNFL Savg and RNFL Iavg of POAG-HTG patients. An increase in HSP 70 by one measurement unit caused a significant decrease in the MD value by 1.37 dB with a confidence interval of 0.01 - 2.73, a significant decrease in the value of RNFL Avg by 4.78 with a confidence interval of 0.11 - 9.45, a significant decrease in the RNFL Savg value of 5.74 with a confidence interval of 0.29 - 11.20, a significant decrease in the RNFL Iavg value of 7.62 with a confidence interval of 0.89 - 14.36, in patients with POAG-HTG (table 3).

Table 3. Results of univariate linear regression analysis and influence evaluation of the HSP 70 on the value of IOP, C/D ratio, MD, RNFL Avg, RNFL Savg and RNFL Iavg of POAG-HTG and XFG patients.

	POAG-HTG					XFG				
HSP 70	t	p	B	95% IP za B		t	p	B	95% IP za B	
IOP (mmHg)	0.50	0.6161	0.30	-0.87	1.47	0.07	0.9446	0.12	-3.28	3.52
C/D ratio	-0.99	0.3255	-0.01	-0.04	0.01	0.51	0.6166	0.03	-0.10	0.16
MD (dB)	**-2.02**	**0.0490**	***-1.37**	**-2.73**	**-0.01**	-1.17	0.2678	-5.29	-15.27	4.69
RNFL Avg	**-2.07**	**0.0450**	***-4.78**	**-9.45**	**-0.11**	-2.07	0.0450	-4.78	-9.45	-0.11
RNFL Savg	**-2.13**	**0.0396**	***-5.74**	**-11.20**	**-0.29**	-0.14	0.8932	-2.90	-50.36	44.56
RNFL Iavg	**-2.29**	**0.0276**	***-7.62**	**-14.36**	**-0.89**	-0.52	0.6174	-8.42	-45.23	28.39

DISCUSSION

Heat shock proteins (HSPs) are ubiquitous intracellular proteins. They are evolutionarily conserved molecules, with the role of chaperone and cytoprotective function (23).

HSP 70 plays an important role in protein metabolism, both under stress and under normal conditions. It facilitates the assembly of newly created proteins, translocation and degradation of damaged proteins, and participates in regulatory processes. In neurons exposed to extremely stressful conditions, such as ischemia and excitotoxicity, the expression of HSP occurs, and it is considered a neuroprotector. In addition, HSPs are highly antigenic, and HSPs or anti-HSP antibodies have a significant pathogenetic effect in glaucoma. The resulting immune response to HSP can have a protective or pathogenic effect. HSP production is up-regulated in RGCs of glaucoma patients. The amount of HSP, as well as HSP 70, is increased in monkeys with experimental glaucoma, and HSP 72 is increased in RGCs in a glaucoma rat model (24-27). However, elevated serum levels of HSP antibodies, detected in glaucoma patients, may activate nerve cell death via apoptosis. Initially, increased HSP expression in the glaucomatous eye may be neuroprotective, protecting against further degeneration and inhibiting apoptosis. Since HSP can also act as an immunostimulatory signal, the breakdown of the immune tolerance and the

loss of their protective effect can occur, which results in the loss of regulation of antiapoptotic processes and accelerates and facilitates apoptosis, and finally disease progression occurs. Thus, glaucomatous optic neuropathy may be a consequence of aberrant autoimmunity (28).

HSP expression determines cell fate. Reduced expressivity leads to ineffectiveness response to stress, and increased expression activates immunodestruction. In both cases, the outcome is the cell death, however, through two mechanisms. The first involves oxidative free radicals, and the second involves cytokines as shown by Tezel et al. (16). The presence of HSP in RGCs and glial cells may be important for the stimulation of cytoprotective events at the beginning and then for neurodegenerative changes (16, 25).

Heat shock proteins 70 (HSP 70) are present in the peripheral circulation of healthy individuals (Pockley et al. 1998), (29).

Previously reported data indicate that HSPs are involved in the pathogenesis of glaucoma and the development of glaucomatous neuropathy due to increased IOP. The involvement of HSP in the development of glaucoma is twofold, it can be neuroprotective or degenerative related to the activation of the autoimmune response during the progression of the disease (16, 17, 30).

A study conducted by Lichtenaur et al. indicates that the serum concentration of HSP 70 in healthy subjects was 49 pg/mL ± 22 (31). Another study showed that HSP 70 was detectable only in 77% of the analyzed samples, whereas the serum concentration of this protein decreases with age (400 ng/ml < 40 years; 20 ng/ml > or = 90 years), while the concentrations of anti-HSP 70 antibodies tend to increase with age, but without mutual dependence. These findings suggest that the stress response potential decreases with age (32).

Our research found very similar plasma values of HSP 70 in all three groups of participants (POAG-HTG, XFG and CONT 2.20 ±1.32 ng/ml). Thus, we can conclude that there is no increase in the plasma concentration of HSP 70 in POAG-HTG, however, not excluding the increase of this protein in the aqueous humor or in the RGC nor the increase of antibodies to HSP 70. This partially supports the hypothesis that the decrease in HSP levels leads to a decrease in their protective role in the pathogenesis of glaucoma. Güler M. et al. showed that the level of HSP 70 in the aqueous humor was increased in patients with pseudoexfoliation without glaucoma compared to patients with cataract without pseudoexfoliation (33), thus supporting the neuroprotective role of HSP before the onset of glaucoma and the increase of anti-HSP antibodies.

We found a significant negative correlation of the serum concentration of HSP 70 and RNFL Sup ($p<0.05$) in patients with POAG-HTG. Hence, plasma concentration of HSP 70 can be a measure of the development and progression of glaucomatous neuropathy in POAG-HTG. Our research has confirmed that HSP 70 concentration has a significant effect on MD and RNFL Avg, RNFL Savg and RNFL Iavg of POAG-HTG patients. However, it remains unclear why it is not the case with XFG.

Due to a limited number of studies invastigating the concentration of HSP 70 in the plasma of glaucoma patients, and almost no research on the correlation between concentration and clinical parameters, we are unable to fully analyze the obtained results. Finally, further research, particularly larger and similarly designed studies should be conducted. Nevertheless, our research has a small contribution to the study of the role of HSP70 in glaucoma and the possibility of using this protein as a biomarker for glaucoma disease.

These results support a mechanism involving the immune response in glaucomatous damage, which may provide a new therapeutic approach in the neuroprotection of glaucomatous optic neuropathy. Understanding genetics and immunity in the pathogenesis of glaucoma is crucial for the development of new treatments.

CONCLUSION

There was no increase in the plasma concentration of HSP 70 in POAG-HTG and XFG patients. However, there was an interdependence between the plasma level of HSP 70 and the examined clinical parameters of POAG-HTG (MD, RNFL Avg, RNFL Savg and RNFL Iavg). This suggests that HSP 70 can be a significant biomarker for glaucoma.

REFERENCES

1. Lu RC, Tan MS, Wang H, Xie AM, Yu JT, Tan L. Heat shock protein 70 in Alzheimer's disease. Biomed Res Int 2014; 2014: 435203.
2. Sooraj K, Shukla S, Kaur R, Titiyal JS, Kaur J. The protective role of HSP27 in ocular diseases. Mol Biol Rep 2022; 49(6): 5107-5115.
3. Chidlow G, Wood JP, Casson RJ. Expression of inducible heat shock proteins Hsp27 and

Hsp70 in the visual pathway of rats subjected to various models of retinal ganglion cell injury. PLoS One 2014; 9(12): 114838.

4. Yamashima T. Hsp70.1 and related lysosomal factors for necrotic neuronal death. J Neurochem 2012; 120(4): 477-94.
5. Park KH, Cozier F, Ong OC, Caprioli J. Induction of heat shock protein 72 protects retinal ganglion cells in a rat glaucoma model. Ophthalmol Vis Sci 2001; 42: 1522-30.
6. Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, et al. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. Mol Cell Biol 2000; 20(19): 7146-59.
7. Caprioli J, Ishii Y, Kwong JM. Retinal ganglion cell protection with geranylgeranyl-acetone, a heat shock protein inducer, in a rat glaucoma model. Trans Am Ophthalmol Soc 2003; 101: 39-50; discussion 50-1.
8. Kim JM, Park KH, Kim YJ, Park HJ, Kim DM. Thermal injury induces heat shock protein in the optic nerve head in vivo. Invest Ophthalmol Vis Sci 2006; 47 (11):4888-94.
9. Tamm ER, Russell P, Johnson DH, Piatigorsky J. Human and monkey trabecular meshwork accumulate β-crystallin in response to heat shock and oxidative stress. Invest Ophthalmol Vis Sci 1996; 37: 2402-13.
10. Caprioli J, Kitano S, Morgan JE. Hyperthermia and hypoxia increase tolerance of retinal ganglion cells to anoxia and excitotoxicity. Invest Ophthalmol Vis Sci 1996; 37: 2376-81.
11. Cao Y, Gao L, Tang R, Zhang W. Hsp70 protects human trabecular meshwork cells injury induced by UVB through Smad pathway. Pharmazie 2017; 72(6): 334-337.
12. Kim YM, Vera ME, Watkins SC, Billar TR. Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor induced apoptosis by inducing heat shock protein 70 expression. J Biol Chem 1997; 272: 1402-11.
13. Amin V, Cumming DVE, Latchman DS. Over-expression of heat shock protein 70 protects neuronal cells against both thermal and ischaemic stress but with different efficiencies. Neurosci Lett 1996; 206: 45-48.
14. Didelot C, Schmitt E, Brunet M, Maingret L, Parcellier A, Garrido C. Heat shock proteins: endogenous modulators of apoptotic cell death. Handb Exp Pharmacol 2006; 172: 171-98.
15. Nowak A, Szaflik JP, Gacek M, Przybylowska-Sygut K, Kamińska A, Szaflik J, et al.

BDNF and HSP gene polymorphisms and their influence on the progression of primary open-angle glaucoma in a Polish population. Arch Med Sci 2014; 10(6): 1206-13.

16. Tezel G, Hernandez R, Wax MB. Immunostaining of heat shock proteins in the retina and optic nerve head of normal and glaucomatous eyes. Arch Ophthalmol 2000; 118: 511-8.
17. Tezel G, Yang J, Wax MB. Heat shock proteins, immunity and glaucoma. Brain Res Bull 2004; 62: 473-80.
18. Rokutan K, Hirakawa T, Teshima S, Nakano Y, Miyoshi M, Kawai T, et al. Implications of heat shock/stress proteins for medicine and disease. J Med Invest 1998; 44: 137-47.
19. Vayssier M, Polla BS. Heat shock proteins chaperoning life and death. Cell Stress Chaperones1998; 3: 221-27.
20. Polla BS, Kantengwa S, François D, Salvioli S, Franceschi C, Marsac C, et al. Mitochondria are selective target for the protective effects of heat shock against oxidative injury. Proc Natl Acad Sci USA 1996; 93: 6458-63.
21. Li CY, Lee JS, Ko YG, Kim JI, Seo JS. Heat shock protein 70 inhibits apoptosis downstream of cytochrome c release and upstream of caspase-3 activation. J Biol Chem 2000; 275: 25665-71.
22. Mosser DD, Caron AW, Bourget L, Denis-Larose C, Massie B. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. Mol Cell Biol 1997; 17: 5317-27.
23. Hightower LE. Heat shock, stress proteins, chaperones, and proteotoxicity. Cell 1991; 66(2): 191-7.
24. Nowak A, Majsterek I, Przybyłowska-Sygut K, Pytel D, Szymanek K, Szaflik J, et al. Analysis of the expression and polymorphism of APOE, HSP, BDNF, and GRIN2B genes associated with the neurodegeneration process in the pathogenesis of primary open angle glaucoma. Biomed Res Int. 2015; 2015:258281.
25. Ou-Yang Y, Liu ZL, Xu CL, Wu JL, Peng J, Peng QH. miR-223 induces retinal ganglion cells apoptosis and inflammation via decreasing HSP-70 in vitro and in vivo. J Chem Neuroanat 2020; 104: 101747.
26. Piri N, Kwong JM, Gu L, Caprioli J. Heat shock proteins in the retina: Focus on HSP70 and alpha crystallins in ganglion cell survival. Prog Retin Eye Res. 2016 May; 52:22-46.
27. Chen H, Tian A, Wu Y, Li R, Han R, Xu X, et al. HSP70 expression before and after

treatment and its clinical value in patients with acute angle-closure glaucoma. Exp Ther Med. 2021 Mar;21(3):253.

28. Tosaka K, Mashima Y, Funayama T, Ohtake Y, Kimura I; Glaucoma Gene Research Group. Association between open-angle glaucoma and gene polymorphism for heat-shock protein 70-1. Jpn J Ophthalmol 2007; 51: 417-23.
29. Meng X, Harken AH. The interaction between Hsp70 and TNF-alpha expression: a novel mechanism for protection of the myocardium against post-injury depression. Shock 2002; 17(5): 345-53.
30. Wax MB, Tezel G, Yang J, Peng G, Patil RV, Agarwal N, et al. Induced autoimmunity to heat shock proteins elicits glaucomatous loss of retinal ganglion cell neurons via activated T-cell-derived fas-ligand. J Neurosci 2008; 28(46): 12085-96.
31. Lichtenauer M, Zimmermann M, Nickl S, Lauten A, Goebel B, Pistulli R, et al. Transient hypoxia leads to increased serum levels of heat shock protein-27, -70 and caspase-cleaved cytokeratin 18. Clin Lab 2014; 60(2): 323-8.
32. Rea IM, McNerlan S, PockleyAG. Serum heat shock protein and anti-heat shock protein antibody levels in aging. Exp Gerontol 2001; 36(2): 341-52.
33. Güler M, Aydın S, Urfalıoğlu S, Yardım M. Aqueous humor heat-shock protein 70, periostin, and irisin levels in patients with pseudoexfoliation syndrome. Arq Bras Oftalmol. 2020 Sep-Oct;83(5):378-382.