Prognostic importance of endothelin-1 and endothelin receptor: a plasma levels in the early perimetric stage of primary open-angle glaucoma

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ABSTRACT
An increasing amount of data suggests a role of the eye vascular system and oxidative stress in glaucoma pathogenesis. Reports have suggested endothelin-1 (ET-1) and its receptor (ETR-A) as possible glaucoma biomarkers. This study explored the diagnostic and prognostic abilities of ET-1 and ETR-A plasma concentrations in primary open angle glaucoma (POAG). Seventy-five participants were divided into three groups: controls, early and advanced POAG stage, graded by a perimetric visual field test. All of them underwent a standard ophthalmological examination including optical coherence tomography. The statistical analysis showed a significant difference between the ET-1 values in the controls (4.88 ± 1.75 pg/mL) and the glaucoma patients, but lack of statistical significance in the glaucoma severity (early POAG: 6.33 ± 2.38 pg/mL and advanced POAG: 6.34 ± 1.56 pg/mL). The mean ETR-A values were significantly different between the three groups (controls 1209.28 ± 314.48 pg/mL, early POAG: 673.44 ± 283.02 pg/mL and advanced POAG: 992.28 ± 264.22 pg/mL). Two mathematical models were developed concerning the two perimetric indices (MD/PSD) and ETR-A in the early glaucoma group. ETR-A showed a very high diagnostic accuracy. Only ETR-A had significant diagnostic ability for advanced glaucoma after the comparison between the two glaucoma groups. Every 1 pg/mL increase in the ET-1 plasma concentration increased the possibility for early glaucoma changes by 2.124 times, whereas every 1 pg/mL increase in the ETR-A level decreased this possibility by 1%. Our results indicate that ET-1 and ETR-A could be two very good diagnostic parameters for early POAG changes.

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Introduction
Glaucoma is a chronic progressive neurodegenerative ocular disorder that is considered the primary cause for irreversible blindness worldwide [1]. The diagnosis and staging of the disease include various techniques such as tonometry, perimetric visual-field examination, optical coherence tomography (OCT), scanning laser tomography, etc. All of them show good discriminating ability between health and disease but a drawback is the difficulty in the correct staging of the glaucoma, as many optic nerve fibres can be lost before any significant changes in the results become present [2]. Research has focused on identifying indicators and methods suitable for adequate early staging of glaucoma such as retinal nerve fibre layer (RNFL) thickness [3, 4] or ganglion cell damages in the area of the macula [3] measured with spectral domain OCT (SD-OCT) or image analysis of optic nerve head (ONH) [5] and others. Considering the glaucoma severity and fast progression when not treated accordingly, a trend has been established in order to identify biomarkers suitable for early detection and staging of glaucoma. Various options have been investigated after isolation from tears, urine, serum/plasma, cerebrospinal fluid, vitreous body, etc. [6]. As possible glaucoma biomarkers, endothelin-1 (ET-1) and its receptor (ETR-A) have been reported in the literature [7, 8]. Recently, there is an increasing amount of data suggesting the role of the eye vascular system and oxidative stress in the pathogenesis of glaucoma [9, 10]. The progression of glaucoma associated ocular neuropathy is significantly affected by the endothelins (ET). These are a family of strong vasoactive peptides comprised of three isoforms (ET-1, ET-2 and ET-3). They exert their effect through two types of G-coupled endothelin receptors A (ETRA) and B (ETRB) [11, 12]. ET-1 is one of the most significant vasoconstrictors secreted by

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The increase in the ET-1 release is related to extended boost in the vascular tonus in numerous pathophysiological processes associated with endothelial dysfunction. In the physiological state, the role of ET-1 in the vascular tone regulation is limited. This can be partially explained by the nitrogen oxide (NO) activity which is simultaneously a vasodilator and an inhibitor of ET-1 synthesis [15]. ET-1 is strongly related to the pathogenesis of glaucoma [16]. It is the most potent constrictor for small and medium size blood vessels [17]. The presence of ET and the expression of their receptors have been established in the following ocular tissues: trabecular meshwork, ciliary body, lamina cribrosa and retina [18]. It is considered that ET-1 takes part in regulating the ocular blood flow, the outflow of aqueous humour from the anterior eye segment and consequently the intraocular pressure (IOP) [7]. There is also evidence that the intravitreal application of ET provokes retinal ganglion cell death [19, 20].

The vessel dysregulation and the endothelin system activity have been investigated in the case of various systemic diseases such as arterial hypertension (AH) with and without accompanying diabetes [21]; obesity (regression analysis) [22]; steroid production, follicles production and ovulation [23]; acute and chronic inflammatory demyelinating polyneuropathy, multiple sclerosis and Alzheimer’s disease [24]; hypertrophic cardiomyopathy (regression analysis) [25]; acute kidney failure, kidney ischemia, heart failure, ischemic heart disease, Prinzmetal angina, primary lung hypertension, Reino’s disease, subarachnoid hemorrhage and migraine [26]. Regression analysis has been applied also in the evaluation of plasma ET-1 levels in glaucoma patients [16]. Thus, the aim of the present study was to investigate the diagnostic and prognostic ability of plasma ET-1 and ETR-A as biomarkers in the early stages of primary open-angle glaucoma (POAG) patients.

Subjects and methods

The participants in this prospective study were recruited from the Eye Clinic at the University Hospital ‘Alexandrovska’ (Sofia, Bulgaria). The study, being a project of the Medical University of Sofia, was also approved by the Bioethics Committee. All the 75 patients signed informed consent forms for the participation in this study.

Based on the POAG stage, three groups were formed: control group (25 healthy volunteers, age range 45–71 years), early stage group (22 patients, age range 45–82 years) and advanced stage group (28 patients, age range 45–82 years). The controls were recruited amongst visitors and staff of the hospital without eye and general diseases. The following inclusion criteria for glaucoma patients were defined: best corrected visual acuity more than 0.2; refractive error in the range between ±4.00 dsph and ±1.00 dcyl; IOP above 21 mmHg (Goldmann applanation tonometry); presence of open anterior chamber angle III–IV grade of the Shaffer classification; glaucomatous changes in the eye fundus and visual field defects corresponding to the damages of the ONH. Both the early and advanced glaucoma stage were determined by the Hodapp–Parrish–Anderson functional perimetric staging system. All eye and general pathological conditions outside the inclusion criteria were excluded. Diseases associated with high plasma ET levels were also taken into account in this research. Only AH was not defined as an exclusion disease. This was because of the impossibility of AH to be avoided as a risk factor for high ET plasma levels and vasospasm syndrome in all patients especially in these over 50 years of age, who predominated in this study.

Each participant underwent a complete ophthalmic examination including detailed case and family history, visual acuity assessment, ONH ophthalmoscopy, visual-field examination and OCT. Standard automated perimetry (SAP) test was performed by Humphrey Field Analyzer II (Carl Zeiss Meditec, Dublin, CA, USA), algorithm SITA Standard, pattern 24-2. With spectral domain OCT examination, we defined the structural changes in the RNFL thickness of the peripapillary (pRNFL) and macular area (mRNFL). It was performed using Topcon 3D OCT 2000 (FAF + FA) (Topcon Corporation, Japan). We worked with two programs: Circle and 3D Macula (V) and examined eight RNFL parameters: Total pRNFL (average thickness in 360°), Sup pRNFL (in the superior 90°), Inf pRNFL (in the inferior 90°), Nas pRNFL (in the nasal 90°), Temp pRNFL (in the temporal 90°), Sup mRNFL (the thickness in the superior half), Inf mRNFL (in the inferior half) and Total mRNFL (average thickness in the whole investigated area).

Blood samples were collected from each fasting patient at early morning in supine position and relaxed condition to prevent changes in ET-1 plasma levels. The vacutainer tubes were centrifuged and the plasma was separated from the blood cell elements, removed and aliquoted into 1.5 mL Eppendorf tubes and stored frozen at -10.00°C until measurement. We used sandwich-type enzyme-linked immunosorbent assay (ELISA) to determine the plasma concentration.
of ET-1 and ETR-A. We used Endothelin-1 Assay Kit – IBL and Human endothelin-1 receptor (EDNRA) Elisa Kit (CASABIO).

The results for each group were analysed using Microsoft Excel (MS Office 2013) and statistical software package SPSS 17.0. Quantitative data were expressed as mean values with standard deviation (±SD). Values of \( p < 0.05 \) were considered to indicate significant differences. Dispersion analysis (ANOVA test) with inter-group variance was used to compare the means of the groups; ROC analysis, correlation analysis and regression analysis were applied also.

**Results and discussion**

The total number of the participants in the present survey was 75 (45–83 years, mean age 63.4 ± 8.6 years, 22 (29.3%) men, 53 (70.7%) women). The control group included 25 healthy participants (6 men, 19 women), at a mean age of 56.5 ± 8.8 years. The early stage glaucoma group included 22 patients (5 men, 17 women), at a mean age of 66.9 ± 8.5 years. The advanced stage glaucoma group included 28 patients (11 men, 17 women; mean age 66.8 ± 8.46 years). The number of women was larger probably due to their higher willingness to participate in clinical investigations. Because of the higher number of women in the study, Chi-square test (\( \chi^2 \) test) of independence was applied (\( \chi^2 = 2.14, df = 2, p = 0.342 \)). The results showed absence of significant relationship between gender and the investigated groups.

In the control group, the mean ET-1 plasma level was 4.88 ± 1.75 pg/mL, whereas in the patients with early glaucoma stage, it was 6.33 ± 2.38 pg/mL, and in the advanced glaucoma patients, 6.34 ± 1.56 pg/mL. We [27] found a statistically significant difference in the ET-1 plasma concentration between the controls and the early glaucoma patients (\( p = 0.029 \)), the control group and the advanced glaucoma patients (\( p = 0.018 \)), but we could not find significant difference between the two glaucoma groups (\( p = 0.998 \)) after application of inter-group comparative analysis. Therefore, it could be suggested that the increase in the ET-1 level may be related with the glaucoma development and may play a role in the pathogenesis of the disease. These results are similar to previous reports by other authors. Two research teams, Chen et al. [16] and Cellini et al. [28] also found a statistically significant increase in the ET-1 plasma levels in patients with POAG as compared to healthy participants. Other authors [29–31] also report an increase in ET-1 plasma levels in patients with POAG and normotensive glaucoma (NTG) but without significance.

In the control group, the mean ETR-A plasma level was 1209.28 ± 314.48 pg/mL; in the patients with early glaucoma stage 673.44 ± 283.02 pg/mL, and in the advanced glaucoma patients 992.28 ± 264.22 pg/mL. In our previous study [27], we found a statistically significant difference in the plasma concentration of ETR-A between the three groups: controls and early glaucoma patients (\( p < 0.001 \)), control group and advanced glaucoma patients (\( p = 0.021 \)) and between the two glaucoma groups (\( p = 0.001 \)).

Significant downregulation of ETR-A is evident in the early stage glaucoma patients, which corresponds to the increased ET-1 levels. The progression of the disease is related to some degree of restoration of ETR-A, whereas ET-1 is permanently high. These observations are related to some theories about the glaucoma pathogenesis [18], but require further investigation.

Our previous results [27] showed a statistically significant negative relationship between ET-1 and RNFL thickness: Inf pRNFL (\( p = 0.027, R = -0.256 \)), Inf mRNFL (\( p = 0.011, R = -0.291 \)) and Total mRNFL (\( p = 0.024, R = -0.260 \)). We could not find any statistical correlation between ETR-A and RNFL thickness.

Correlation analysis in all 75 participants was used to assess the relationship of MD and PSD with ET-1 and ETR-A. We found a weak but statistically significant negative relationship between MD and ET-1 (\( N = 75 \) participants, \( p = 0.049, R = -0.229 \)). The increase of ET-1 levels leads to a decrease in MD, which means that ET-1 is related to glaucoma progression. In order to explain this correlation, we aimed at developing a mathematical model. We used the following linear equation \( y = a + bx \), where \( y \) is the dependent variable (MD), \( a \) is a constant also known as the y-intercept, \( b \) is the slope of the regression line and \( x \) is the independent variable (ET-1). In order to develop a mathematical model which can explain the relationship, the two coefficients, \( a \) and \( b \), in the formula must be values significantly different from zero. In this case, the \( a \) constant was not statistically significant (\( p = 0.638 \)) and it did not allow us to develop a model. When we applied the same correlation analysis separately in the groups, we found only two statistically significant correlations in the early glaucoma patients. One was between MD and ETR-A (\( N = 22, p = 0.036, R = 0.450 \)), indicating that the ETR-A plasma levels decreased with the progression of glaucoma changes, whereas, at the same time, the MD values were on a decrease. Another significant relationship...
was found between PSD and ETR-A \((N = 22, \ p = 0.046, \ R = -0.429)\). It showed a decrease in the ETR-A plasma level with the further progress of glaucoma changes in the early stage of the disease, while at the same time, the PSD values increased. The rest of the relationships were not statistically significant. The presence of these two significant correlations was a reason to try mathematical modelling. In the first example of modelling, MD was a dependent variable and ETR-A was an independent variable, \(R^2 = 0.203\), adequate model \(- F (1; 20) = 5.08, \ p = 0.036\), significant constant \((p < 0.001)\).

The mathematical model in the group of early stage of POAG was: \(MD = -4.524 + 0.003 \times ETR-A\).

In the second example of modelling, PSD was the dependent variable and ETR-A was the independent variable, \(R^2 = 0.184\), adequate model \(- F (1; 20) = 4.51, \ s = 0.046\), significant constant \((p < 0.001)\).

The model in the group of early stage of POAG was: \(PSD = 6.714 - 0.004 \times ETR-A\).

In this case, the coefficient of determination was \(R^2 = 0.184\), indicating that 18.4% of the dependent variable PSD changes could be explained by the changes of the independent variable ETR-A. These two models could allow prediction of MD and PSD in early stage glaucoma based on ETR-A serum levels.

Chen et al. [16] also applied correlation and regression analysis to investigate the plasma ET-1 levels in patients with POAG, NTG and primary angle-closure glaucoma. The correlations of ET-1 with MD and the average RNFL thickness were compared after adjusting the effects of age and refraction using multiple linear regression, but none of them showed significance.

We also applied binary logistic regression analysis and quantified the factors ET-1, ETR-A and age. The dependent variable was the group, which consisted of two categories, healthy controls and patients with glaucoma, with two subcategories, early and advanced changes. We applied the analysis for the both cases of disease stages.

In this multiple factor analysis, we investigated the independent influence of ET-1, ETR-A and age on the early stage of POAG (Table 1). Values of OR > 1 show that increasing the factor values leads to an increase of the possibility for early glaucomatous changes to appear. Two of the investigated factors had a value of OR > 1. The results showed that each year added to the age increases the possibility for early glaucoma changes to appear 1.243 times. It is well established that glaucoma is an age-related disease [18], which was supported by our results. Every 1 pg/mL increase in the ET-1 plasma concentration increased the possibility for early glaucoma changes to appear by 2.124 times. Values of OR < 1 show that increasing the factor values leads to a decrease of the possibility for early stage of glaucoma. Every 1 pg/mL increasing of the ETR-A plasma concentration decreased by 1% (100%–99% = 1%) the possibility for early glaucomatous changes to appear. It may be considered that the ET-1 and ETR-A levels are important in the early glaucoma stages. Therefore, they show potential for development of an ETR-A antagonist that may be applied in early glaucoma and prevent the progression of the disease, as suggested by other researchers as well [18].

In the stage of advanced POAG, again the same two factors, age and ET-1, had OR > 1, and ETR-A had OR < 1 (Table 2). All differences showed significance in the two regression analyses.

Applied stepwise regression analysis could explain the impact of the independent variables, ET-1, ETR-A and age, on the RNFL values. The RNFL parameters which we analysed were those most significant of early glaucoma changes: Inf pRNFL and Total mRNFL. In all the 75 participants, the age and ET-1 significantly affected the Inf pRNFL values: \(R^2 = 0.263\); adequate model \(F(2;74) = 12.83\); age \((p < 0.001)\), ET-1 \((p = 0.029)\). The same test was used for Inf pRNFL in the two separate glaucoma groups. We obtained a significant model only for the early glaucoma stage for ET-1: \(R^2 = 0.221\); adequate model \(F(2;74) = 5.66\); \(p = 0.027\). The test was also applied for the second OCT parameter, Total mRNFL, in all participants. The model was significant \((p < 0.001)\) only for age: \(R^2 = 0.244\); adequate model \(F(2;74) = 23.61\). However, it did not show an adequate model in different glaucoma groups for Total mRNFL.

The association of glaucoma with the ET-1 levels has been discussed in the literature, even though variable results have been reported [16, 18]. Our data suggest that there is likely to be a relationship in the

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**Table 1.** Binary logistic regression of the analysed factors in the group of early glaucoma.

| Factor          | OR   | 95% CI for OR | \(p\) |
|-----------------|------|---------------|------|
| Age (years)     | 1.243| 1.054–1.466   | 0.010|
| ET-1 (pg/mL)    | 2.124| 1.027–4.394   | 0.042|
| ETR A (pg/mL)   | 0.990| 0.984–0.997   | 0.004|

OR: odds ratio; 95% CI: 95% confidence interval.

**Table 2.** Binary logistic regression of the analysed factors in the group of advanced glaucoma.

| Factor          | OR   | 95% CI for OR | \(p\) |
|-----------------|------|---------------|------|
| Age (years)     | 1.152| 1.058–1.255   | 0.001|
| ET-1 (pg/mL)    | 1.688| 1.032–2.761   | 0.037|
| ETR A (pg/mL)   | 0.997| 0.991–0.999   | 0.049|
pathogenesis of the disease but the exact biochemical mechanism has remained elusive and is still to be determined.

ROC analysis was also used to determine the diagnostic ability of ET-1 and ETR-A for early POAG staged by MD values (Table 3). The two investigated parameters showed significant diagnostic ability for early POAG. In comparison, the two parameters also had significant discriminating ability between the control group and the advanced POAG group: ET-1 (AUC = 0.741, p = 0.003) and ETR-A (AUC = 0.706, p = 0.010). When the analysis was applied to define the diagnostic ability for advanced glaucoma after comparison between early and advanced glaucoma, only ETR-A had significant diagnostic ability (AUC = 0.807, p < 0.001). ET-1 and ETR-A can be used as diagnostic biomarkers for glaucoma in its early stages.

ET-1 and ETR-A are important biomarkers for a lot of diseases, including glaucoma. In the present study, they could be applied as a diagnostic tool in the early glaucoma stage. The data showed correlation between their levels and the main parameters tested in glaucoma patients (MD and PSD). Even though some of the findings are not in complete concordance with already published results [16, 27], it is noteworthy that ET-1 likely plays a role in the pathogenesis and pathophysiology of glaucoma. This could be used in the development of ETR-A antagonists for topical administration to be applied in the management of early glaucoma.

Conclusions

The results from the present study showed that the ET-1 and ETR-A levels had some significant correlation with the development of glaucoma. This was more predominant in the early stage of the disease as compared with the healthy control group. These biomarkers possess a potential to be used as diagnostic biomarkers of POAG in the early stages. This could be very contributory for the early and adequate onset of treatment and would possibly reduce the irreversible sight impairment.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] European Glaucoma Society (EGS). Terminology and guidelines for glaucoma. 4th ed. Savona (Italy): PublComm; 2014.
[2] Jonas JB, Aung T, Bourne R, et al. Glaucoma. Lancet. 2017;390(10108):2183–2193.
[3] Elbendary A, Helal R. Discriminating ability of spectral domain optical coherence tomography in different stages of glaucoma. Saudi J Ophthalmol. 2013; 27:19–24.
[4] Tatham A, Mederos F. Detecting structural progression in glaucoma with optical coherence tomography. Ophthamology. 2017;124(12):557–565.
[5] Soltani A, Battikh T, Jabri I, et al. A new expert system based on fuzzy logic and image processing algorithms for early glaucoma diagnosis. Biomed Signal Proc Control. 2018;40:366–377.
[6] Ban N, Siegfried C, Apte R. Monitoring neurodegeneration in glaucoma: therapeutic implications. Trends Mol Med. 2018;24(1):7–17.
[7] Wróbel-Dudzińska D, Kosior-Jarecka E, Łukasik U, et al. Risk factors in normal-tension glaucoma and high-tension glaucoma in relation to polymorphisms of endothelin-1 gene and endothelin-1 receptor type A gene. J Ophthalmol. 2015: 368792. DOI:10.1155/2015/368792.
[8] Kosior-Jarecka E, Wróbel-Dudzińska D, Łukasik U, et al. Plasma endothelin-1 and single nucleotide polymorphisms of endothelin-1 and endothelin type A receptor genes as risk factors for normal tension glaucoma, Mol Vis. 2016;22:1256–1266.
[9] Flammer J, Konieczka K, Flammer AJ. The primary vascular dysregulation syndrome: implications for eye diseases. EMJA J. 2013;4(1):14. DOI 10.1186/1878-5085-4-14
[10] Mroczkowska S, Benavente-Perez A, Nég A, et al. Primary open-angle glaucoma vs normal-tension glaucoma: the vascular perspective. JAMA Ophthalmol. 2013;131(1):36–43.
[11] Houde M, Desbiens I, D’Orléans-Juste P. Endothelin-1: biosynthesis, signaling and vasoreactivity. Adv Pharmacol. 2016;77:143–175.
[12] Blanco R, Martínez-Navarrete G, Valiente-Soriano F, et al. The S1P1 receptor-selective agonist CYM-5442 protect retinal ganglion cells in endothelin-1 induced retinal ganglion cell loss. Exp Eye Res. 2017; 164:37–45.
[13] Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature. 1988;332(6163):441–445.
[14] Mikhail M, Vachon P, D’Orléans-Juste P, et al. Role of endothelin-1 and its receptors ETA and ETB in the survival of human vascular endothelial cells. Can J Physiol Pharmacol. 2017;95(10):1298–1305.
[15] De Mey J, Vanhoutte P. End l’ the line revisited: moving on from nitric oxide to CGRP. Life Sci. 2014; 118:120–128.

Table 3. ROC analysis to define the diagnostic ability of ET-1 and ETR A.

| Parameter   | AUC | p     | Cut-off | Sensitivity | Specificity |
|-------------|-----|-------|---------|-------------|-------------|
| ET-1 (pg/mL)| 0.765 | 0.002 | 7.069   | 0.727       | 0.920       |
| ETR A (pg/mL)| 0.905 | <0.001 | 972.849 | 0.840       | 0.818       |

AUC: area under the curve; cut-off, boundary between health and disease.
[16] Chen H, Chang Y, Chen W, et al. Association between plasma endothelin-1 and severity of different types of glaucoma. J Glaucoma. 2013;22(2):117–122.

[17] López-Riquelme N, Villalba C, Torno C, et al. Endothelin-1 levels and biomarkers of oxidative stress in glaucoma patients. Int Ophthalmol. 2015;35(4):527–532.

[18] McGrady N, Minton A, Stankowska D, et al. Upregulation of the endothelin A (ETA) receptor and its association with neurodegeneration in a rodent model of glaucoma. BMC Neurosci. 2017;18:27. DOI:10.1186/s12868-017-0346-3

[19] Ko A, Hyun H, Min S, et al. Endothelin-1 induces LIMK2-mediated programmed necrotic neuronal death independent of NOS activity. Mol Brain. 2015;8:58. DOI 10.1186/s13041-015-0149-3.

[20] He S, Park Y, Yorito T, et al. Endothelin-mediated changes in gene expression in isolated purified rat retinal ganglion cells. Invest Ophthalmol Vis Sci. 2015;56:6144–6161.

[21] Skalska A, Pietrzycka A, Stepniewski M. Correlation of endothelin 1 plasma levels with plasma antioxidant capacity in elderly patients treated for hypertension. Clin Biochem. 2008;42(4–5):358–364.

[22] Weil B, Westby C, Guilder G, et al. Enhanced endothelin-1 system activity with overweight and obesity. Am J Physiol Heart Circ Physiol. 2011;301(3):H689–H695.

[23] Ervin J, Schütz L, Spicer L. Current status of the role of endothelins in regulating ovarian follicular function: a review. Anim Reprod Sci. 2017;186:1–10.

[24] Chang Ch, Wu H, Lyu R, et al. Elevate serum levels of endothelin-1 in patients with chronic inflammatory demyelinating polyneuropathy. Clin Chim Acta. 2018;476:49–53.

[25] Wang Y, Tang Y, Zou Y, et al. Plasma level of big endothelin-1 predicts the prognosis in patients with hypertrophic cardiomyopathy. Int J Cardiol. 2017;243:283–289.

[26] Jain SK, Yadava RK, Raikar R. Role of endothelins in health and disease. J Indian Acad Clin Med. 2002;3:59–64.

[27] Mihaylova B, Petkova I, Rankova-Yotova CH, et al. Plasma endothelin-1 and endothelin-A receptor concentrations in patients with primary open-angle glaucoma. Biotechnol Biotechnol Equip. 2017;31(4):782–787.

[28] Cellini M, Strobbe E, Gizzi C, et al. Endothelin-1 plasma levels and vascular endothelial dysfunction in primary open angle glaucoma. Life Sci. 2012;91:699–702.

[29] Nicolela MT, Ferrier SN, Morrison ChA, et al. Effects of cold induced vasospasm in glaucoma: the role of endothelin-1. Invest Ophthalmol Vis Sci. 2003;44(6):2565–2572.

[30] Tezel G, Kass MA, Kolker AE, et al. Plasma and aqueous humor endothelin levels in primary open angle glaucoma. J Glaucoma. 1997;6:83–89.

[31] Kunimatsu S, Mayama C, Tomidokoro A, et al. Plasma endothelin-1 level in Japanese normal tension glaucoma patients. Curr Eye Res. 2006;31(9):727–731.