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Correlation of EPO and VEGF levels in serum and aqueous humor with ocular hemodynamics and oxidative stress − mitochondrial function in patients with glaucoma

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ABSTRACT

Objective: To study the correlation of EPO and VEGF levels in serum and aqueous humor with ocular hemodynamics and oxidative stress - mitochondrial function in patients with neovascular glaucoma (NVG). **Methods:** A total of 47 patients who were diagnosed with neovascular glaucoma in Mianyang Wanjiang Ophthalmic Hospital between April 2015 and April 2017 were selected as the NVG group of the study, and 75 patients who were diagnosed with cataract in Mianyang Wanjiang Ophthalmic Hospital over the same period were selected as the CAT group of the study. The aqueous humor and serum samples were collected to determine the contents of EPO, VEGF, oxidative stress molecules and mitochondrial function molecules; the intraocular vascular hemodynamic parameters were detected. **Results:** EPO and VEGF contents in serum as well as EPO and VEGF contents in aqueous humor of NVG group were significantly higher than those of CAT group; EDV and PSA levels of central retinal artery as well as EDV and PSA levels of short posterior ciliary artery in NVG group were significantly lower than those in CAT group and negatively correlated with EPO and VEGF contents in aqueous humor; MDA, AOPP, Drp1, CytC, Apaf-1 and caspase-3 contents in aqueous humor of NVG group were significantly higher than those of CAT group and positively correlated with EPO and VEGF contents in aqueous humor while SOD, GSH-Px, CAT, Mfn1 and Mfn2 contents were significantly lower than those of CAT group and negatively correlated with EPO and VEGF contents in aqueous humor. **Conclusion:** The abnormal increase of EPO and VEGF contents in serum and aqueous humor of patients with glaucoma is closely related to the increase of ocular blood resistance, activation of oxidative stress reaction and mitochondrial dysfunction.

1. Introduction

Neovascular glaucoma (NVG) is the clinical common secondary glaucoma, and the iris surface and anterior chamber angle neovascularization is the main local pathological characteristic of NVG. The local angiogenesis of NVG is secondary to central retinal vein occlusion and diabetic retinopathy, and local hypoxic stimulation is an important factor causing the activation of angiogenesis. The constant generation of local new blood vessels will cause trabecular meshwork and adjacent iris adhesion as well as fibrous connective tissue formation and cause elevated intraocular pressure, and it is also accompanied by intraocular vascular hemodynamic changes[1,2]. Erythropoietin (EPO) and vascular endothelial growth factor (VEGF) are important angiogenesis regulation molecules induced by hypoxia factors, and they are closely related to the abnormal formation of NVG local new blood vessels[3]. In the following studies, we specifically analyzed the correlation of EPO and VEGF levels in serum and aqueous humor with ocular hemodynamics and oxidative stress - mitochondrial function in patients with neovascular glaucoma.

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2. Case information and research methods

2.1 General case information

A total of 47 patients who were diagnosed with neovascular glaucoma in Mianyang Wanjiang Ophthalmic Hospital between April 2015 and April 2017 were selected as the NVG group of the study, and the patients were with intraocular pressure was > 21 mmHg, with visible new blood vessels shown in slit lamp microscope examination and gonioscopy, and with central retinal vein occlusion, diabetic retinopathy and other medical history. 75 patients who were diagnosed with cataract in Mianyang Wanjiang Ophthalmic Hospital over the same period were selected as the CAT group of the study, and the patients were with hypopsia and lens opacity, and without eye trauma, glaucoma, fundus lesions and other medical history. NVG group included 22 men and 25 women that were 49-65 years old; CAT group included 36 men and 39 women that were 47-63 years old. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Serum and aqueous humor index detection

About 2 mL cubital venous peripheral blood was collected from NVG and CAT group 1 d before surgery, about 0.2 mL aqueous humor was collected during the operation, enzyme-linked immunosorbent assay kit was used to detect the content of EPO and VEGF in serum and aqueous humor, radioimmunoprecipitation kit was used to detect the contents of MDA, AOPP, SOD, GSH-Px and CAT in aqueous humor, and enzyme-linked immunosorbent assay kit was used to detect the contents of Mfn1, Mfn2, Drp1, CytC, Apaf-1 and caspase-3 in aqueous humor.

2.3 Ocular hemodynamic index detection

Model Affiniti50 color Doppler diasonograph from Philips Company was used to measure the end-diastolic velocity (EDV) and peak systolic velocity (PSA) of central retinal artery and short posterior ciliary artery.

2.4 Statistical methods

SPSS 22.0 software was used to input data, differences in data between two groups were by t test and P<0.05 indicated statistical significance in differences in test results.

3. Results

3.1 EPO and VEGF contents in serum and aqueous humor

Analysis of EPO (pg/mL) and VEGF (U/L) contents in serum and aqueous humor between two groups of patients was as follows: EPO and VEGF contents in serum as well as EPO and VEGF contents in aqueous humor of NVG group were significantly higher than those of CAT group. Differences in EPO and VEGF contents in serum and aqueous humor were statistically significant between NVG group and CAT group (P<0.05).

Table 1.

Comparison of EPO and VEGF contents in serum and aqueous humor were statistically significant between two groups of patients.

Groups	n	Serum		Aqueous humor	
		EPO	VEGF	EPO	VEGF
NVG group	47	31.56±5.62	468.22±54.73	214.95±3.49	1 048.65±14.85
CAT group	75	16.61±2.52	184.41±20.35	58.52±7.35	252.31±33.58
T		9.387	11.756	24.482	27.028
P		<0.05	<0.05	<0.05	<0.05

3.2 Ocular hemodynamic parameters

Analysis of hemodynamic parameters EDV and PSA of central retinal artery and short posterior ciliary artery between two groups of patients was as follows: EDV and PSA levels of central retinal artery as well as EDV and PSA levels of short posterior ciliary artery in NVG group were significantly lower than those in CAT group. Differences in EDV and PSA levels of central retinal artery as well as EDV and PSA levels of short posterior ciliary artery were statistically significant between NVG group and CAT group (P<0.05). Pearson correlation analysis showed that EPO and VEGF contents in aqueous humor of patients with NVG were negatively correlated with EDV and PSA levels of central retinal artery as well as EDV and PSA levels of short posterior ciliary artery.

Table 2.

Comparison of ocular hemodynamic parameters between two groups of patients (cm/s).

Groups	n	Central retinal artery		Short posterior ciliary artery	
		EDV	PSA	EDV	PSA
NVG group	47	2.56±0.36	8.03±0.98	2.78±0.36	9.21±1.03
CAT group	75	3.77±0.52	12.42±1.73	3.61±0.49	14.85±1.94
T		7.029	7.867	8.182	8.651
P		<0.05	<0.05	<0.05	<0.05

3.3 Oxidative stress index contents in aqueous humor

Analysis of oxidative stress indexes MDA (nmol/mL), AOPP (nmol/mL), SOD (U/mL), GSH-Px (U/mL) and CAT (U/mL)

contents in aqueous humor between two groups of patients was as follows: MDA and AOPP contents in aqueous humor of NVG group were significantly higher than those of CAT group while SOD, GSH-Px and CAT contents were significantly lower than those of CAT group. Differences in MDA, AOPP, SOD, GSH-Px and CAT contents in aqueous humor were statistically significant between NVG group and CAT group (P<0.05). Pearson correlation analysis showed that EPO and VEGF contents in aqueous humor of patients with NVG were positively correlated with MDA and AOPP contents in aqueous humor, and negatively correlated with SOD, GSH-Px and CAT contents in aqueous humor.

3.4 Mitochondrial function index contents in aqueous humor

Analysis of mitochondrial function indexes Mfn1, Mfn2, Drp1, CytC, Apaf-1 and caspase-3 contents in aqueous humor between two groups of patients was as follows: Mfn1 and Mfn2 contents in aqueous humor of NVG group were significantly lower than those of CAT group while Drp1, CytC, Apaf-1 and caspase-3 contents were significantly higher than those of CAT group. Differences in Mfn1, Mfn2, Drp1, CytC, Apaf-1 and caspase-3 contents in aqueous humor were statistically significant between NVG group and CAT group (P<0.05). Pearson correlation analysis showed that EPO and VEGF contents in aqueous humor of patients with NVG were negatively correlated with Mfn1 and Mfn2 contents in aqueous humor, and positively correlated with Drp1, CytC, Apaf-1 and caspase-3 contents in aqueous humor.

4. Discussion

Abnormal activation of angiogenesis process is an important pathological feature of neovascular glaucoma (NVG), and the local hypoxia caused by central retinal vein occlusion, diabetic retinopathy and other diseases is an important cause inducing abnormal activation of angiogenesis process. The abnormally generated new blood vessels in iris surface and the anterior chamber Angle will extend to the surrounding tissues and cause trabecular meshwork and iris adhesion, which can block the outflow pathway of aqueous humor and lead to the increase of intraocular pressure[4,5]. The angiogenesis in intraocular local tissue is dependent on the regulation of various molecules, and the VEGF and EPO are the currently known molecules that can clearly promote angiogenesis. Local hypoxia is the important cause to induce the expression and secretion of VEGF and EPO, the local hypoxia caused by central retinal vein occlusion, diabetic retinopathy and other diseases can promote the retina epithelial cells and Muller cells to produce a large number of VEGF and EPO[6,7]. In the study, analysis of the contents of the above two angiogenesis molecules in serum and aqueous humor of NVG patients showed that EPO and VEGF contents in serum as well as EPO and VEGF contents in aqueous humor of NVG group were significantly higher than those of CAT group. This indicates that the abnormal increase in the contents of EPO and VEGF is closely related to the occurrence of NVG, and promoting local angiogenesis is the specific way for EPO and VEGF to participate in NVG.

The VEGF effect on promoting angiogenesis depends on the receptor VEGFR on the surface of the endothelial cell membrane, and the combination of VEGF and VEGFR can on the one hand, promote the proliferation, migration and vascularization of endothelial cells, and on the other hand, increase the vascular permeability, promote vascular dilation and cause the basement membrane of the vascular wall to degrade, which is beneficial to the chemotaxis of endothelial cells and the formation of vascular structures[8,9]. EPO is an endogenous hormone that promotes red blood cell differentiation and maturation, and increases the oxygen carrying capacity of red blood cells, and hypoxia can enhance EPO gene transcription and extend the EPO mRNA half-lives to increase the production of EPO; in recent years, it has been found that EPO has the independent role of promoting angiogenesis in the eyes[10,11]. The increase of angiogenesis in the eyes can lead to trabecular meshwork and iris adhesion, affect the blood supply of normal blood vessels and increase vascular resistance. In the study, analysis of

Table 3.

Comparison of oxidative stress indexes in aqueous humor between two groups of patients.

Groups	n	MDA	AOPP	SOD	GSH-Px	CAT
NVG group	47	3.52±0.53	2.37±0.35	22.31±3.25	17.60±2.06	9.28±1.08
CAT group	75	1.77±0.21	1.14±0.14	58.64±7.28	42.37±5.62	23.52±3.26
T		10.286	11.639	13.569	12.592	15.216
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

Table 4.

Comparison of mitochondrial function indexes in aqueous humor between two groups of patients (ng/mL).

Groups	n	Mfn1	Mfn2	Drp1	CytC	Apaf-1	Caspase-3
NVG group	47	0.84±0.11	0.71±0.09	3.46±0.56	1.73±0.22	0.93±0.12	1.42±0.18
CAT group	75	2.25±0.37	1.87±0.26	2.03±0.35	0.57±0.08	0.45±0.07	0.71±0.10
T		13.495	12.561	7.947	20.394	11.216	10.082
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

the ocular hemodynamic parameters of patients with NVG showed that the EDV and PSA levels of central retinal artery and short posterior ciliary artery in NVG group were significantly lower than those in CAT group. This indicates that the increase of intraocular vascular resistance is an important feature of NVG. Further analysis of the correlation between angiogenesis and intraocular vascular hemodynamics showed that EPO and VEGF contents in aqueous humor of patients with NVG were negatively correlated with EDV and PSA levels of central retinal artery as well as EDV and PSA levels of short posterior ciliary artery. This indicates that the increase of angiogenesis caused by the increase of intraocular EPO and VEGF in patients with NVG can lead to increased intraocular vascular resistance.

Hypoxia is an important pathological factor to stimulate the EPO and VEGF secretion and increase the angiogenesis, and in the pathological process of NVG, hypoxia can not only promote angiogenesis, but can also increase the production of free radicals and cause oxidative stress injury to retinal ganglion cells so as to participate in optic atrophy and hypopsia evolution[12,13]. When the formation of free radicals increases in the intraocular local tissue, the lipid, protein and other compositions in cell structure will have oxidizing reaction with free radicals, which damages the cell structure and also generate a large number of oxidative stress products MDA and AOPP[14]. SOD, GSH-Px and CAT are important antioxidant enzymes in intraocular local tissue that can remove free radicals such as reactive oxygen and hydrogen peroxide via catalytic deoxidizing reaction and reduce the damage of oxidative stress to cells; in hypoxia state, the excessive generation of free radicals can cause massive consumption of antioxidant enzymes[15,16]. MDA and AOPP contents in aqueous humor of NVG group were significantly higher than those of CAT group while SOD, GSH-Px and CAT contents were significantly lower than those of CAT group. This indicates that the activation of local oxidative stress response and the significant consumption of antioxidant enzymes are closely related to the occurrence of NVG. Further analysis of the correlation between oxidative stress response and angiogenesis showed that the increase of angiogenesis caused by the increase of intravascular EPO and VEGF in patients with NVG is closely related to the activation of oxidative stress response.

The activation of oxidative stress is closely related to the injury of mitochondrial function. On the one hand, mitochondrial function injury can block the biological process of oxidative respiration and increase the formation of free radicals, and on the other hand, the mitochondria is an important target of free radicals, and the excessively generated free radicals can aggravate the mitochondria damage[17,18]. Mfn1 and Mfn2 are important mitofusin that can mediate mitochondrial fusion process to ensure the normal function of mitochondria; Drp1 is a protein that participates in the process of mitochondrial division, which gathers in mitochondrial outer membrane and hydrolyzes the GTP to make the mitochondria break and cause the mitochondria damage[19]. After the mitochondrial function is damaged, a large amount of CytC enters the cytoplasm, causes the cascade activation of caspase-3 through the mediation of Apaf-1, and finally results in apoptosis[20]. In the study, analysis of the contents of the mitochondrial function indexes in aqueous humor showed that Mfn1 and Mfn2 contents in aqueous humor of NVG group were significantly lower than those of CAT group while Drp1, CytC, Apaf-1 and caspase-3 contents were significantly higher than those of CAT group. This indicates that the injury of mitochondrial function and the activation of mitochondrial apoptosis are closely related to the occurrence of NVG. Further analysis of the correlation between oxidative stress and mitochondrial function showed that EPO and VEGF contents in aqueous humor of patients with NVG were negatively correlated with Mfn1 and Mfn2 contents in aqueous humor, and positively correlated with Drp1, CytC, Apaf-1 and caspase-3 contents in aqueous humor. This indicates that the increased angiogenesis caused by the increased intraocular EPO and VEGF is closely related to the injury of mitochondrial function.

The contents of EPO and VEGF increase remarkably in the serum and aqueous humor of patients with NVG; the abnormally elevated EPO and VEGF can mediate angiogenesis, and cause the increased intraocular blood flow resistance, the activated oxidative stress and the mitochondrial dysfunction.

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