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ERETHROPOIETIN AND SOLUTION CD44 LEVELS IN PATIENTS WITH PRIMARY OPEN-ANGLE GLAUCOMA

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Abstract:

Purpose*: To evaluate the levels of erethropoietin (EPO) and soluble CD44 (sCD44) in the aqueous humor and plasma of patients with primary open-angle glaucoma and the relation to visual fields loss.* ***Patients and methods****: Thirty patients with primary open-angle glaucoma (POAG) and twenty five patients with senile cataracts (control group) of matched age and gender were included in the study. Aqueous humor samples were obtained by paracentesis from glaucoma and cataract patients who were undergoing elective surgery. Aqueous humor and corresponding plasma samples were analysed for EPO and sCD44 concentrations by enzyme linked immunosorbent assay.* ***Results:*** *EPO and sCD44 levels revealed a high significant rise in aqueous humor of POAG patients and non-significant rise in plasma of the same patients, in comparison with cataract patients. A high positive correlation between EPO and sCS44 in aqueous humor of POAG patients. In addition, sCD44 concentration was greater in patients with visual field loss.* ***Conclusion:*** *Increased levels of aqueous humor EPO and sCD44 may play an important role in the pathogenesis of POAG. Positive correlation between sCD44 in aqueous of POAG and severity of visual field loss concluding that sCD44 concentration in aqueous humor is a possible protien biomarker foe visual field deterioration in POAG patients.*

Key words: Glaucoma, Erythropoietin, soluble CD44.

INTRODUCTION: Primary open-angle glaucoma is a common neurodegenerative ocular disease in which selective cell death of retinal ganglion cells results in a characteristic clinical pattern of visual field loss and excavated appearance of the optic nerve head. To date, at least 20 genetic foci for POAG have been reported although the underlying patho-physiology of POAG candidate genes remains to be elucidated [1].

In recent decades, there has been increasing acceptance of the premise that glaucoma in primarily a blinding optic neuropathy as opposed to a disease of elevated intraocular pressure [2]. As a result, current glaucoma research has shifted much of its attention from ocular hypotensive agents to the discovery of therapeutic modalities that can preserve optic nerve function independent of intrauocular pressure control.

Erythropoietin (EPO) is an oxygen-regulated hormone produced in the fetal liver and the adult kidney and released in the blood-stream. The systemic function of EPO is to stimulate erythrocyte formation in the bone marrow in response to hypoxia [3]. EPO is a 165 amino acid sialoglycoprotein that has demonstrated a remarkable tissue-protective ability in numerous animal and cell culture studies of ischemia and neuronal degeneration as well as stroke patients [4].

Erythropoitein is the first target gene for hypoxia inducible factor-1 (HIF-1) to be identified and still one of the best-characterised genes activated by reduced oxygen levels. HIF-1 has been shown to have, either clinically or experimentally, a mediating or contributing role in several oxygen-dependent retinal diseases such as glaucoma [5]. Meanwhile, EPO inhibits apoptosis, reduces glutamate and/or reactive oxygen species, regulates the Bcl XL Bcl 2 genes, reduces proinflammatory cytokines, and maintains vascular autoregulation [4].

Soluble CD44 (sCS44), the ectoderm fragment of transmembrane protein receptor CD44, is a cytotoxic protein to trabecular meshwork and retinal ganglion cells in vitro [6]. sCD44 toxicity was prevented by an pancaspase inhibitor, indicating that sCD44 is a proapototic factor. sCS44 toxicity was also prevented by coadministration of hyaluronic acid, indicating that sCD44 binding to hyaluronic acid blocks sCD44 activity. However, hypo-phosphorylated sCD44, which is present only in POAG and normal pressure glaucoma, was significantly more toxic to trabecular meshwork and retinal ganglion cells than the standard sCD44 [7].

The purpose of our study was to determine the aqueous humor and serum levels of EPO and sCD44, a neuroprotective agents, in patients with primary open-angle glaucoma and to asses their relation to visual field loss.

MATERIALS AND METHODS. This is a prospective, comparative randomized study.

Thirty patients with primary open-angle glaucoma and twenty five patients with senile cataract of age matched controls are included in this prospective clinical study. For statistical analysis, one eye of each participant was randomly chosen. Patients were recruited inpatient ward from Mansoura University Ophthalmic Center.

***Exclusion Criteria*. Exclusion criteria include patients with any type of glaucoma except POAG such as pseudoexfoliative glaucoma, angle-closure glaucoma, pigmentary glaucoma, neovascular glaucoma, and secondary glaucoma. Patients with**

previous laser surgery or history of intraocular surgery were excluded. Diseases that could influence the EPO and sCD44 levels such as diabetes mellitus, kidney diseases, hematologic diseases (polycythemia vera, anemia), arterioscherotic diseases (myocardial infarction, coronary artery disease), systemic disease (autoimmune disease, endocrinopathy), and history of drug usage (chemotherapeutic agents, iron preparations) were excluded.

All patients were subjected to ophthalmic examination included slit-lamp biomicroscopy, Goldmann applanation tonometry, gonioscopy using Goldmann three mirror contact lens, and Humphrey visual field analyzer (stages of visual field loss are mild, arcuate defect; moderate, abnormal in one hemifield and not within 5 degrees of fixation; and, severe, abnormal in both hemifields or within 5 degrees of fixation). In addition, clinical data on age, gender, history of intraocular surgery, diabetes mellitus, and anti-glaucoma medical regimen were collected.

***The selected patients were classified into*: *Group I:* This group included 30 patients with primary open-angle glaucoma (12 males and 18 females) with their ages ranged from 50 to 65 years.**

***Group II:* This group included 25 patients with senile cataract (11 males and 14 females) with their ages ranged from 52 to 65 years (control group).**

***Sample collection:* Collection of aqueous humor was performed during cataract or glaucoma surgery. Aqueous humor 0.1-0.2 ml was collected at the beginning of surgery through a paracentesis using a 27 gauge neddle on a tuberculin microsyringe. Blood contamination was meticulously avoided. Aqueous humor was immedietly cooled at −80°C. All sample were protected from light. Venous blood sample (two millimeters) were also collected (without anticoagulant) from patients before surgery. The sample were centrifuged within 1 hour and stored at −80°C untis the time of assay.**

***Determination of Erythropoietin*. The concentration of EPO in aqueous humor and serum samples was measured by sandwich «double antibody» enzyme-linked immunosorbent assay (ELISA) with the Biomerica ELISA kit. The EPO concentrations were determined by measuring the optical density at 450 nm with TRITURUS.**

***Determination of soluble CD44*. The concentration of sCD44 in aqueous humor and serum samples was measured using the standard commercially avaliables CD44 soluble ELLISA kit as measured by Asplund and Heldin[8].**

***Statistical analysis*. Was done using SPSS program version 10. Student t-test was for comparing means of quantitative data. Persons' correlation (r) was used for correlation coefficient. P value of ≤ 0.05 was considered statistically significant.**

The study was approved by the local ethics committee and all patients signed informed consent before entering the study. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

RESULTS. The study included 30 eyes of POAG patients and 25 eyes with senile cataract but without glaucoma. The mean age of the POAG group was 60.61 ± 5.72, whereas the mean age of the control group was 61.27 ± 6.21 and there was no statistically significant difference between the groups (p = 0.481). There was no statistically significant difference between the POAG group and the control group regarding gender and IOP [p = 0.931, p = 0.561, respectively] (Table 1).

Table 1

Clinical data of the studied patients

Parameter / Group	POAG	Senile cataract	*p. value
No.	30	25	
Age (year)	60.61 ± 5.72	61.27 ± 6.21	0.481
Gender (M/F)	12/18	11/14	0.931
IOP (mmhg)	20.6 ± 5.1	19.5 ± 6.1	0.561

No. = Number, mmhg = milimeter mercury, M/F = Male/Female.
*p = ≤ 0.05 statistically significant.

Aqueous humor concentration of EPO was highly significant increased in the POAG group when compared with of the control group — (11.81 ± 4.21) mU/ml Vs (6.56 ± 1.56) mU/ml; p = 0.007 (Table 2).

Table 2

EPO and sCD44 in aqueous humor of the studied patients

Parameter / Group	POAG	Senile cataract	*p. value
Aqueous humor EOP X±SD (mU/ml)	11.81 ± 4.21	6.56 ± 1.56	0.007
Aqueous humor sCD44 X±SD (ng/ml)	12.56 ± 0.51	6.85 ± 0.37	0.008

*p = ≤ 0.05 statistically significant.

Also, aqueous humor concentration of sCD44 was highly significant increased in the POAG group when compared with that of the control group — (12.56 ± 0.51) ng/ml Vs (6.85 ± 0.37) ng/ml, p = 0.008 (Table 2). EPO concentration in plasma of the POAG group was non-significantly higher when compared with that of the control group (24.37 ± 9.36) mU/ml Vs (23.28 ± 7.86) mU/ml; p = 0.376 (Table 3.). Also, sCD44 concentration in plasma of the POAG group was non-significantly increased when compared with that of the control group — (18.27 ± 0.25) ng/ml Vs (16.86 ± 0.36) ng/ml; p = 0.253 (Table 3).

Table 3

EPO and sCS44 in plasma of the studied patients

Parameter / Group	POAG	Senile cataract	*p. value
Plasma EOP X±SD (mU/ml)	24.37 ± 9.36	23.28 ± 7.86	0.376
Plasma sCD44 X±SD (ng/ml)	18.27 ± 0.25	16.86 ± 0.36	0.253

*p = ≤ 0.05 statistically significant.

Aqueous humor concentration of EPO in the POAG patients was singificantly correlated with those of sCD44 [r = 0.308, p = < 0.02] (Table 4).

No significant correlation between aqueous and plasma levels of EPO and sCD44 (Table 4).

Aqueous humor concentration of EPO and sCS44 and visual field loss (mild, moderate, severe) in POAG patients, revealed that sCD44 was greater in patients with visual field loss (Table 5).

Table 4

Kendall's correlation between EPO and sCD44 in aqueous humor and plasma of the POAG and senile cataract patients

Correlation between studied variable	r	p	Level of significant
EPO (aqueous) versus EPO (plasma)	0.261	0.179	Non significant
sCD44 (aqueous) versus sCD44 (plasma)	0.175	0.207	Non significant
EPO (aqueous) versus sCD44 (aqueous)	0.308	0.02	High significant
EPO (plasma) versus sCD44 (plasma)	0.351	0.081	Non significant

Table 5

Aqueous humor EPO and sCD44 concentration and visual field loss in POAG patients

Parameter/Group	Normal V. F. (n = 15)	Mild V. F. loss (n = 19)	Moderate V. F. loss (n = 8)	Severe V. F. loss (n = 3)
Aqueous EPO X±SD (mU/ml)	6.79 ± 1.23	7.19 ± 2.51	8.41 ± 2.71	8.81 ± 1.75
Aqueous sCD44 X±SD (ng/ml)	5.12 ± 1.35	8.21 ± 3.52	11.51 ± 3.51	14.61 ± 2.51

V.F = Visual fiels.

DISCUSSION: Because intraocular pressure (IOP) alone can not accurately predict POAG, it is critical to identify associated risk factors for the development and/or progression of POAG. Approximately, one third of all patients have what are considered to be normal IOPs, and it is now well — established that the visual field deficit associated with all forms of glaucoma are due to retinal ganglion cell apoptosis [9].

Thus, there is much hope that neuroprotective agents can only day become an important adjunct or even primary therapy in the management of glaucoma.

Erythropoietin, characterised be haematopoietic, angiogenicand neuroprotective properties. Its hematopoietic function dependent on its interaction with the homodimeric receptor on erythrocyte progenitor cells within the bone-marrow. While, its neuroprotective function dependent on its ability to traverse the intact blood-brain and blood-retinal barrier in therapeutic amounts [10].

Our study revealed a statistically significant increase in the aqueous humor EPO levels in POAG patients compared with control patients but there was no significant increase in the serum levels. This findings are consistent with Cumurcu et al. [11] and Auricchio et al. [12].

The cause of the elevated aqueous humor EPO concentration in eyes with POAG may be related to the ischemia, hypoxia, or elevated reactive oxygen species caused by glaucomatous damage [5, 13]. Also, EPO may increase with a compensatory mechanism owing to the increase in glutamate, nitric oxide, and the free radicals after the glaucomatous damage [14]. Some experimental studies showed that exogenous EPO protected retinal ganglion cells in glaucomatous eyes through an antiapoptosis mechanism [4]. Also, a subsequent study to evaluate the possibility of dose-dependent EPO toxicity showed that intravitreal injection of EPO (at dose up to 625 ng) did not impair retinal function in rats as assessed by electroretinography [15].

Brines et al. [16], suggested that EPO is a multifunctional, pro-angiogenic, and pro-survival growth factor that does much more than stimulated erythrogenesis. Oxygen regulates EPO production not only in the kidney but also in the retina. Local retinal production of EPO was found to be as critical as vascular endothelial growth factor (VEGF). Watanabe et al. [17], reported that both EPO and VFGF found in high concentration in the vitreous in diabetic retinopathy. This stated that EPO has angiogenic and neuroprotective properties like VEGF. Also, EPO like VEGF, is responsible for the regulation and activation of hypoxia-inducible factor 1 which is one of the best-characterised gene activated by oxygen-dependent retinal diseases such as glaucoma [18].

Our study revealed a statistically significant increase in the aqueous humor sCD44 levels in POAG patients compared with control patients but there was no significant increase in the serum level. This finding is consistent with Nolan et al. [19]. sCD44 functions as apart of the regulation of immuno-mediated inflammation and is released from the cell surface by metalloproteases in response to metabolic stress, and hypoxia [20]. sCD44 is most likely shed from the ciliary epithelium into aqueous humor because the ciliary body has the highest concentration of CD44. The increase in sCD44 concentration in POAG aqueous is secondary to the underlying heterogenous pathophysiology, that is, genetic anomaly, aberrant protein, or metabolic stress, which impact the severity and clinical course of the POAG disease process [19].

The bioavailability of sCD44 depends on its binding to hyaluronic acid, and the results of the binding of sCD44 to hyaluronic acid are influenced by pressure. The apparent change in the hyaluronic acid polymer with increased pressure and the decreased binding of sCD44 to hyaluronic acid with increased pressure may be a reason why increased IOP is a clinical risk factor in POAG [7].

Miller et al. [21], reported that metabolic stress using lactate administration to trabecular meshwork cells causes activation and the release of sCD44 in cultured trabecular meshwork cells. Moreover, VEGF secretion, COX-2 activation, and prostacyclin formation in endothelial cells, occurs by CD44 binding to hyaluronic acid [22].

CD44 was identified as a primary phagocytic receptor that mediates the internalization of large particles. Because sCD44 binding to CD44 receptor inactivates the CD44 receptor, a consequence of increased sCD44 concentration is the accumulation of extracelluar matri-

al which may increase the outflow resistance, and thus, increase IOP [19].

Knepper et al. [23], reported that soluble CD44 isoforms may influence the activity of the transmembrane CD44H by acting as inhibitor of CD44H and, thereby, adversely influence the cell survival of trabecular meshwork and retinal ganglion cells in primary open-angle glaucoma. In addition, sCD44 has been shown to impair tumor metastasis by inhibiting the function of CD44H as a cell-adhesion molecule and induce apoptosis by binding to membrane-bound CD44H. Thus, elevated levels of sCD44 will be accompanied by programmed cell death.

In the present study, there was no correlation between the aqueous humor and plasma levels of EPO and sCD44. These results suggested that EPO and sCD44 levels in aqueous humor were not related to breakdown of blood-retinal barrier and/or ocular blood.

In the current study, the EPO level in aqueous humor was significantly correlated with a aqueous humor of sCD44. This correlation supported that both EPO and sCD44 may be important in pathogenesis of glaucoma. However, there was no significant correlation between plasma levels of EPO and sCD44.

A positive correlation between sCD44 concentration and extent of visual field loss was observed in POAG patients. This is consistent with Nolan et al. [19]. Thus sCD44 in the first aqueous humor protein reported as a possible biomarker for the extent of visual field loss in POAG.

In contrast, no positive correlation between EPO concentration and extent of visual field loss was observed in POAG.

CONCLUSION

Increased levels of aqueous humor EPO and sCD44 may play an important role in the pathogenesis of POAG. Positive correlation between sCD44 in aqueous of POAG and severity of visual field loss concluding that sCD44 concentration in aqueous humor is a possible protein biomarker for visual field deterioration in POAG patients.

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УРОВНИ ЭРИТРОПОЭТИНА И РАСТВОРИМОГО СД44 У ПАЦИЕНТОВ С ПЕРВИЧНОЙ ОТКРЫТОУГОЛЬНОЙ ГЛАУКОМОЙ

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Изучались уровни эритропоэтина (ЭПО) и растворимого СД44 (рСД44) во влаге передней камеры и плазме крови у больных первичной открытоугольной глаукомой (ПОУГ) в соответствии с нарушениями поля зрения.

В исследование включено 30 больных ПОУГ и 25 пациентов со старческой катарактой соответствующего возраста и пола (контрольная группа). Отбор камерной влаги проводили в момент парацентеза у глаукомных больных и у больных катарактой при антиглаукоматозной операции и при экстракции катаракты.

Анализ концентрации ЭПО и рСД44 в образцах влаги передней камеры и сыворотки крови проводили методом энзимосвязанных иммуносорбентов. Исследование показало, что уровни ЭПО и рСД44 были значительно повышены в камерной влаге больных ПОУГ и незначительно — в плазме крови по сравнению с таковыми у больных катарактой. Отмечена высокая положительная корреляция между ЭПО и рСД44 в камерной влаге у пациентов с ПОУГ. Кроме того, концентрация рС44 оказалась повышенной у пациентов с нарушением поля зрения, что может свидетельствовать о зависимости степени выпадения поля зрения от содержания в камерной влаге рСД44 как возможного белкового маркера нарушений поля зрения у больных ПОУГ.

Сделан вывод о значительной роли изученных показателей в патогенезе первичной открытоугольной глаукомы.