

## Role of Matrix Metalloproteinase-2 and its Inhibitor and Erythropoitin in the Pathogenesis of Pseudoexfoliative Glaucoma

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**Abstract:** To determine the levels of matrix metalloproteinase-2 (MMP2) and its endogenous inhibitor, tissue inhibitor metalloproteinase-2 (TIMP2) and erythropoitin (EPO) in the aqueous humour and serum of patients with pseudo- exfoliative glaucoma (PEXG) in relation to samples derived from cataract control patients. Aqueous humour and serum samples were collected from patients with PEXG and cataract (25 glaucoma samples and 15 cataract samples). Glaucoma and cataract subjects underwent routine glaucoma trabeculectomy and cataract extraction surgeries respectively. The levels of MMP-2, TIMP2 and erythropoitin were determined by enzyme linked immunosorbent assay (ELISA). Whereas serum samples showed no significant differences, total MMP2 was detected in significantly higher concentration in aqueous samples from PEXG patients compared to cataract patients. The ratio of MMP2 to its principle inhibitor TIMP2 was balanced in cataract patients but was increased in samples from PEXG patients resulting in excess of MMP2 over TIMP2. The mean aqueous humor EPO concentration in eyes with PEXG was significantly higher than that from eyes with cataract. Also there was significant increase in the serum EPO concentrations of PEXG patients when compared to the control group. There was no correlation between the EPO aqueous humor concentration and the EPO serum concentration in both PEXG and cataract patients. Also a positive correlation was detected between total MMP2 and EPO in aqueous of PEXG patients. These findings suggest that complex changes in the local MMP-TIMP balance in aqueous humor may promote the abnormal matrix accumulation characteristic of PEX syndrome and may be involved in the pathogenesis of PEX glaucoma. Also our results indicate that EPO may play a role in the pathogenesis of PEXG.

**Key words:**

### INTRODUCTION

Pseudoexfoliation (PEX) syndrome is a clinical systemic disorder of the extracellular matrix, (Ritch, R., U. Schlötzer - Schrehardt, 2001). Which represents not only the most common identifiable cause of open-angle glaucoma but also a risk factor for cardiovascular disease (Naumann, G.O.H. *et al.* 1998). Increasing evidence suggests that PEX syndrome is a type of fibrosis associated with the excessive synthesis and deposition of an abnormal elastic fibrillar material in many intra- and extraocular tissues (Ritch, R., 1996). Active involvement of the trabecular meshwork in this abnormal matrix process leading to progressive accumulation of PEX material in the juxtacanalicular tissue is considered to be the primary cause of chronic pressure elevation in eyes with PEX syndrome (Mitchell, P. *et al.* 1997). Excessive production and accumulation of abnormal matrix components may be due to increased de novo synthesis, decreased turnover of matrix components, or both (Schumacher, S. *et al.* 2001).

The principal ocular cells implicated in PEX material production are those closely associated with the aqueous humor circulation (i.e., nonpigmented ciliary epithelium, iris pigment epithelium, iridal vascular cells, equatorial lens epithelial cells, and trabecular endothelial cells) and are thus influenced by the substances contained therein. The composition of the aqueous humor may therefore play an important role in influencing the matrix metabolism of adjacent tissues (Schlötzer-Schrehardt, U. *et al.* 1999).

Matrix metalloproteinases (MMPs) are a group of genetically distinct but structurally related endoproteinases capable of degrading almost all essential extracellular matrix (ECM) and basement membrane components (Brinckerhoff, C.E., L.M. Matrisian, 2002). Common to MMPs is that they contain zinc atoms in their active site and are irreversibly inhibited by their specific endogenous inhibitors, tissue inhibitors of metalloproteinases

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(TIMPs) usually in 1:1 molar ratio (Fassina, G., *et al.*, 2000; Alexander, J.P., *et al.*, 1991). Currently well over 20 MMP family members have been characterized, and in the body MMPs and TIMPs are widely distributed exerting strictly controlled expression pattern in both health and disease (Huang, S.H., *et al.*, 1996). MMPs have been shown to be involved in the normal TM tissue turnover (Bill O., A. Maepea, 1994). Therefore, MMPs and TIMPs are likely candidates to be involved in the abnormal extracellular matrix metabolism characteristic of PEX syndrome/glaucoma (PEXG) (Ueda, J., 2002).

Elevated intraocular pressure (IOP) causes death of retinal ganglion cells (RGCs) in patients with chronic glaucoma (Agar, A., *et al.*, 2000). Hypoperfusion-induced ischemia at the optic nerve head owing to the dysregulation of the ocular microcirculation is another pathogenic mechanism that contributes to the visual deficit in glaucomatous optic neuropathy (Cioffi, G.A., 2005).

Erythropoietin (EPO) is the first target gene for hypoxia-inducible factor 1 (HIF-1) to be identified and still one of the best-characterized genes activated by reduced oxygen levels (Tezel, G., M.B. Wax, 2004). 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 (Wenger, R.H., 2002). EPO has been shown to protect primary cultured neurons from N-methyl-D-aspartate receptor-mediated glutamate toxicity and against ischemia-induced neuronal death (Arjamaa, O., M. Nikinmaa, 2006). In addition, EPO has been shown to directly inhibit the release of glutamate from neurons (Kawakami, M., *et al.*, 2001).

The aim of our work was to study extracellular matrix (ECM) metabolism by matrix metalloproteinases (MMP2) and their tissue inhibitor (TIMP2) and EPO, a neuroprotective agent in aqueous humor (AH) and serum samples collected from pseudoexfoliation glaucoma (PEXG) in relation to samples derived from cataract control patients.

## MATERIALS AND METHODS

Table 1 shows Characteristics of PXFG patients and controls. This study involved 25 patients with pseudo- exfoliative glaucoma (PEXG) attending glaucoma clinic of the Research Institute of Ophthalmology, and 15 patients with senile cataract serving as control subjects. Each subject underwent a detailed ophthalmologic examination included: Best corrected visual acuity, slit lamp biomicroscopy and gonioscopy to assess the anterior chamber angle, intraocular pressure (IOP) measurement using Goldman applanation tonometry, visual field (VF) examination using the Humphrey Field Analyzer, fundoscopic examination using a 90 diaptor lens with determination of the C/D ratio, OD rim, lamina cribrosa, blood vessels, pallor, hemorrhages and retinal nerve fiber defect.

**Table 1:** Characteristics of PXFG patients and controls, included in this study.

	Controls	PXFG
Number (n)	15	25
Sex (M/F)	7/8	15/10
Mean age	56- 65	57- 60
Duration of disease	-	12.6 ± 8.4

A detailed medical history was obtained to identify patients with risk factors for vascular disease such as, hypertension, diabetes mellitus, hyperlipidemia, smoking statues, and also the presence of cardiovascular and cerbero vascular disease.

### **Exclusion Criteria Included:**

Patients with diabetes mellitus, major systemic illness (myocardial infarction), evidence of renal or hepatic disease and gastro-intestinal diseases. Patients with ocular disease other than glaucoma and senile cataract were also excluded.

Aqueous humor samples were carefully collected at the beginning of surgery through a paracentesis, using a 27- gauge needle on a tuberculin syringe under an operating microscope with special care to avoid blood contamination.

Fasting blood samples were obtained from patients before the operation on EDTA and were immediately centrifuged and serum was separated and stored at -80 until assayed.

Levels of MMP2 and TIMP2 were assessed in aqueous humor and serum with commercially available sandwich enzyme immunoassay kits (Dong, Z., 2001; Davidson, B., 2001).

Levels of EPO were measured using a sandwich enzyme-linked immunosorbent assay kit.

**Statistical Analysis:**

SPSS software (version 10) was used for data management and analysis. Quantitative data was expressed as mean  $\pm$  SD. As most of the data were normally distributed continuous variables, Student's-t test was used to assess whether a statistical significance is present between the studied groups. The degree of association between the variables was assessed using Pearson's correlation coefficient (r), where values of  $p < 0.05$  were considered significant.

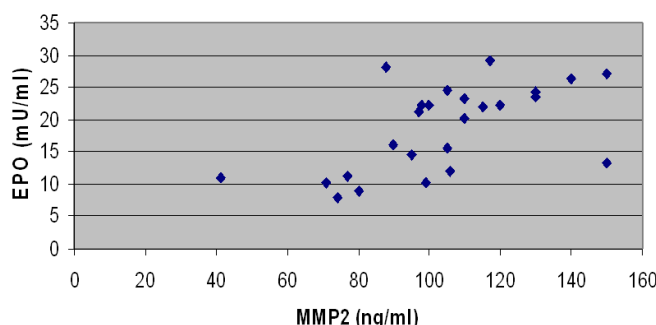
**RESULTS AND DISCUSSION**

**Results:**

The results of this study were illustrated in Table 2 and Fig. (1). Total MMP-2 was detected in significantly higher concentration in aqueous humor samples from PEXG eyes compared to cataractous eyes ( $p < 0.001$ ). Also TIMP-2 was detected in significantly higher concentration in aqueous humor samples from PEXG eyes compared to cataractous eyes ( $p < 0.05$ ). While Serum samples showed no significant difference in MMP2 and TIMP2 levels between PEXG patients and controls.

**Table 2:** Mean  $\pm$  SD of laboratory findings in controls and PEXG groups.

	Controls	PEXG	p value
Aqueous MMP2(ng/ml)	33.8 $\pm$ 9.1	103 $\pm$ 26.3	$p < 0.001$
Aqueous TIMP2(ng/ml)	31.9 $\pm$ 15.6	60.9 $\pm$ 19.5	$p < 0.05$
Aqueous EPO (mU/ml)	9.4 $\pm$ 2.4	18.7 $\pm$ 6.7	$p < 0.01$
Serum MMP2 (ng/ml)	401 $\pm$ 88.9	431 $\pm$ 136	NS
Serum TIMP2 (ng/ml)	123 $\pm$ 20.6	128 $\pm$ 29.8	NS
Serum EPO (mU/ml)	24.9 $\pm$ 3.1	32.8 $\pm$ 9.9	$p < 0.05$



**Fig. 1:** Correlation between total aqueous humor MMP2 and EPO in PEXG patients. ( $r=0.61$   $p<0.01$ ).

The ratio of MMP2 to its principle inhibitor TIMP2 was balanced in cataract samples (1:1) but was increased in samples from PEXG patients (1.72) resulting in excess of MMP2 over TIMP2. The EPO concentration in PEXG patients was significantly higher in aqueous and serum of PEXG patients in comparison to cataract patients. ( $p < 0.01$  and  $p < 0.05$ ) respectively.

There was a positive correlation between total MMP2 and EPO concentrations in aqueous of PEXG patients ( $r=0.61$   $p<0.01$ ). (Fig. 1)

**Discussion:**

Exfoliation syndrome is characterized by accumulation of abnormal fibrillar ECM material mainly to a lens, cornea, and TM often leading to severe secondary glaucoma (Ritch, R., U. Schlotzger-Schrehardt, 2001). Extensive pathological changes occur in the trabecular meshwork (TM) and juxtacanalicular tissue of the chamber angle (Naumann, G.O.H., *et al* 1998). Aqueous humor drainage is disturbed due to the accumulation of extracellular matrix (ECM) material in the outflow system. Matrix metalloproteinases (MMPs) remodel ECM material and, thus, they may have a role in regulating outflow facility and intraocular pressure (El-Shabrawi, Y.G., *et al.* 2000).

The present study demonstrated high levels of MMP2 and TIMP2 in aqueous humor of patients with PEXG compared to cataract patients. In contrast, serum levels of MMP2 and TIMP2 did not show any significant differences between the two groups. In PEXG, accumulation of abnormal extracellular material in the trabecular meshwork, either in the form of plaque material or PEX material may be responsible for increased outflow resistance and chronic elevation of pressure. The increased aqueous levels of endogenous MMP-2 activity may

contribute to the abnormal matrix accumulation found in the juxtacanalicular meshwork of PEXG eyes (Bradley, J.M.B., *et al.*, 2001).

In eyes with PEX syndrome and PEXG, the excess de novo production of various extracellular matrix components results in a progressive accumulation of an abnormal fibrillar material in most anterior segment tissues (Bradley, J.M.B., *et al.*, 2001), including the juxtacanalicular region of the trabecular meshwork. The findings of this study suggest that both the increased levels of activated MMP-2 and TIMP-2 in aqueous humor from PEXG eyes are causally related to inappropriate matrix degradation and progressive matrix accumulation. A decreased degradability of PEX material from increased cross-linking processes preventing access of proteases or MMP activators may further play a role (Kim C.Y., *et al.*, 2000). Our results agreed with previous results of Schrehardt *et al* (2003) and Maatta *et al* (2005). Increased deposition of matrix components may provide a signal for increased MMP synthesis. Therefore, the significantly increased levels of total MMP-2 in PEX samples cannot be causally related to pathogenesis, and up regulation of total MMP2 is more likely to be a consequence of matrix remodeling and accumulation than a cause (Alexander, J.P., 1991).

Normal tissue homeostasis requires a balanced interaction of MMP-2 and TIMP-2, and the ratio of enzyme to inhibitor is normally 1:1 (Schrehardt *et al* 2003). Any disturbance in the balance may result in excessive or insufficient matrix degradation and matrix accumulation. An excess of proteases over inhibitors or an excessive MMP activity is associated with abnormal matrix degradation, as seen in inflammatory diseases (Schrehardt *et al* 2003). Our results reveal an imbalance between MMP2 and their endogenous tissue inhibitor in aqueous samples from patients with PEXG. The ratio of MMP2/TIMP2 was significantly higher in samples from PEXG compared to those of cataract patients.

We also found a statistically significant higher serum and anterior chamber EPO concentration in PEXG patients compared to controls. The cause of the elevated aqueous EPO concentration in eyes with POAG may be related to the hypoxia, ischemia, or elevated reactive oxygen species caused by glaucomatous damage (Wenger, R.H., 2002). The EPO may also increase with a compensatory mechanism owing to the increase in glutamate, nitric oxide, and free radicals after the glaucomatous damage (Arjamaa, O., M. Nikinmaa, 2006). Dirnagl *et al* (2003) have defined this mechanism as "ischemic tolerance" and stated that practically any stimulus capable of causing injury to a tissue or organ can, when applied near to (but below) the threshold of damage, activate endogenous protective mechanisms. Some studies mention blood-aqueous barrier breakdown especially in pseudoexfoliative and uveitic glaucoma (Cumurcu, T., *et al.* 2007; Nguyen, N.X., *et al.* 2005). Finally, it was believed that EPO, like VEGF, is responsible for the regulation and activation of HIF-1 (Dirnagl *et al.* 2003).

Our results demonstrated a positive correlation between total MMP2 and EPO in aqueous humor of PEXG patients. MMPs can process various chemokines, cytokines, growth factors, serpins, and cell surface receptors thus exerting abilities to modify the course of inflammatory and immunological reactions (El-Shabrawi, Y.G., *et al* 200). It must be kept in mind that EPO levels may increase in glaucoma owing to the disturbance in the aqueous humor outflow (Nguyen, N.X., *et al.* 2005).

### **Conclusion:**

The findings of this study suggest that complex changes in the local MMP2-TIMP2 balance and increase EPO concentrations in the aqueous humor may promote the abnormal matrix accumulation characteristic of PEX syndrome and may be causally involved in the pathogenesis of PEXG. As the importance of MMP-TIMP involvement, in particular in PEX syndrome and PEXG, becomes increasingly apparent, these enzymes and inhibitors may become targets for pharmacotherapeutic intervention.

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