Regulatory cytokines prescribe the outcome of the inflammation in the process of pseudoexfoliation production

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Abstract

Background: The purpose of this study is to reveal the participation of different regulatory cytokines within the process of pseudoexfoliation (PEX).

Methods: Our study included 140 patients referred to cataract surgery with early and late stage of pseudoexfoliation syndrome (XFS) or pseudoexfoliation glaucoma (XFG). Humor and serum levels of cytokines: transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), IL-8 and interferon-inducible T cell alpha chemoattractant (ITAC) were measured in a sample using high sensitivity enzyme-linked immunoabsorbent assay (ELISA) kit.

Results: Our results indicate that profibrotic action induced by increasing TGF-β and PDGF locally activates fibrous tissue production in the early XFS with a prolonged effect of PDGF (late XFS) and finally (XFG stage) it is dominantly controlled by EGF and IGF. ITAC overrides angiogenetic effects of IL-8 in XFG.

Conclusion: Based on our findings, local chronic inflammation in the eye is accompanied by the secretion of different profibrotic cytokines (TGF-β, PDGF, EGF, IGF, IL-8) without angiogenesis due to effects of ITAC.

Keywords: Cytokines; Fibrosis; Pseudoexfoliation

1. INTRODUCTION

Pseudoexfoliation syndrome (XFS) is a genetically determined generalized disease of the extracellular matrix leading to a progressive deposition of abnormal fibrillar material in various intraocular and extraocular tissues including trabecular meshwork.[1]

Evidence increasingly suggests that cellular stress conditions, such as oxidative stress and ischemia/hypoxia, constitute major mechanisms involved in the pathophysiology of fibrotic process in XFS. Oxidative stress changes local conditions, leading to the activation of local inflammation. It is believed that chronic exposure to oxidative stress factors leads to their cumulative effect which cannot be stopped resulting in permanent changes in tissues.[2]

Products of oxidation stress cause changes in the function of the endothelium of blood vessels, and consequently tissue inflammation.[3] Chronic inflammation triggers the synthesis of profibrotic cytokine initiating a complex activation process of other regulatory (profibrotic, angiogenic, anti-angiogenesis) cytokine synthesis in tissues. The final product of the entire process is the synthesis of mutually connected fibrous fibers and the creation of XFS. Fibrous tissue production is the consequence of the earlier stage.[4] Regulatory cytokines participate in a compound process of fibrous fibres production and accumulation in the tissue.[4] Despite the fact that oxidative stress parameters are elevated in aqueous humor and that angiogenetic factors are present (vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF], transforming growth factor beta [TGF-β], platelet-derived growth factor [PDGF], angiopoietin 1 [Ang-1], and IL-8), there is no relevant research on vasculogenesis and angiogenesis in the anterior segment of the eye.[5,6]

The purpose of this study is to indicate the significance of different regulatory cytokine actions in stress conditions, as well as to reveal their roles in different stadiums of pseudoexfoliation (PEX) production and in the accumulation process. The mechanism of the development of XFS via cytokine formation will provide new therapeutic insights for the treatment.

2. METHODS

One hundred forty patients were recruited into the study. The study design was approved by the local Ethics Committee, and all enrolled patients gave their written consent at the beginning

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of the study. All study patients were referred to the Clinic for Ophthalmology (Clinical Center Kragujevac, Kragujevac, Serbia) for cataract surgery.

All patients underwent a complete ophthalmic examination including visual acuity, intraocular pressure (IOP) measurement, slit lamp and fundus examination, gonioscopio and perimetric examination for glaucomatous patients. A detailed slit lamp examination was performed for each individual in mydriasis with the aim of examining the signs of XFS. We divided all patients into four age-matched groups according to PEX deposits and IOP levels: early stage of XFS (I group, n = 35), late stage of XFS (II group, n = 35), pseudoexfoliation glaucoma (XFG) (III group, n = 35), and control group (IV group, n = 35) without PEX deposition. The first group with early XFS had diffuse precipitation of PEX material on the anterior lens capsule and/or on the pupillary margin, mild pigment liberation after pupillary dilation with deposition on iris, lens and chamber angle, discreet pupillary ruff atrophy, and slightly reduced mydriasis. Late stages of XFG were described by massive PEX material deposits on pupillary margin and/or lens revealing the "ring" imprint on the anterior lens capsule, hard pigment deposition on the structures of the anterior segment (including chamber angle), extensive pupillary ruff atrophy, and intensive restricted mydriasis. The group of patients with XFG involved individuals with elevated IOP (>20 mmHg), an open chamber angle with PEX, characteristic glaucomatous visual field defects and glaucomatous changes of the nervous optic head with the presence of PEX material deposits in the anterior segment of the eye. Patients with a history of previous trauma, intraocular inflammation, diabetes mellitus, myopia, earlier laser photocoagulation, cryotherapy, or intraocular surgery were excluded from the study.

2.1. Collecting aqueous humor and serum

Aqueous humor samples were collected during paracenthesis and serum samples were collected from patients before the cataract surgery. The samples were immediately frozen in liquid nitrogen and stored at −80°C until final examination.

2.2. Enzyme-linked immunosorbent assay

Sera (undiluted) and aqueous humor (diluted 1/10) were collected by single needle stick from cubital vein and from the anterior chamber of the eye from selected patients and stored at −80°C until thawed for assay. Serum and humor levels of cytokines were measured in a sample with high sensitivity enzyme-linked immunoabsorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) specific for human cytokines. Labeling procedure was conducted according to R&D Systems instructions manual. All measurements were performed in triplicate.

2.3. Statistical analysis

The unpaired ANOVA test and Mann–Whitney test were performed using SPSS 19.0 statistical software package (SPSS Inc., Chicago, IL, USA). The results were expressed as the Mean ± SE. All p values were 1-sided and a p value <0.05 was considered statistically significant (significance levels as indicated in figure legends). Exact p values and the number (n) of patients are given in the Results section and Figure legends of the respective study.

3. RESULTS

The main purpose of our study was to investigate the role of selected cytokines in the complex process of PEX production. According to their role in the process of PEX production these cytokines were divided into three groups: profibrotic (TGF-β, PDGF, EGF, and IGF), angiogenetic (IL-8), and antiangiogenetic cytokines (ITAC).

The measurements of regulatory cytokines in the aqueous humor indicated statistically significant increased levels ($p < 0.05$) of TGF-β in the early stage of XFS (3346.89 ± 95.53 pg/mL) in comparison to patients with developed XFG (3123.86 ± 96.29 pg/mL), the late stage of XFS (3054.63 ± 109.37 pg/mL), and the control group (3134.83 ± 73.01 pg/mL). Levels of PDGF were statistically significantly elevated in patients with the early (395.16 ± 20.21 pg/mL) and late stage (395.61 ± 19.92 pg/mL) of XFS in comparison to patients with XFG (323.34 ± 11.48 pg/mL), $p<0.001$; and the control group (350.95 ± 5.84 pg/mL), $p < 0.05$. Thus, EGF level was enhanced in patients with XFG (24.44 ± 4.67 pg/mL) with statistically significant difference ($p < 0.001$) in comparison to patients with the early (3.78 ± 2.27 pg/mL) and late stage of XFS (7.12 ± 3.56 pg/mL) and the control group (2.38 ± 1.2 pg/mL). In the aqueous humor of the patients with XFS (early and late stage), IGF was not detected, and there is a statistically significant level ($p < 0.001$) of this regulatory cytokine in the control group (152.88 ± 15.18 pg/mL) in comparison to its level (1.88 ± 1.38 pg/mL) in XFG group (Fig. 1A).

The serum level of TGF-β was statistically significantly ($p < 0.001$) higher in the early stage of XFS (776.19 ± 117.93 pg/mL) than its level in the late stage of XFS (63.95 ± 34.0 pg/mL) or XFG (78.47 ± 48.50 pg/mL) or the control group without detected TGF-β. The level of PDGF in XFG patients (988.69 ± 60.59 pg/mL) was statistically significantly lower ($p < 0.05$) than its level in the late stage of XFS (1422.05 ± 57.33 pg/mL), but with no statistically significant differences between the early stage of XFS (1216.18 ± 57.42 pg/mL) and the control group (1312.37 ± 47.67 pg/mL). Our results indicated statistically significant ($p < 0.05$) decrease in the level of EGF in XFG (43.89 ± 7.94 pg/mL) in comparison to the late stage of XFS patients (72.74 ± 15.67 pg/mL), but without statistically significant differences ($p > 0.05$) in comparison to the early stage (49.81 ± 10.96 pg/mL) of XFS and the control group (58.68 ± 8.63 pg/mL). We found that the level of IGF was statistically significantly increased in XFG patients (160.96 ± 21.48 pg/mL) in comparison to the early (99.86 ± 18.65 pg/mL; $p < 0.05$) and late stage (33.31 ± 9.23 pg/mL; $p < 0.001$) XFS patients, and no statistically significant differences ($p > 0.05$) with the control group (113.23 ± 22.61 pg/mL) (Fig. 1B).

Patients with XFG showed statistically significant ($p < 0.05$) increased level of IL-8 (1132.57 ± 78.56 pg/mL) in comparison to patients with the early stage (667.24 ± 87.67 pg/mL) of XFS and control group patients (666.95 ± 78.49 pg/mL), but without statistically significant differences ($p > 0.05$) with patients of the late stage of XFS group (785.14 ± 59.43 pg/mL) (Fig. 2A). The aqueous humor levels of ITAC were statistically significantly ($p < 0.05$) increased in XFG (418.93 ± 22.67 pg/mL) in comparison to patients from early XFS (362.46 ± 28.5 pg/mL) and the control group (351.97 ± 18.34 pg/mL) (Fig. 2A).

Serum levels of IL-8 were not detected in the group of early stage XFS patients, and it was statistically significantly ($p < 0.001$) increased in patients with the late stage (10.91 ± 1.29 pg/mL) of XFS, XFG (10.35 ± 1.45 pg/mL); but not in the control group (0.56 ± 0.14 pg/mL). Chemokine ITAC levels showed statistically significant ($p < 0.001$) increase in XFG patients (76.01 ± 6.26 pg/mL) in comparison to patients from the early (5.98 ± 0.98 pg/mL) and late stage (8.43 ± 3.45 pg/mL) of XFS and the control group (7.39 ± 2.13 pg/mL) (Fig. 2B).

In the group of regulatory cytokines (Fig. 3), we found a strong positive correlation ($r = 0.839$, $p < 0.001$) between the serum levels of IGF in patients with developed XFG and patients with the early stage of XFS (Fig. 3A). We also established strong positive correlations ($r = 0.718$, $p < 0.001$) between serum and

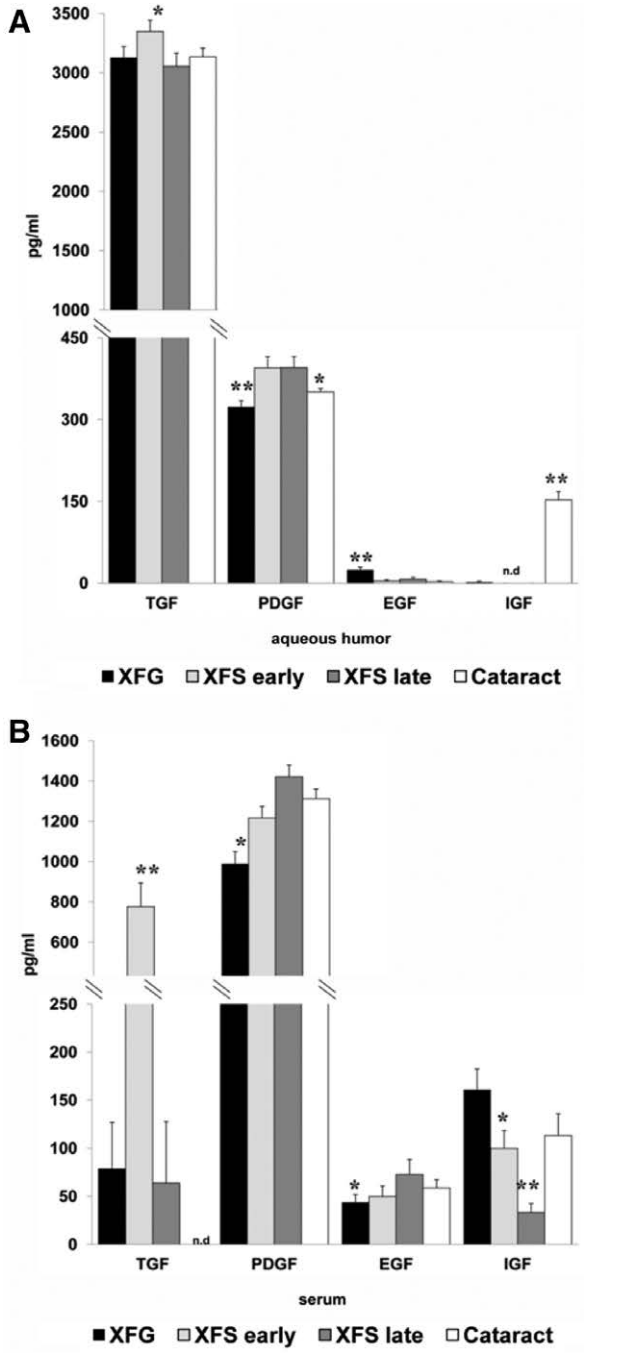

Fig. 1 Aqueous humor and serum levels of TGF-β, PDGF, EGF, and IGF. TGF-β level in aqueous humor was significantly increased ($p < 0.05$) in patients with the early stage of XFS compared with patients in other groups. PDGF level was significantly increased in patients with the early and late stage of XFS in comparison with other examined groups. Patients with XFG had significantly enhanced levels of EGF and IGF (A). Serum level of TGF-β was significantly increased in patients with the early stage of XFS in comparison to other three groups of examinees, $^{**}p < 0.001$. PDGF and EGF levels indicated significantly increased level in patients with the late stage of XFS, $^{*}p < 0.05$. Patients with XFG had significantly increased levels of IGF (B), $^{*}p < 0.05$, $^{**}p < 0.001$. PDGF, platelet-derived growth factor; TGF-β, transforming growth factor beta; XFG, pseudoexfoliation glaucoma; XFS, pseudoexfoliation syndrome.

aqueous humor levels of ITAC in patients with XFG (Fig. 3B). Additionally, we established a strong positive correlation ($r = 0.859$, $p < 0.001$) between EGF levels at serum samples of

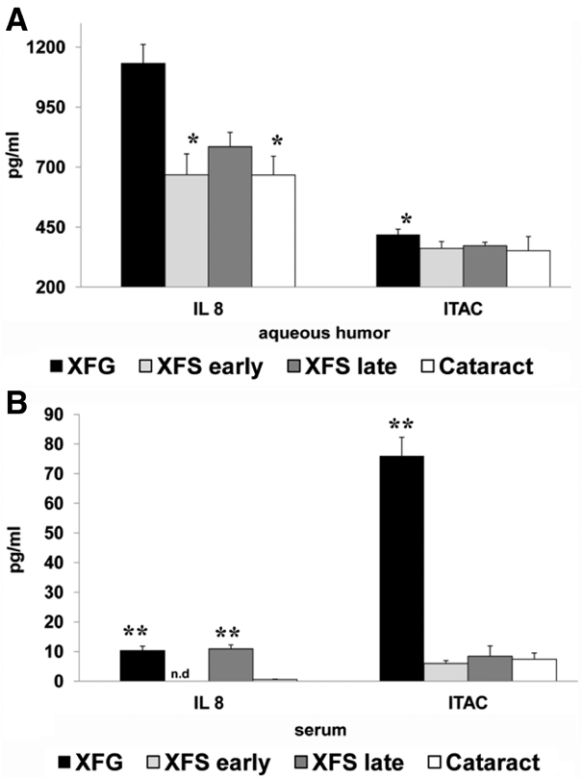

Fig. 2 Aqueous humor and serum levels of IL-8 and ITAC. IL-8 level in aqueous humor was significantly increased ($p < 0.05$) in patients with XFG compared with patients with early XFS and control group patients. Patients with XFG had significantly increased levels of ITAC, $p < 0.05$ (A). Serum level of IL-8 was significantly increased in late XFS and in XFG compared with early PEX and control group patients ($p < 0.001$). XFG patients had significantly high level of ITAC in comparison with patients from other three groups, $p<0.001$, (b), $^{*}p<0.05$, $^{**}p<0.001$. PEX, pseudoexfoliation; XFG, pseudoexfoliation glaucoma; XFS, pseudoexfoliation syndrome.

patients with the early stage of XFS and IGF serum levels of patients with XFG (Fig. 3C). Serum levels of TGF-β of patients with XFG were in a strong positive correlation ($r = 0.865$, $p < 0.001$) with serum levels of PDGF in patients with XFG (Fig. 3D).

4. DISCUSSION

Ophthalmologists made PEX manifestation gradual scale from XFS to XFG according to PEX deposits, IOP levels, and optic head glaucomatous changes.[1]

Based on three stages of fibrosis process, we can make a presumption of the cytokine profile in the PEX production process.[4] Earlier studies indicated important roles of some cytokines in this process.[5,6] In the first phase local tissues of the eye react to the ultraviolet radiation, infection, oxidative stress, etc.[2,7] PEX is in tight connection with older population, so alternated local hypoxic conditions trigger this compound process.[8] Also, inflammation in the tissue is the initial step in the process of the PEX production in the eye.[5] Local inflammation, provoked by aging and disturbed oxidative stress, continues consequently with fibrous tissue production. Some other studies indicated that the inflammation is one of the important phases in the process of the PEX production.[5,9] Our study indicated very important roles of some regulatory cytokines in PEX production as in fibrosis process. We divided regulatory cytokines according to their roles: profibrotic (TGF-β, PDGF, EGF, and IGF), angiogenetic

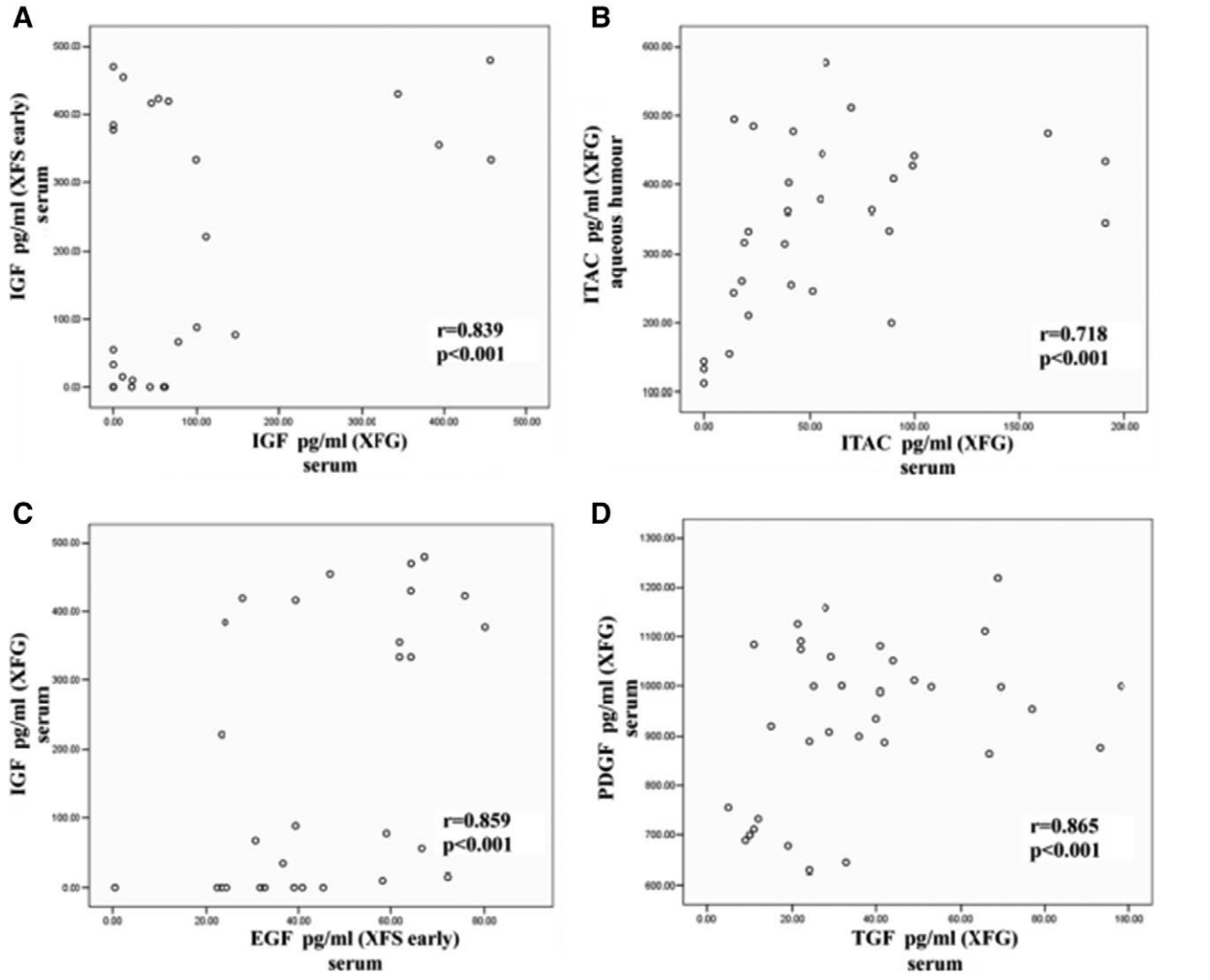

Fig. 3 Correlations between regulatory cytokines in XFS/XFG. In the group of regulatory cytokines, we found a strong positive correlation between: serum levels of IGF in patients with developed XFG and patients with the early stage of XFS (A); serum levels and humor levels of ITAC in patients with XFG (B); EGF levels in serum of patients with early stages of XFS and IGF serum levels of patients with XFG (C); and serum levels of TGF-β of patients with XFG with serum levels of PDGF in patients with XFG (D), $^{**}p<0.001$. PDGF, platelet-derived growth factor; TGF-β, transforming growth factor beta; XFG, pseudoexfoliation glaucoma; XFS, pseudoexfoliation syndrome.

(IL-8), and antiangiogenetic cytokines (ITAC). Increased aqueous levels of TGF-β and PDGF were recorded in early stages of PEX production as the first step of fibrotic tissue reparation. A previous study indicates the elevated level of TGF-β in patients with PEX with no effect on PEX production stage.[10] Furthermore, TGF-β can activate fibroblasts to settle in the tissue and to activate their further proliferation and differentiation by PDGF.[5] During the late stage of XFS development, PDGF level is high and the process of activation of fibroblasts is continued by amplifying fibroblast proliferation and differentiation. Generalized chronic inflammation with consequently fibrosis process is controlled and maintained by increased levels of PDGF, EGF, and IL-8 in circulation (sera levels) which results in this complex process in the eye. High levels of profibrotic cytokines in the sera influence their levels in aqueous humor by maintaining their local action. Regulatory cytokines can activate fibrous fiber production and accumulation in the eye. Increased aqueous humor levels of TGF-β and PDGF activate fibrous tissue production in early stage of XFS with prolonged effect of PDGF (late stage of XFS) and fibrous production (XFG stage) is finally dominantly controlled by EGF and IGF. Thus, fibrous tissue production is amplified. Koliakos et al have reported elevated levels of some growth factors.[11] Local chronic inflammation maintains fibrous tissue production process. EGF and IL-8 are responsible for maintaining fibrous tissue production,[12–14] so they are locally increased in the eye in the next phase of disease development-XFG. Our study indicated elevated IL-8 aqueous level in XFG. We can presume that IL-17 can promote local synthesis of IL-8 in the tissue.[15] IL-17 makes changes in IL-8 expression in human fibroblasts. The level of IL-8 produced by fibroblasts was increased by the rise in IL-17 concentration.[16] Our results, from earlier study for the levels of proinflammatory cytokines indicated the elevated level of IL-17 in aqueous humor.[15] We showed the increased level of TGF-β in aqueous humor at the early stage of XFS. TGF-β enhances production of CTGF.[17] CTGF increases local synthesis of EGF and IGF.[18] CTGF level is increased in the patients with PEX, which was recorded in some earlier studies.[5,6] Additionally, PDGF can promote IGF synthesis and their higher level and activation are maintained in the circulation.[19,20] Fibroblast proliferation can

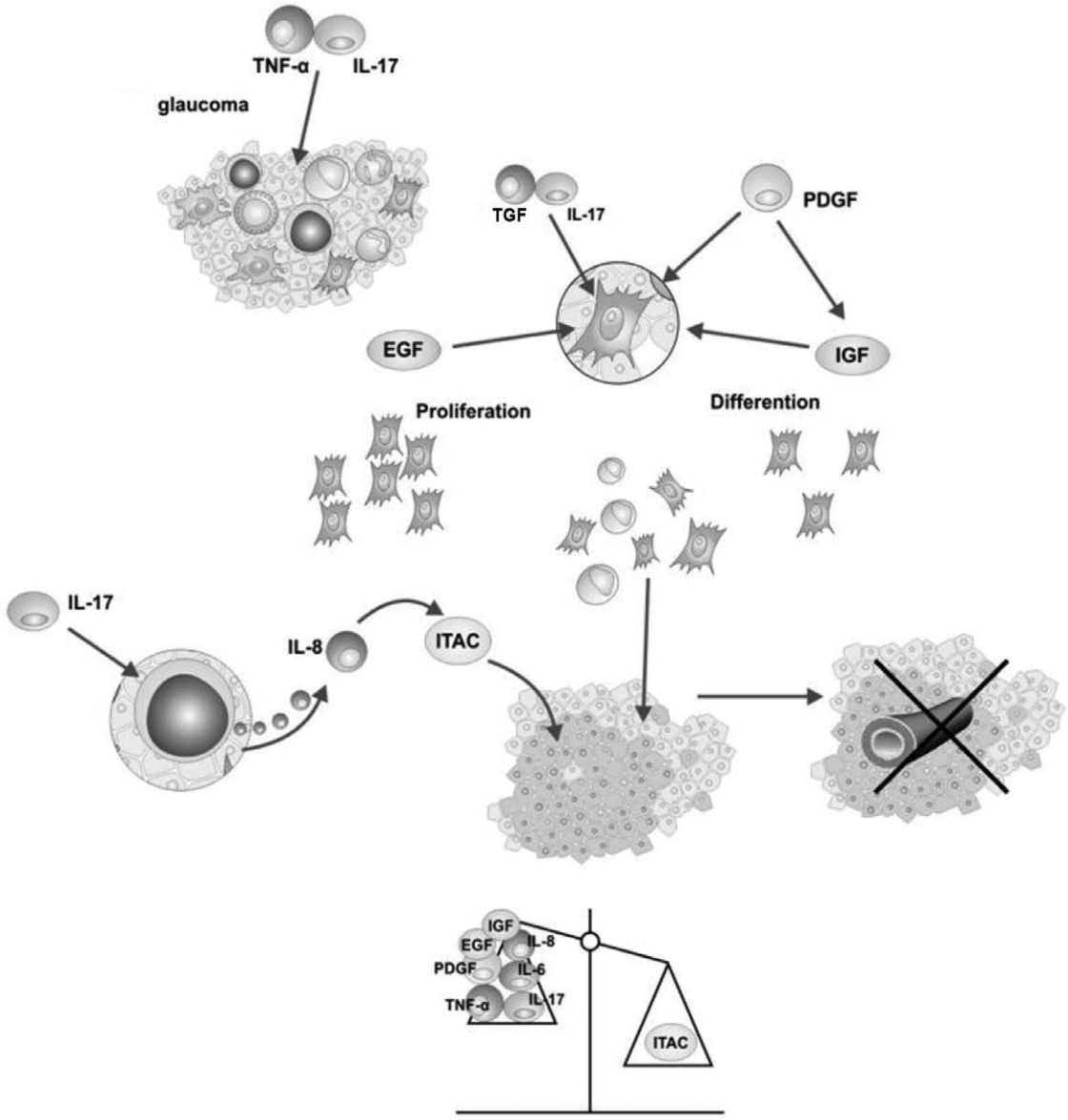

Fig. 4 XFG stage. PDGF activates EGF and IGF synthesis, which promotes proliferation and differentiation of fibroblasts. IL-17 acts on fibroblasts to secrete IL-8, but its action can be restrained by the action of ITAC. Final result of this process is PEX production and accumulation in the tissue without vasculogenesis and angiogenesis. PDGF, platelet-derived growth factor; PEX, pseudoexfoliation; XFG, pseudoexfoliation glaucoma.

be controlled by EGF and myofibroblast differentiation can be controlled by IGF.[18] Our investigation indicates elevated levels of EGF, IL-8 in XFG in humor aqueous, while IGF was detected only in sera of the patients with XFG. According to our results no IGF local action was recorded throughout the early and late stage, but we recorded its action in the sera through all phases of the PEX development with increased action in the final phase, XFG. These results can indicate that in the final process of the XFG development IGF is the main profibrotic cytokine with an important action in the final phase. It promotes a great differentiation of fibroblasts and maintains the process of fibrous tissue production. We can observe the effects of these cytokines in the process of PEX deposition in the eye through levels and correlation. We established a positive correlation between the serum levels of the IGF in patients with the early stage of XFS and developed XFG, and a positive correlation between serum levels of EGF in patients with the early stages of XFS and serum levels IGF of the patients with XFG. Additionally, we showed low levels of TGF-β and PDGF in humor aqueous and serum and a positive correlation between serum levels of TGF-β and PDGF of the patients with XFG. The analysis of correlation test results leads to the conclusion that the main action of profibrotic cytokines (TGF-β, PDGF) is in the early phase of XFS and it is followed by an intensive action of other regulatory cytokines (EGF, IGF) in the next step of the disease development (XFG). Increased levels in the tissue are probably maintained by their action in the serum in the late stage of XFS and their increased levels were preserved during the glaucoma phase.

During a chronic injury, endothelial cells enter a process of vasculogenesis (*de novo* blood vessel formation) and angiogenesis (new capillary branches from existing blood vessels).[21] Angiogenesis can be controlled by several angiogenesis factors including VEGF, FGF, TGF-β, PDGF,[22] and other cytokines (IL-8 etc.) and chemokines with similar roles.[19,23] Our investigation

signified elevated levels of TGF-β, PDGF in humor aqueous of the patients in the early stage of XFS; and elevated levels of IL-8 in the serum and humor aqueous of patients with developed XFG. However, there is no clear evidence about new vessels in the eye with XFS/XFG. Thus, some factors which restrain angiogenesis must exist. Earlier studies indicated that there is no significant increase of the levels of some antiangiogenetic factors.[6] In our study, we measured humor aqueous and serum levels of ITAC, which indicated elevated levels in humor aqueous and serum of the patients with developed XFG. ITAC overrides angiogenetic effects of IL-8 in XFG stage. We also affirmed positive correlations between serum levels and humor levels of ITAC in patients with XFG. These results indicate that the final step in fibrous tissue production with angiogenesis is prevented by increased secretion of ITAC in the body. The final result is fibrous tissue deposition with no new vessels in the body and in the eye (Fig. 4).

According to our findings, the local conditions (oxidative stress) triggers chronic inflammation activation in the eye accompanied by the secretion of different profibrotic cytokines (TGF-β, PDGF, EGF, IGF, and IL-8). Activated tissue fibroblasts are main contributors in fibrous tissue production. Accumulated PEX material in all parts of the eye and decreased blood flow cause XFG development without vasculogenesis and angiogenesis due to the increased level of ITAC (humor aqueous and sera) in XFG.

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REFERENCES

1. Schlötzer-Schrehardt U. New pathogenetic insights into pseudoexfoliation syndrome/glaucoma. Therapeutically relevant?. *Ophthalmologe* 2012;**109**:944–51.
2. Schlötzer-Schrehardt U. Oxidative stress and pseudoexfoliation glaucoma. *Klin Monbl Augenheilkd* 2010;**227**:108–13.
3. Chambers RC, Scotton CJ. Coagulation cascade proteinases in lung injury and fibrosis. *Proc Am Thorac Soc* 2012;**9**:96–101.
4. Wilson MS, Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. *Mucosal Immunol* 2009;**2**:103–21.
5. Zenkel M, Lewczuk P, Jünemann A, Kruse FE, Naumann GO, Schlötzer-Schrehardt U. Proinflammatory cytokines are involved in the initiation of the abnormal matrix process in pseudoexfoliation syndrome/glaucoma. *Am J Pathol* 2010;**176**:2868–79.
6. Yildirim Z, Yildirim F, Uçgun NI, Sepici-Dinçel A. The role of the cytokines in the pathogenesis of pseudoexfoliation syndrome. *Int J Ophthalmol* 2013;**6**:50–3.
7. Schlötzer-Schrehardt U. Molecular pathology of pseudoexfoliation syndrome/glaucoma–new insights from LOXL1 gene associations. *Exp Eye Res* 2009;**88**:776–85.
8. Amini H, Daneshvar R, Eslami Y, Moghimi S, Amini N. Early-onset pseudoexfoliation syndrome following multiple intraocular procedures. *J Ophthalmic Vis Res* 2012;**7**:190–6.
9. Sarenac Vulovic TS, Pavlovic SM, Jakovljevic VLj, Janicijevic KB, Zdravkovic NS. Nitric oxide and tumour necrosis factor alpha in the process of pseudoexfoliation glaucoma. *Int J Ophthalmol* 2016;**9**:1138–42.
10. Amini H, Daneshvar R, Eslami Y, Moghimi S, Amini N. Matrix metalloproteinase-2, tissue inhibitor of matrix metalloproteinase-2, and transforming growth factor beta 1 in the aqueous humor and serum of patients with pseudoexfoliation syndrome. *Clin Ophthalmol* 2014;**8**:305–9.
11. Djordjević-Jocić J, Zlatanović G, Veselinović D, Jovanović P, Djordjević V, Zvezdanović L, et al. Transforming growth factor beta1, matrix-metalloproteinase-2 and its tissue inhibitor in patients with pseudoexfoliation glaucoma/syndrome. *Vojnosanit Pregl* 2014;**69**:231–6.
12. Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys* 2004;**59**(2 Suppl):21–6.
13. Reeves EP, Williamson M, O'Neill SJ, Greally P, McElvaney NG. Nebulized hypertonic saline decreases IL-8 in sputum of patients with cystic fibrosis. *Am J Respir Crit Care Med* 2011;**183**:1517–23.
14. Sarenac Vulovic TS, Pavlovic SM, Zdravkovic NS. Proinflammatory cytokines induce XFG development. *Ocul Immunol Inflamm* 2016;**24**:671–7.
15. Zdravkovic N, Rosic M, Lutovac M, Zdravkovic V. Physiology and Pathology of Cytokine: Commercial Production and Medical Use. In: Rezaei N, editor: *Physiology and Pathology of Immunology*. Rijeka: InTech; 2018, p. 33–53.
16. Niu YZ, Gong GQ, Chen S, Chen JJ, Kong WJ, Wang YJ. Effects of IL-17 on expression of GRO-α and IL-8 in fibroblasts from nasal polyps. *J Huazhong Univ Sci Technolog Med Sci* 2014;**34**:591–5.
17. Yoneda K, Nakano M, Mori K, Kinoshita S, Tashiro K. Disease-related quantitation of TGF-beta3 in human aqueous humor. *Growth Factors* 2007;**25**:160–7.
18. Ponticos M, Holmes AM, Shi-Wen X, Leoni P, Khan K, Rajkumar VS, et al. Pivotal role of connective tissue growth factor in lung fibrosis: MAPK-dependent transcriptional activation of type I collagen. *Arthritis Rheum* 2009;**60**:2142–55.
19. Strieter RM, Gomperts BN, Keane MP. The role of CXC chemokines in pulmonary fibrosis. *J Clin Invest* 2007;**117**:549–56.
20. Halper J. Advances in the use of growth factors for treatment of disorders of soft tissues. *Adv Exp Med Biol* 2014;**802**:59–76.
21. Semenza GL. Vasculogenesis, angiogenesis, and arteriogenesis: mechanisms of blood vessel formation and remodeling. *J Cell Biochem* 2007;**102**:840–7.
22. Montorfano I, Becerra A, Cerro R, Echeverría C, Sáez E, Morales MG, et al. Oxidative stress mediates the conversion of endothelial cells into myofibroblasts via a TGF-β1 and TGF-β2-dependent pathway. *Lab Invest* 2014;**94**:1068–82.
23. Antoniou KM, Soufla G, Lymbouridou R, Economidou F, Lasithiotaki I, Manousakis M, et al. Expression analysis of angiogenic growth factors and biological axis CXCL12/CXCR4 axis in idiopathic pulmonary fibrosis. *Connect Tissue Res* 2010;**51**:71–80.