

The Association Between Aqueous Connective Tissue Growth Factor and the Severity of Age related Cataracts as Graded by the Lens Opacities Classification System III

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Introduction

- Transforming growth factor b (TGF-b)
 - Identified as a key regulator of many pathological conditions in the lens
 - Induces anterior sub-capsular cataracts, anterior polar cataracts , causative factor in posterior capsule opacification
- Connective tissue growth factor (CTGF)
 - Induced by TGF-b
 - CTGF mediates - downstream effects of TGF-b in proliferation, migration, and extracellular matrix (ECM) production
 - Proposed that CTGF acts mainly as a matricellular protein, modulating and integrating the role of the TGF-b superfamily
 - Important factor in physiological wound healing and in the pathological conditions of fibrosis
- CTGF levels in the aqueous humor might be increased in patients with age-related cataracts as the severity of cataracts increases

Introduction

- No previous study has demonstrated a relationship between CTGF and cataracts
- Thus, we investigated the concentration of CTGF in the aqueous chamber
 - According to cataract severity
 - As graded by the Lens Opacities Classification System (LOCS) III
 - LOCS III is a standardized system for grading and comparing cataract severity and type

Purpose : To evaluate the relationship between aqueous humor concentrations of connective tissue growth factor(CTGF) and the severity of age-related cataracts

Methods

- Prospective clinical study - 43 eyes of 43 patients with senile cataracts
- Routine phacoemulsification surgery
 - Before surgery, all patients were graded for cataract severity
Using the Lens Opacities Classification System III in terms of four features:
Nuclear opalescence (NO), nuclear color (NC), cortical cataracts (C), and posterior sub capsular cataracts (P)
- During surgery, aqueous humor samples were obtained from all patients
 - Sandwich enzyme-linked immunosorbent assays (ELISAs)
: Used to determine CTGF concentrations.
- Various correlation analyses and multiple linear regression
 - Assess any relationship between cataract severity and CTGF levels of the aqueous humor

Methods

Patient samples and cataract grading

Routine phacoemulsification and posterior chamber intraocular lens (IOL) implantation
overall cataract grade was calculated as follows: $NO+NC+C+P$



Sampling of aqueous humor

Via clear corneal paracentesis as the first intraocular maneuver at the beginning of cataract surgery



Measurement of CTGF by Enzyme immunoassay

Using CTGF-specific monoclonal antibodies that specifically bind to distinct epitopes on the CTGF protein



Statistical Analysis

Polyserial correlation test

:relationship between the overall cataract grade & each feature of the cataract grade and the aqueous CTGF level

Pearson's correlation method :Age

Point-biserial correlation method :Gender and diabetes

Multiple regression analysis

Results

TABLE 1. Baseline patient characteristics

Characteristics	Mean \pm SD	Minimum	Maximum
Age	64.70 \pm 11.96	41	88
Diabetes	20		
Gender (Male/Female)	21/22		
Nuclear opalescence	3.33 \pm 1.19	2	6
Nuclear color	3.35 \pm 1.19	2	6
Cortical cataract	3.77 \pm 1.15	2	6
Posterior sub-capsular cataract	3.58 \pm 1.30	1	6
Overall cataract grade	14.02 \pm 3.87	8	24
CTGF concentration (pg/mL)	643.31 \pm 183.5	400.78	1233.63

TABLE 2. Univariate association of our eight variables with the significance values predicting the aqueous CTGF concentration (pg/mL) by various correlation tests

Variables	Correlation test	Correlation coefficient (r)	Standard error	Chi-square	p value
Age	Pearson	0.038	ns	ns	0.810
Gender (Male/Female)	Point-biserial	0.040	ns	ns	0.799
Diabetes	Point-biserial	0.063	ns	ns	0.689
Nuclear opalescence	Polyserial	0.408	0.129	10.003	<0.001
Nuclear color	Polyserial	0.428	0.126	11.538	<0.001
Cortical cataract	Polyserial	0.426	0.125	11.614	<0.001
Posterior sub-capsular cataract	Polyserial	0.403	0.127	10.069	0.001
Overall cataract grade	Polyserial	0.443	0.117	14.336	<0.001

TABLE 3. Final step-wise, multiple regression model for predicting the aqueous CTGF concentration

Variables	Unstandardized coefficients		Standardized coefficients		
	Beta	Standard error	Beta	t	p value
Age	3.344	2.649	0.218	1.262	0.215
Gender (Male/Female)	36.219	51.871	0.100	0.698	0.490
Diabetes	35.056	52.261	0.096	0.671	0.507
Nuclear opalescence	75.277	25.461	0.488	2.957	0.005
Nuclear color	78.671	24.883	0.511	3.162	0.003
Cortical cataract	71.030	24.348	0.446	2.917	0.006
Posterior sub-capsular cataract	51.072	21.592	0.360	2.365	0.023
Overall cataract grade	25.729	6.998	0.542	3.682	0.001

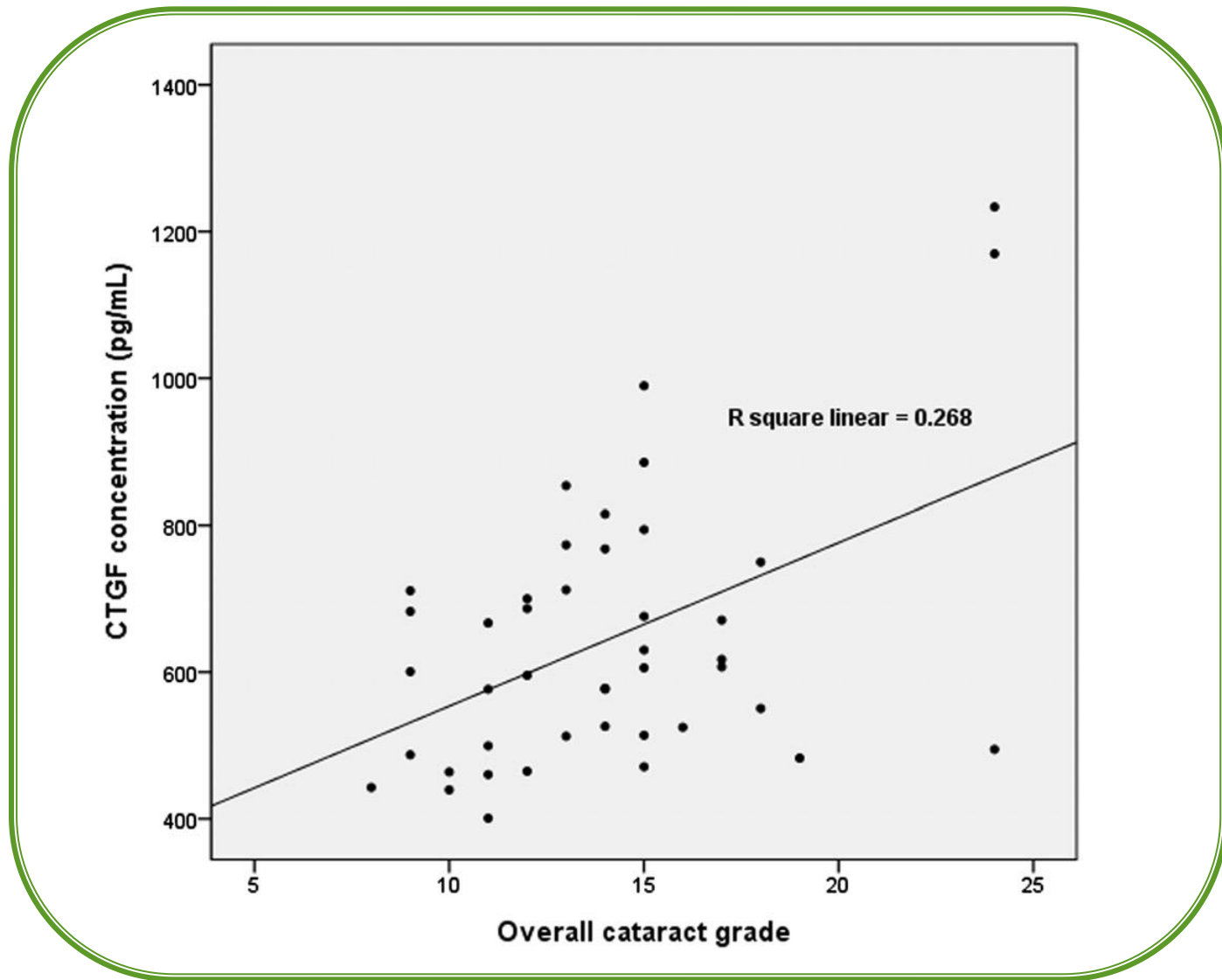


FIGURE 1. Linear regression analysis of overall cataract grade with aqueous CTGF concentration (pg/mL). The line represents the regression line demonstrating the relationship between the overall cataract grade and aqueous CTGF level ($R^2 = 0.268$, $p = 0.001$)

Results

- Baseline patient data are shown (Table 1)
- Positive correlation between the overall cataract grade and aqueous CTGF level
($R = 0.443$, $p = 0.001$, polyserial correlation test) (Table 2)
- 4 features of the cataract grade (NO, NC, C, and P)
 - Positively correlated with the aqueous CTGF concentration
($R = 0.408$, 0.428 , 0.426 , and 0.403 , respectively, $p = 0.001$ for NO, NC, and C and $p = 0.001$ for P, polyserial correlation test) (Table 3)
- Multiple linear regression analyses
 - To identify the best set of independent predictors for the aqueous CTGF concentration
 - The final regression model
: Overall cataract grade as an independent predictor of increased CTGF levels in the aqueous humor
($B = 25.729$, adjusted $R^2 = 0.268$, $p = 0.001$) (Figure 1)

Discussion

- TGF- β induces an epithelial-to-mesenchymal transition(EMT)
 - Results in lens opacities found in some forms of cataract
 - MAPK cascade and extracellular signal-regulated kinases(ERK) pathways are involved in EMT in the lens
 - Activated TGF- β induces CTGF expression
- CTGF
 - Involved in the pathogenesis of diseases of lens epithelial cells(LECs) and vascular smooth muscle
- Established role of CTGF as a modulator of ECM production in many fibrotic diseases
& Increased CTGF tended to be positively correlated with the severity of age-related cataracts

- CTGF tends to increase in the aqueous humor as the severity of age-related cataracts increases
→ this cytokine may play an important role in the pathogenesis of age-related cataracts