Quantitative relationships between transforming growth factor beta mRNA isoforms in congenital and traumatic cataracts

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Purpose: The aim of this study was to determine differences in the expression profiles of transforming growth factor (TGF) β isoforms in the fragments of anterior lens capsules (ALCs) and peripheral blood mononuclear cells (PBMCs) of pediatric patients with congenital and traumatic cataracts.

Methods: Forty children with congenital cataracts (19 girls and 21 boys) and 22 children with traumatic cataracts (six girls and 16 boys) participated in the study. Fragments of ALCs obtained during cataract surgery and whole blood samples were analyzed. Quantification of TGFβ1, TGFβ2, and TGFβ3 mRNA was performed by real-time quantitative reverse transcription (QRT)-PCR using SYBR Green I chemistry.

Results: TGFβ1, TGFβ2, and TGFβ3 mRNA was detected in all the studied samples. Significant differences were found for TGFβ1 and TGFβ2 expression profiles in PBMCs between the patients with congenital and traumatic cataracts. The expression profiles of TGFβ isoforms in ALCs did not differ significantly between the groups.

Conclusions: Overexpression of TGFβ1 and TGFβ2 in the PBMCs of patients with congenital cataracts might indicate that these cytokines are involved in the development of lens opacity.

Cataracts represent a degenerative disorder of the lens, and remain a leading cause of blindness worldwide [1]. However, the molecular mechanisms of the formation of congenital and traumatic cataracts are still poorly understood. The identification of molecular and genetic events involved in these diseases may be essential for the better understanding of cataracts, as well as for the development of diagnostic markers and novel treatment strategies.

Transforming growth factor (TGF) β is a strong candidate inducer of the epithelial–mesenchymal transition and extracellular matrix production, which characterize congenital and traumatic cataracts in the human lens [2,3]. In addition, the evidence from animal studies in vitro and in vivo supports the hypothesis of the cataractogenic potential of TGFβ [2,4-6].

Five members of the TGFβ family have been identified. However, only TGFβ1, TGFβ2, and TGFβ3 have been demonstrated to be expressed in mammals [7]. Each isoform is encoded by unique genes of different chromosomal location [8], and reveals a 64–85% amino acid sequence homology [9]. All TGFβ isoforms have a similar biologic effect in vitro, but in vivo they are generally characterized by varied expression levels and different functions. Their biologic activity depends on quantitative relationships between individual isoforms [10,11].

Members of the TGFβ family regulate fundamental aspects of cellular functions, including cell growth, differentiation, inflammation, and wound healing [9-15]. Additionally, substantial evidence also implicates the role of TGFβ in many human diseases [2,4,11], including fibrotic diseases of the eye [5,6,16-18]. The relationship between TGFβ3 levels and a degree of fibrosis in various organs is well documented [19,20].

Most researchers determine only the TGFβ protein level using immunoenzymatic methods [20-26]. It should be mentioned that the change in the protein level is preceded by the alteration of gene transcriptional activity encoding this protein. Many attempts have been made to identify proteins in serum or mRNA in peripheral blood mononuclear cells (PBMCs), which could be easily accessed and act as markers of intratissue processes in various diseases [19,27]. However, there are no published data regarding differences between mRNA levels of all three TGFβ isoforms in the anterior lens capsules (ALCs) and PBMCs of pediatric patients with congenital and traumatic cataracts.

In the present study, real-time quantitative reverse transcription (QRT)-PCR was applied to investigate the changes in TGFβ1, TGFβ2, and TGFβ3 gene expression in
fragments of ALCs and PBMCs from pediatric patients with congenital and traumatic cataracts. Quantitative relationships between mRNA levels of these three TGFβ isoforms were analyzed.

**METHODS**

The patient group comprised 40 individuals (19 girls and 21 boys, mean age 9.8 years; range 4.7–17.6 years) with clinically diagnosed congenital cataracts. The comparison group consisted of 22 individuals (six girls and 16 boys, mean age 11.4; range 3.9–17.9 years) with clinically diagnosed traumatic cataracts (Table 1), treated in the Department of Ophthalmology, University Hospital No. 5, Medical University of Silesia, Katowice, Poland. The diagnosis of traumatic cataracts was based on the Birmingham Eye Trauma Terminology System [16]. The mean time interval between injury and cataract surgery was 14.9 months (range 0.2–156.2 months).

The criteria for inclusion in the molecular analysis were as follows: age ≤18 years, no inflammatory conditions (before the surgery, all children were examined by an anesthesiologist, pediatrician, ear, nose, and throat specialist, and dentist), and no systemic disease. In the case of children with congenital cataracts, we also excluded local disorders such as persistent hyaloid vessels, small cornea, and microphthalmia.

The study was approved by the Bioethics Committee of the Medical University in Katowice (KNW/0022/KB1/63/I/09) in accordance with the Declaration of Helsinki regarding medical research involving human subjects. The study and its purpose were explained to each participant or his or her legal guardian, who gave informed written consent.

**Tissues:** Circular sections of ALCs with attached anterior lens epithelial cells were obtained during cataract surgery, and were stored for 48 h at −70 °C until RNA extraction. Venous blood samples were collected into EDTA-containing tubes, and a 7.5 ml sample from each patient was centrifuged on a Ficoll-Conray gradient (specific gravity 1.077; Immunobiological Co., Gumma, Japan) immediately after blood collection.

**Ribonucleic acid extraction from tissue specimens:** Total RNA was extracted from PBMCs and from ALCs using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA extracts were treated with DNase I (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer’s instructions. The quality of extracts was checked electrophoretically using 0.8% agarose gel stained with ethidium bromide. The results were analyzed and recorded using the 1D Bas-Sys gel documentation system (Biotech-Fisher, Perth, Australia). Total RNA concentration was determined by spectrophotometric measurement in 5 μl capillary tubes using the Gene Quant II RNA/DNA Calculator (Pharmacia Biotech, Cambridge, UK).

**Real-time quantitative reverse transcription polymerase chain reaction assay:** Gene expression of TGFβ1, TGFβ2, TGFβ3, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were evaluated using real-time QRT–PCR and SYBR Green I chemistry (SYBR Green Quantitect RT–PCR Kit; QIAGEN, Valencia, CA). The analysis was performed using an Opticon™ DNA Engine Continuous Fluorescence Detector (MJ Research, Watertown, MA). All samples were tested in triplicate. GAPDH was included to monitor the QRT–PCR efficiency. Oligonucleotide primers specific for TGFβ1, TGFβ2, TGFβ3, and GAPDH were described previously by Strzalka et al. [9,15] and Ercolani et al. [28] (Table 2). The thermal profile for one-step RT–PCR was as follows: reverse transcription at 50 °C for 30 min, denaturation at 95 °C for 15 min, and 50 cycles consisting of

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**Table 1. Selected clinical features of the patients with clinically diagnosed congenital or traumatic cataracts.**

| Characteristic                      | Congenital cataract (n=40) | Traumatic cataract (n=22) |
|------------------------------------|-----------------------------|----------------------------|
| Gender                             | F  19                       | 6                          |
|                                   | M  21                       | 16                         |
| Age (years)                        | 9.8 (4.7–17.6)              | 11.3 (3.9–17.9)            |
| **Eye**                            |                             |                            |
| Right                              | 18                          | 9                          |
| Left                               | 22                          | 13                         |
| Mean time interval between injury and cataract surgery (months) | -                          | 14.9 (0.2–156.2) |
| Mean age at the time of injury (years) | -                          | 10.2 (3.7–17.6) |
| **Type of trauma (BETTS*)**        |                             |                            |
| Penetrating                        | -                           | 22                         |

Values of clinical parameters are expressed as means (minimum-maximum). Abbreviations M and F stand for male and female participants, respectively. *BETTS - Birmingham Eye Trauma Terminology System.
Sequence of primers | Length of amplicon (bp) | Tm (°C)  
--- | --- | ---  
**GAPDH**  
Forward: 5'-GAAGGTGAAGGTCGGAGTC-3'  
Reverse: 5'-GAAGATGGTGATGGTTCAGGATT-3'  
226 | 80  
**TGFβ1**  
Forward: 5'-TGAACCGGCTGCTTGCTCATG-3'  
Reverse: 5'-GCGGAAGTCAATGTACAGCTGCCG-3'  
151 | 85  
**TGFβ2**  
Forward: 5'-TACTACGCAGGAAGCTTTGACGACTCGCTGGTTCATG-3'  
Reverse: 5'-TTGCTACGCAGCATCTGGTTCATG-3'  
201 | 80  
**TGFβ3**  
Forward: 5'-CTGGGATTGTGTTCCATGCA-3'  
Reverse: 5'-TCCCCGAATGCTCACAT-3'  
121 | 81

bp – base pairs; Tm - melting temperature.

Quantification of the expression of target genes: To quantify the results obtained by RT–PCR for TGFβ1, TGFβ2, TGFβ3, and GAPDH, a standard curve method was employed [9,15]. Commercially available standards of β-actin (ACTB) cDNA (TagMan® DNA Template Reagent Kit; PE Applied Biosystems, Inc., Foster, CA) were used at five different concentrations (0.6, 1.2, 3.0, 6.0, and 12.0 ng/µl) to simultaneously detect the expression profile of each investigated gene. For standards, the calculation of copy number values was based on the following relationship: 1 ng of DNA=333 genome equivalents (PE Applied Biosystems). Amplification plots for each dilution of a commercially available standard template were used to determine Ct values [9,15]. A standard curve was generated by plotting Ct values against the log of the known amount of ACTB cDNA copy numbers. Correlation coefficients for standard curves ranged from 0.988 to 0.995, indicating a high degree of confidence for measurement of the copy number of molecules in each sample. The copy numbers of analyzed mRNAs were calculated from linear regression of the standard curve.

Statistical analyses: Statistical analyses were performed using Statistica 8.0 software (StatSoft, Tulsa, OK), and the level of significance was set at p<0.05. Values were expressed as median (Me) with the 25th and 75th quartiles. Nonparametric tests were used for statistical analyses because the Shapiro–Wilk test indicated that the data were not normally distributed. The Kruskal–Wallis test and post hoc multiple test based on average ranks were applied to assess differences in the expression of TGFβ isoforms. Intergroup comparison of the gene expression under investigation was performed with the Mann–Whitney U test. Correlations were evaluated using the Spearman rank correlation test.

RESULTS

Specificity of the real-time reverse transcription polymerase chain reaction assay: RT–PCR specificity for the target genes was confirmed experimentally on the basis of amplimers’ melting temperatures. For each RT–PCR product, a single peak at expected temperatures was observed: TGFβ1, 85.4°C; TGFβ2, 80.0°C; TGFβ3, 80.6°C; and GAPDH, 80.1°C (data not shown). Gel electrophoresis also revealed the presence of a single product of predicted length (data not shown).

Differences in transforming growth factor β1, β2, and β3 messenger ribonucleic acid between anterior lens capsules and peripheral blood mononuclear cells: In this part of the study, the expression of TGFβ1, TGFβ2, and TGFβ3 was analyzed by real-time QRT–PCR. Then, the quantitative relations between the mRNA of these three isoforms in congenital and traumatic cataracts were evaluated.

TGFβ1, TGFβ2, and TGFβ3 isoforms were detected in ALC and PBMC samples obtained from patients with congenital and traumatic cataracts (Figure 1A,B).

A comparative analysis of all TGFβ mRNA copies/µg of total RNA revealed that TGFβ1 was a predominant isoform in the ALCs of patients with congenital cataracts. There was no statistically significant difference between TGFβ1 (Me=9162) and TGFβ2 (Me=5427) mRNA levels (p=0.113, post hoc test). However, the mRNA level of TGFβ3 (Me=2470) was significantly lower than those of TGFβ1 (Me=9162, p<0.001, post hoc test) and TGFβ2 (p<0.001, post hoc test). In the PBMCs of patients with congenital cataracts, the expression of TGFβ3 (Me=9395) was the highest and significantly greater than those of TGFβ1 (Me=4475, p=0.015, post hoc test) and TGFβ2 (p=0.001, post hoc test). The mRNA level of TGFβ1 was also significantly greater than that of TGFβ2 (p<0.001, post hoc test).

The quantitative relations between mRNA of these three isoforms were similar in the patients with clinically diagnosed traumatic cataracts. TGFβ1 was also the main isoform in
ALCs. The mRNA levels of TGFβ1 (Me=10501) and TGFβ2 (Me=7380) were not statistically significant (p=0.226, post hoc test). However, the expression of TGFβ3 (Me=1573) was found to be significantly lower than those of TGFβ1 (p<0.001, post hoc test) and TGFβ2 (p=0.013, post hoc test) in ALCs. For PBMCs, the relationships were as follows: the TGFβ3 mRNA level (Me=17650) was the highest and significantly greater than those of TGFβ1 (Me=1459, p=0.002, post hoc test) and TGFβ2 (Me=190, p=0.012, post hoc test). The mRNA level of TGFβ1 was also significantly greater than that of TGFβ2 (p<0.001, post hoc test).

Correlations between transforming growth factor β1, β2, and β3 messenger ribonucleic acid for anterior lens capsules and peripheral blood mononuclear cells: All three isoforms were positively correlated with each other in the ALCs of patients with congenital cataracts. However, there was only a significant association between mRNA levels of TGFβ1 and TGFβ2 for PBMCs (Table 3).

For traumatic cataracts, a positive correlation was only observed between the expression of TGFβ1 and TGFβ2 in ALCs, whereas for PBMCs, a positive correlation was only affirmed between the expression of TGFβ1 and TGFβ3 (Table 3).

Differences in transforming growth factor β1, β2, and β3 messenger ribonucleic acid between congenital and traumatic cataracts: In ALCs, there was no significantly different expressions of TGFβ1, TGFβ2, and TGFβ3 between congenital and traumatic cataracts (TGFβ1 p=0.78, TGFβ2 p=0.75, TGFβ3 p=0.23, Mann–Whitney U test). In PBMCs, the expression of TGFβ1 and TGFβ2 was about twofold higher in the congenital cataract patients compared to the patients with traumatic cataracts, and a statistical significance was found (TGFβ1 p<0.0001, TGFβ2 p<0.0001, Mann–Whitney U test). However, no statistically significant relationship was found for TGFβ3 isoforms (p=0.23, Mann–Whitney U test; compare Figure 1A and Figure 1B).
**TABLE 3.** The correlations between TGFβ isoforms (copies/µg total RNA) in the ALCs and PBMCs of patients with clinically diagnosed congenital and traumatic cataracts.

| Variable                  | n   | TGFβ1* | p**       | TGFβ2* | p**       | TGFβ3* | p**       |
|---------------------------|-----|--------|-----------|--------|-----------|--------|-----------|
| **Anterior lens capsules (ALCs)** |     |        |           |        |           |        |           |
| Gender                    |     |        |           |        |           |        |           |
| M                         | 21  | 9682 (4554–19139) | NS | 5119 (1967–15660) | NS | 1915 (1494–2845) | NS |
| F                         | 19  | 9162 (5792–24306) | NS | 8034 (2154–19968) | NS | 3082 (2062–8230) | NS |
| Eye                       |     |        |           |        |           |        |           |
| Right                     | 18  | 11122 (5790–15423) | NS | 7161 (2518–18149) | NS | 2524 (1564–5017) | NS |
| Left                      | 22  | 8942 (4621–24306) | NS | 5119 (1967–18430) | NS | 2295 (1527–3888) | NS |
| **Peripheral blood mononuclear cells (PBMCs)** |     |        |           |        |           |        |           |
| Gender                    |     |        |           |        |           |        |           |
| M                         | 21  | 3640 (1519–8691) | NS | 356 (295–529) | NS | 8998 (7261–15053) | NS |
| F                         | 19  | 4475 (2749–5010) | NS | 330 (265–506) | NS | 16768 (4119–35775) | NS |
| Eye                       |     |        |           |        |           |        |           |
| Right                     | 18  | 4475 (1787–7761) | NS | 356 (300–552) | NS | 11827 (6327–35775) | NS |
| Left                      | 22  | 4070 (2121–7105) | NS | 308 (266–513) | NS | 9788 (6943–19801) | NS |

*Median with 25th and 75th quartiles (mRNA copies/µg total RNA). **U Mann–Whitney test. NS – not significant.

Abbreviations M and F stand for male and female participants, respectively.

**TABLE 4.** Factors associated with TGFβ isoforms’ expression changes in the ALCs and PBMCs of patients with clinically diagnosed congenital cataracts.

| Variable                  | n   | TGFβ1* | p**       | TGFβ2* | p**       | TGFβ3* | p**       |
|---------------------------|-----|--------|-----------|--------|-----------|--------|-----------|
| **Anterior lens capsules (ALCs)** |     |        |           |        |           |        |           |
| Gender                    |     |        |           |        |           |        |           |
| M                         | 21  | 9682 (4554–19139) | NS | 5119 (1967–15660) | NS | 1915 (1494–2845) | NS |
| F                         | 19  | 9162 (5792–24306) | NS | 8034 (2154–19968) | NS | 3082 (2062–8230) | NS |
| Eye                       |     |        |           |        |           |        |           |
| Right                     | 18  | 11122 (5790–15423) | NS | 7161 (2518–18149) | NS | 2524 (1564–5017) | NS |
| Left                      | 22  | 8942 (4621–24306) | NS | 5119 (1967–18430) | NS | 2295 (1527–3888) | NS |
| **Peripheral blood mononuclear cells (PBMCs)** |     |        |           |        |           |        |           |
| Gender                    |     |        |           |        |           |        |           |
| M                         | 21  | 3640 (1519–8691) | NS | 356 (295–529) | NS | 8998 (7261–15053) | NS |
| F                         | 19  | 4475 (2749–5010) | NS | 330 (265–506) | NS | 16768 (4119–35775) | NS |
| Eye                       |     |        |           |        |           |        |           |
| Right                     | 18  | 4475 (1787–7761) | NS | 356 (300–552) | NS | 11827 (6327–35775) | NS |
| Left                      | 22  | 4070 (2121–7105) | NS | 308 (266–513) | NS | 9788 (6943–19801) | NS |

*Median with 25th and 75th quartiles (mRNA copies/µg total RNA). **U Mann–Whitney test. NS – not significant.

Abbreviations M and F stand for male and female participants, respectively.

**Relationships between transforming growth factor β1, β2, and β3 messenger ribonucleic acid, gender, and left- or right-sided cataracts:** No statistically significant correlations were found for congenital cataracts between the mRNA levels of TGFβ isoforms and gender or left- or right-sided cataracts (Table 4).

**Correlations between transforming growth factor β1, β2, and β3 messenger ribonucleic acid and patient’s age or time interval between trauma and surgery:** There was no correlation between the age and transcriptional activity for any of the TGFβ isoforms in the ALCs of the congenital cataract samples collected during surgery (ALCs: TGFβ1, r=0.180, not significant (NS); TGFβ2, r=0.168, NS; TGFβ3, r=0.011, NS). For PBMCs, a significant negative correlation was only determined between the mRNA levels of TGFβ1 and age (PBMCs: TGFβ1, r=-0.395, p<0.05; TGFβ2, r=-0.025, NS; TGFβ3, r=-0.051, NS). Correspondingly to the results obtained for congenital cataracts, in ALCs from the patients with traumatic cataracts, no correlations between the expression of TGFβ genes and age (ALCs: TGFβ1, r=0.158, NS; TGFβ2, r=0.110, NS; TGFβ3, r=0.046, NS) were detected. As for PBMCs, a positive correlation was only observed between TGFβ3 mRNA and age (PBMCs: TGFβ3, r=0.657, p<0.05; TGFβ1, r=-0.395, NS; TGFβ2, r=-0.042, NS).

Furthermore, correlations between TGFβ1, TGFβ2, and TGFβ3 mRNA levels and time interval between trauma and surgery were evaluated in our study. There was a significant positive correlation only between the mRNA levels of
DISCUSSION

TGFβ isoforms and their receptors are expressed in both normal and pathological lenses [2,18,29]. Determination of differences between mRNA levels in cataractous lens capsules compared and normal ones seems to be important. Previous research has revealed elevated levels of TGFβ1 in cataractous lenses in comparison to those in noncataractous ones [30]. Therefore, this report focused only on quantitative relations between TGFβ isoforms in congenital and traumatic cataracts. In the present study, real-time QRT–PCR was used to evaluate copy numbers of TGFβ1, TGFβ2, and TGFβ3 mRNA. All three TGFβ isoforms were detected in ALC and PBMC samples, which is partially consistent with the results obtained by Gordon-Thomson et al. [2]. These authors demonstrated the presence of TGFβ1 and TGFβ2 mRNA in various ocular tissues, including the lens. TGFβ3 mRNA was only detected in the retina and choroid. However, they observed mRNA distribution in ocular tissues using in situ hybridization methods.

Our results revealed that TGFβ1 was a predominant isoform in ALCs, whereas TGFβ3 was a major isoform in PBMCs in patients with congenital and traumatic cataracts. Our observation is supported by the data published by Lee et al. [30], who indicated the strongest expression of TGFβ1 mRNA in human lens epithelial cells from patients with anterior polar cataracts. Similar results were demonstrated by Carrington et al. [31], who found TGFβ1 to be a predominant isoform in the bovine cornea during wound healing. However, the real-time QRT–PCR technique was not used in these studies.

Shirai et al. [32] also performed studies where epithelial mesenchymal transition lens cells expressed TGFβ1, TGFβ2, and TGFβ3 isoforms. This remains consistent with current results showing the presence of all three TGFβ isoforms in both types of cataracts. In ALCs, similar mRNA levels of predominant TGFβ1 and TGFβ2 isoforms were found. Whereas Shirai et al. [32] only determined the increase of both active and total TGFβ2 in the injured rat lens and aqueous humor, according to Yoneda et al. [33], the concentration of TGFβ3 was much lower than the content of other two isoforms in the aqueous humor, which confirms our observations for ALC tissues.

Xiao et al. [34] revealed an overaccumulation of TGFβ1 and basic fibroblast growth factor mRNA in the lens with anterior subcapsular congenital cataracts when compared to transparent lenses. In our study, it was observed that TGFβ1 mRNA levels in ALC patients were raised, but there were no significant differences in its levels between congenital and traumatic cataracts, which partially corresponds to the research results of Xiao et al. [34]. The dissimilarity might have resulted from the selection of our control group, i.e., including pediatric patients who had sustained an eye injury rather than patients with clear lenses. The lack of significant differences between the two types of cataractous lenses suggests that TGFβ may mediate at least some part of cataractogenesis for both types.

Previous studies indicated that traumatic events could result in the activation of the TGFβ signaling pathway [17, 18]. Wallentin et al. [35] demonstrated the activation of the TGFβ superfamily signaling pathway following experimental cataract surgery in rabbits. Furthermore, these authors showed that the level of total TGFβ in postoperative aqueous humor was continually increasing. Likewise, our results revealed higher mRNA levels of TGFβ1 and TGFβ2 in ALCs of traumatic cataract patients. However, these results were not statistically significant when compared to those obtained for congenital cataracts. The results shown above may indicate that cataract surgery increases the level of TGFβs.

TGFβ has been found in human plasma, platelets, and circulating leukocytes [36-38]. It is known that the expression of TGFβ is constitutive in leukocytes and does not depend on activation [36]. Therefore, it seems a proper biologic material for the determination of TGFβ levels in blood. Our results for the quantitative relationships between three TGFβ isoforms in PBMCs are in accordance with other studies’ results [38]. Interestingly, the subjects with congenital cataracts showed a significant elevation of TGFβ1 and TGFβ2 expression in PBMCs when compared to those with traumatic cataracts, which suggests that traumatic cataractogenesis is mediated differently from congenital cataractogenesis. The most striking difference concerned TGFβ1. Chan et al. [39] studied the plasma level of the TGFβ1 transgene in hybrid mice. Although the expression of the TGFβ1 transgene was only targeted to the liver, its overexpression resulted in hepatic and multiple extrahepatic lesions. In the eye, there was a progressive cortical cataract formation. In our research, the upregulation of TGFβ1 and TGFβ2 gene expression in the PBMCs of patients with congenital cataracts might suggest that the processes occurring in the lens are affected by systemic expression levels of these cytokines, especially during fetal life when the lens is vascularized.

The second part of this study focused on the correlations between TGF isoforms in congenital and traumatic cataracts. Interestingly, in the ALCs of congenital cataract patients, all three TGFβ isoforms were positively correlated, but in traumatic cataract patients, there were only positive correlations between the mRNA levels of TGFβ1 and TGFβ2. Moreover, in the PBMCs of congenital cataract patients, only TGFβ1 and TGFβ2 exhibited positive association, whereas in the traumatic cataract patients, the TGFβ1 and TGFβ3 isoforms were correlated. The relations between the levels of TGFβ1, TGFβ2, and TGFβ3 and their biologic effects may be explained through TGFβ binding to
the three cell surface receptors. Although TGFβ isoforms share the same receptors for their signal transduction, a specific pathological role played by TGFβ might result from their different activity or potency [33].

In the studies performed by Rostkowska-Nadolska et al. [40], TGFβ1 transcriptional activity was accompanied by TGFβ2 transcriptional activity in nasal polyps, which is in accordance with the observations of their activity in the lenses of our study. The TGFβ2 isoform is structurally similar to TGFβ1, but the biologic responses to these cytokines differ depending on the cell type. The promoter sequences of the genes coding different TGFβ isoforms are not homologous. The analysis of TGFβ1 and TGFβ3 promoters revealed the presence of several binding sites for the transcriptional factors Sp-1 and AP-1. Fragments of the TGFβ1 and TGFβ3 promoters may compete for binding of Sp-1 to DNA. The TGFβ2 promoter does not contain binding sites for the Sp-1 transcriptional factor [41]. The lack of correlation between TGFβs may result from similar regulation of the expressions of these genes and competition for binding Sp-1.

Our data regarding congenital cataract revealed age-dependent differences in the mRNA levels of TGFβ1 in PBMCs. These observations correspond to those of other researchers, who demonstrated that serum TGFβ1 levels tend to decrease with age [24,25]. However, Rungger-Brändle et al. [42] did not show any correlation between the TGFβ2 mRNA levels and the age of the donors of the lenses without visible opacity, lenses with mature cataracts, and cataractous lenses with posterior subcapsular opacity or anterior subcapsular fibrosis, which is consistent with our results. On the other hand, it was found that rodent lens cells showed enhanced TGFβ receptors expression and increased susceptibility to the cataractogenic effects of TGFβ with age. This may suggest that the age-dependent increase in TGFβ signaling is connected with the responsiveness of the target tissue rather than with TGFβ expression itself [43,44].

Correlations between TGFβ2 levels and history of cataract surgery (time interval between previous cataract surgery and the evaluation of TGFβ2 concentrations) were observed in aqueous humor [45]. Taking into account Wallentin’s results [35], which show a continuous increase in total TGFβ after cataract surgery throughout the study period (30 days), the correlations between TGFβ gene expression and the time interval between trauma and surgery were evaluated in our studies. Our results revealed similar positive correlations between TGFβ1 mRNA level and time interval between trauma and surgery for PBMCs. These observations might suggest that the accumulation of TGFβ promotes cataractogenesis.

At present, it is difficult to determine whether systemic TGFβ1 and TGFβ2 overexpression in congenital cataracts might act as a causative or concomitant factor. For instance, Marfan syndrome is believed to result from mutations in the gene encoding fibrillin-1. However, recent research has shed new light on the role of TGFβ in the pathogenesis of this disease. Interference of TGFβ signaling has been found to be involved in aortic aneurysm formation and progression. What is more, the disruption of TGFβ pathways might provide new diagnostic and therapeutic options [46,47]. Perturbations of TGFβ signaling might also underlie some proportion of congenital cataracts. Further studies are needed to conclusively confirm the cataractogenic influence of TGFβ.

In conclusion, all three TGFβ isoforms were found to be differentially expressed in the ALCs and PBMCs of pediatric patients with congenital and traumatic cataracts. Overexpression of TGFβ1 and TGFβ2 in the PBMCs of patients with congenital cataracts might indicate the involvement of these cytokines in the development of lens opacity.

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