Cx43, ZO-1, alpha-catenin and beta-catenin in cataractous lens epithelial cells

Anshul I Arora, Kaid Johar, Devarshi U Gajjar, Darshini A Ganatra, Forum B Kayastha, Anuradha K Pal, Alpesh R Patel, S Rajkumar and Abhay R Vasavada*

Iladevi Cataract and IOL Research Centre, Gurukul Road, Memnagar, Ahmedabad 380 052

**Corresponding author (Fax, 91-79-27411200; Email, icirc@abhayvasavada.com)*

Specimens of the anterior lens capsule with an attached monolayer of lens epithelial cells (LECs) were obtained from patients (n=52) undergoing cataract surgery. Specimens were divided into three groups based on the type of cataract: nuclear cataract, cortical cataract and posterior subcapsular cataract (PSC). Clear lenses (n=11) obtained from donor eyes were used as controls. Expression was studied by immunofluorescence, real-time PCR and Western blot. Statistical analysis was done using the student's t-test. Immunofluorescence results showed punctate localization of Cx43 at the cell boundaries in controls, nuclear cataract and PSC groups. In the cortical cataract group, cytoplasmic pools of Cx43 without any localization at the cell boundaries were observed. Real-time PCR results showed significant up-regulation of Cx43 in nuclear and cortical cataract groups. Western blot results revealed significant increase in protein levels of Cx43 and significant decrease of ZO-1 in all three cataract groups. Protein levels of alpha-catenin were decreased significantly in nuclear and cortical cataract group. There was no significant change in expression of beta-catenin in the cataractous groups. Our findings suggest that ZO-1 and alpha-catenin are important for gap junctions containing Cx43 in the LECs. Alterations in cell junction proteins may play a role during formation of different types of cataract.

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1. Introduction

Cell junctions play an important role in the normal functioning of the cell. The three main types of cell junctions are gap junctions (GJs), tight junctions (TJs) and adherens junctions (AJs). The passive diffusion of ions, metabolites and cell signaling molecules occurs through the gap junctions (Harris 2001; Boswell *et al.* 2010). TJs are apical in nature and act as a paracellular barrier (Denker *et al.* 1996). AJs form a physical association between neighboring cells and hold them together (Meng and Takeichi 2009). The lens is an avascular organ in the anterior segment of the eye. It comprises of three compartments: the outer basement membrane called the capsule, a monolayer of epithelial cells lining the anterior capsule and fibre cells that form the bulk of the lens. Lens epithelial cells (LECs) act as the metabolically active compartment of the lens (Khurana and Khurana 2007). The circulation of current and fluid flow, maintained by the gap junctions, is crucial for sustaining homeostasis in the cells of the lens. Connexin 43 (Cx43) is a GJ protein expressed in the LECs (Boswell *et al.* 2009; Musil *et al.* 1990). Zonula Occludens-1 (ZO-1) is a TJ-associated protein that mediates interactions between TJ proteins and the cytoskeleton (Fanning *et al.* 1998). Interactions between ZO-1 and Cx43 are known to occur in many tissues; and during the assembly of GJs, ZO-1 plays a crucial role in the transportation of Cx43 to the plasma membrane (Laing *et al.* 2005; Rhett *et al.* 2011). Cadherins, which are the classical proteins of AJs, are bound to alpha-catenin and beta-catenin at the cytoplasmic domains. The strengthening of intercellular cadherin bonds in a mechanism independent of the actin cytoskeleton is facilitated by recruitment of alpha-catenin to cadherin complexes (Bajpai *et al.* 2008). Catenins are expressed throughout the lens. During early stages of fibre cell differentiation, the Wnt/β-catenin pathway plays key roles in regulating proliferation of lens progenitor cells (Martinez *et al.* 2009).

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Also, formation of a catenin–ZO-1–Cx43 complex and binding of catenins to ZO-1 plays an important role in the transportation of Cx43 during gap junction formation in cardiomyocytes (Wu *et al.* 2003).

GJs play a very important role in maintaining lens transparency (Kistler *et al.* 1988). GJs are protein membranes containing clusters of intercellular channels that are permeable to ions and molecules up to 1 kDa (Boswell *et al.* 2010). An extensive network of GJs facilitates intercellular lens communication. Epithelial and fibre cells contain morphologically and physiologically distinct gap junctions made up of Connexin (Cx) proteins (Musil *et al.* 1990). Cx43 is associated with LECs, Cx46 with fibres and Cx50 with both fibres and lens epithelial cells. As the lens is an avascular tissue, it depends on the aqueous and the vitreous for nutrition (Eldred *et al.* 2011). The central and metabolically active regions of the lens, i.e. the LECs, face the aqueous humour, and thus it is believed that they face oxidative stress as well as other forms of stress (Long *et al.* 2004).

Cataract is a clouding or opacity of the lens. Depending on the location of opacity, cataracts can be classified as nuclear, cortical and PSC. Mutations in GJ proteins are known to cause cataract (Bennett and Shiels 2011; Wang *et al.* 2011). It has been reported that the fibre cells of the lens with cortical opacities have degenerated GJs (Vrensen 2009). It has been speculated by an *in vitro* study that Cx43 and functional GJs may play a role in the modulation of cellular differentiation in canine LECs (Long *et al.* 2010). Another study has shown that the osmotic balance within the lens is markedly altered in Cx43-null animals (Gao and Spray 1998). ZO-1 and catenins are known to act as key regulatory proteins for Cx43 GJ in cardiac cells. However, ZO-1 and catenins have not been studied as regulatory proteins of Cx43 per se in the lens. There is very limited knowledge of catenins in human lens and they have not been studied extensively in relation to cataract. There are no reports on Cx43 and its regulatory proteins ZO-1 and catenins in different types of cataracts that occur in human patients. In the present study, the expression and localization of the cell junction proteins, Cx43, ZO-1, alpha-catenin and beta-catenin were studied from the LECs of cataractous patients.

2. Materials and methods

2.1 *Patient selection*

A total of 52 patients undergoing cataract surgery at Iladevi Cataract and IOL Research Centre (ICIRC), Ahmedabad, were included in the study. The type of cataract was determined preoperatively by a slit lamp examination using the LOCS III classification. Patients with pseudoexfoliation, glaucoma, uveitis, traumatic cataract or any other systemic disorder were not included in the study. Patients were divided into 3 groups: the nuclear cataract group, the cortical cataract group and the posterior subcapsular cataract (PSC) group. Non-cataractous lenses (n= 11) obtained from donor eyes from the local eye bank (Red Cross Eye bank, Dholka, India) were dissected and anterior lens capsules were used as controls. This study was in accordance with the Declaration of Helsinki and ethical clearance for the study was obtained from the institutional ethical committee. table 1 shows data of patients included in the study.

2.2 *Sample collection and processing*

A continuous capsulorhexis was performed during phacoemulsification. The central portion of the lens capsule (approximately 5 mm in diameter) harbouring LECs was collected in a balanced salt solution. The samples for immunofluorescence were processed within 30 min of surgery. The samples for quantitative real-time polymerase chain reaction were collected in TRIZOL reagent (Invitrogen) for RNA expression within 30 min of surgery and stored at −80°C until processed further. The samples for Western blot were stored at −80°C until processed further.

2.3 *Immunofluorescence*

The anterior capsule specimens were placed on silane-coated (amino-propyl-triethoxy silane) slides such that the LECs were facing the slide and cell specimens were prepared as described earlier (Alapure *et al.* 2012). These cells were used for immunolocalization of Cx43, ZO-1, alpha-catenin and beta-catenin. Antigen retrieval of Cx43 was done by incubating the cells with 10 mM Tris pH 12 containing 8 M urea for 5 min at 37°C. Antigen retrieval of ZO-1 and catenins was done using 0.25% Triton X-100 (Sigma-Aldrich) in PBS (phosphate buffered saline) for 10 min and 20 min respectively. The cells were then incubated overnight at 4°C with commercially available primary antibodies against Cx43 (BD Transduction Laboratories™), ZO-1 (Zymed laboratories®, CA), beta-catenin (BD Transduction Laboratories™),and beta-catenin (BD Transduction Laboratories™). After three rinses of PBS containing 0.05% Tween-20 (PBST) for 3 min each, the cells were incubated with fluorescent-tagged secondary antibodies (Alexa fluor 546 Goat Anti-Rabbit and Alexa fluor 488 Goat Anti-Mouse obtained from Invitrogen). An epifluorescence microscope (Axioskope II; Carl Zeiss) was used to observe the cells and images were documented with a CCD camera (Cohu, San Diego, CA).

2.4 *Real-time PCR quantification*

Total RNA was extracted from samples using a TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. 500 ng of total RNA was reverse-transcribed at 25°C for 5 min, at 42°C for 60 min, and at 70°C for 5 min in 20 μL of 0.5 μg of oligo (dT), 20 pmol primer,

Table 1. Patient detail

Types of cataract	No. of samples			Mean age of patients* (years±SD)
	Total	Male	Female	
Clear donor cadaver lens	11	7	4	57.09±07.24
Nuclear cataract	17	12	5	59.29±09.06
Cortical cataract	18	9	9	61.88±09.75
PSC	17	10	7	57.00±10.01

*There was no statistically significant difference in the age of patients with different types of cataract.

3 mM $MgCl_2$, 0.05 mM of dNTPs, 4 μL of Improm-II™ 5× reaction buffer (Promega, Madison, WI), 1 unit of Recombinant RNasin® Ribonuclease Inhibitor, and 1 μl of Improm-II™ Reverse Transcriptase (Promega). For quantitative PCR, amplifications were performed on the LightCycler®480 (Roche Applied Science) in 20 μL of reaction using 2 μL of cDNA, 10 μL of SYBR Green I Master (Roche Molecular Biochemicals) and gene-specific primers of 2.5 μM concentration. The amplification was carried out at 95°C for 10 min before the first cycle, 95°C for 15 s, and 60°C for 60 s repeated 40 times. Quantitative PCR for Cx43, ZO-1, alpha-catenin and beta-catenin was performed and beta-actin was used as the housekeeping gene. The sequence of primers was obtained from qPrimerDepot online software. The sequences of primers used are listed in table 2. Relative Expression, normalized to the housekeeping gene, was calculated using LightCycler®480 Software (Roche Molecular Biochemicals).

2.5 *Western blot*

Seven specimens of each type of cataract were pooled and stored at −80°C until used further. Samples were lysed in cell lysis buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton-X 100, 0.1% SDS, 0.5% sodium deoxycholate, protease inhibitor cocktail, and phosphatase inhibitor cocktail. The total protein was quantified using the bicinchoninic acid (BCA) method. Equal amounts of proteins were loaded in all the lanes and proteins were separated by SDS-PAGE (8% or 12% polyacrylamide gel). After electrophoresis, the protein was transferred to the nitrocellulose membrane using a standard protocol. The membrane was blocked in 5% non-fat dry milk in TBST (tris-buffered saline containing 0.05% Tween-20). The membranes were incubated overnight in appropriate primary antibodies Cx43, ZO-1, alpha-catenin, beta-catenin, and beta-actin at 1:1000 dilution at 4°C. The membranes were washed with TBST and incubated with HRP-conjugated secondary antibodies (CST) at 1:1500 dilution. After washing with TBST, the bands were developed by the chemiluminescence technique using the Pierce ECL Western blotting substrate (Thermo Scientific). Blots were stripped using a mild buffer (0.2 M glycine, 0.1% SDS, 1% Tween 20, pH 2.2), washed with PBS and TBST and blocked with 5% non-fat dry milk in TBST. The blots were then re-probed twice with different antibodies and developed again using the same method. Each experiment was repeated twice. The densitometry analysis for relative band intensity was done using Image J software (NIH Image).

2.6 *Statistical analysis*

Data is expressed in mean±SE and analysed using student's *t*-test to evaluate the significance between control and cataractous groups. Differences at $p<0.05$ were considered significant.

3. Results

3.1 *Controls*

From immunolocalization studies, we found that Cx43 was localized in cytoplasm and at the cell boundaries where it

Table 2. Details of primers used in qRT-PCR

No.	Protein	Gene Name	Forward primer	Reverse primer
1.	Cx43	GJA1	5′GAGTTTGCCTAAGGCGCTC3′	5′AGGAGTTCAATCACTTGGCG3′
2.	ZO-1	TJP1	5′CCCCACTCTGAAAATGAGGA3′	5′GGGAACAACATACAGTGACGC3′
3.	alpha-catenin	CTNNA1	5′GAGCTGTCTACGCAAGTCCC3′	5′TTTCGGAGTACATGGGCAAT3′
4.	beta-catenin	CTNNB1	5′ATTGTCCACGCTGGATTTTC3′	5′TCGAGGACGGTCGGACT3′
5.	beta-actin	ACTB	5′GTTGTCGACGACGAGCG3′	5′GCACAGAGCCTCGCCTT3′

was seen in punctate form (figure 1a). ZO-1, alpha-catenin and beta-catenin were uniformly distributed at cell boundaries (figures 1b and 2a and b).

3.2 *Nuclear cataract group*

Immunolocalization showed that the distribution of Cx43, alpha-catenin and beta-catenin in the LECs of nuclear cataract group remained similar to that of control group (figures 1c and 2c and d). ZO-1 staining was very weak in the LECs of nuclear cataract group (figure 1d). Real-time PCR results showed

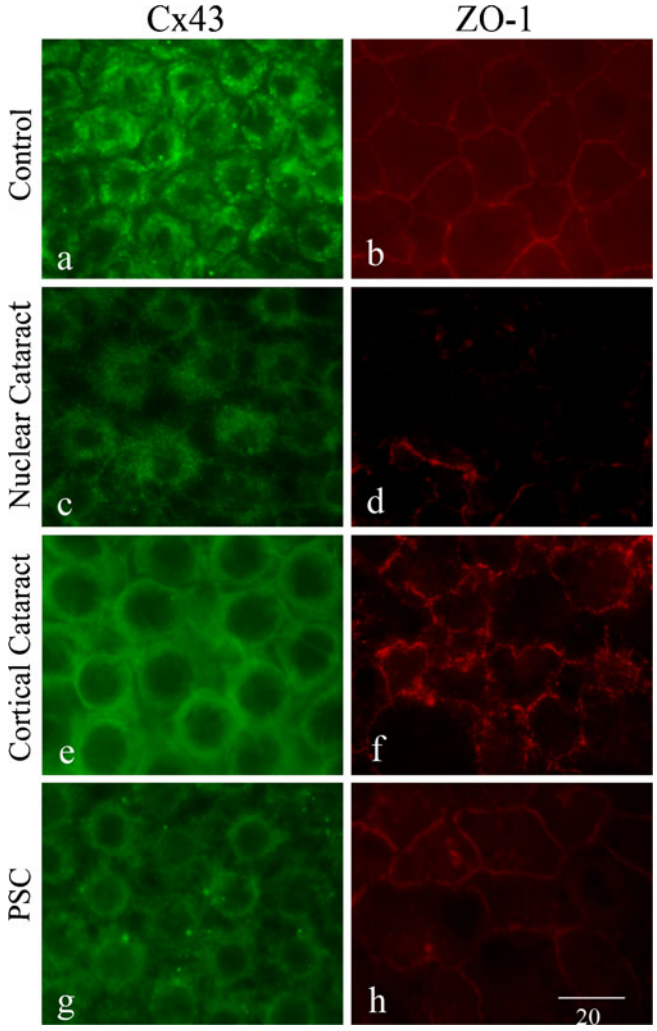

Figure 1. Immunolocalization of Cx43 (**a**, **c**, **e** and **g**) and ZO-1 (**b**, **d**, **f** and **h**) in LECs of control, nuclear cataract, cortical cataract and PSC. Cx43 is localized both in cytoplasm as well in punctate form on cell boundaries in control, nuclear cataract and PSC. Cortical cataract shows high cytoplasmic Cx43 with no localization at cell boundaries. ZO-1 staining is very faint in nuclear cataract, disturbed in cortical cataract. ZO-1 is uniformly localized at cell boundaries in control and PSC (scale bar=20 μm).

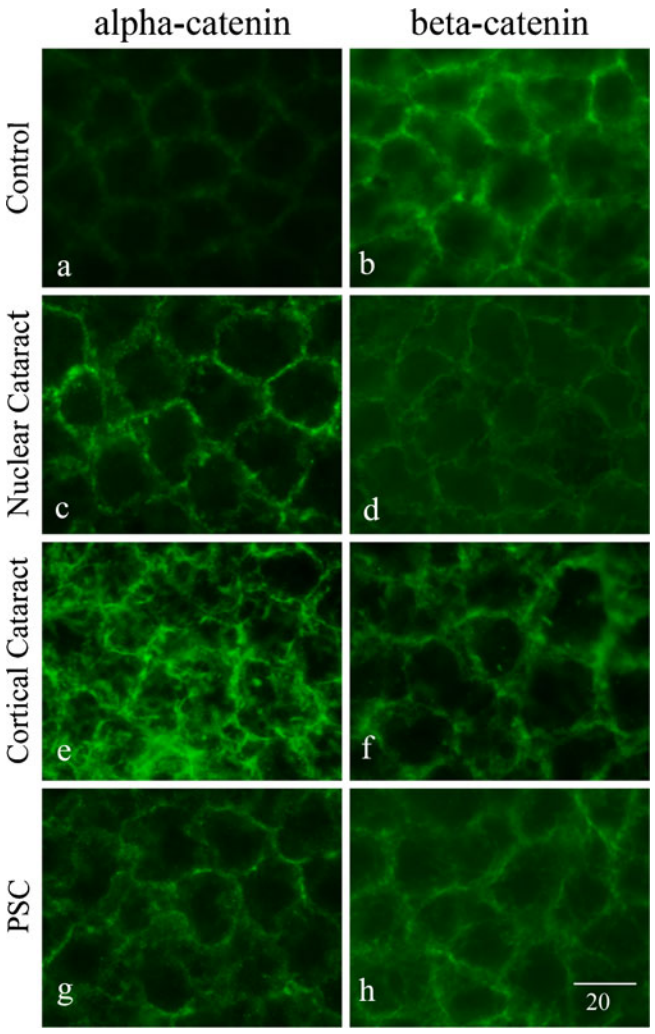

Figure 2. Immunolocalization of alpha-catenin (**a**, **c**, **e** and **g**) and beta-catenin (**b**, **d**, **f** and **h**) in LECs of control, nuclear cataract, cortical cataract and PSC. Alpha-catenin is disturbed in cortical cataract. Beta-catenin is localized uniformly at cell boundaries in all cataract types (scale bar=20 μm).

significant ($p<0.05$) up-regulation of Cx43 while ZO-1 and alpha-catenin showed significant ($p<0.01$ and $p<0.001$ respectively) down-regulation in this group when compared to control (figure 3). Western blot results showed that the protein levels of Cx43 were increased significantly ($p<0.001$) by 2.5-fold and those of ZO-1 and alpha-catenin were significantly ($p<0.05$) decreased by 1.2- and 1.8-fold respectively (figures 4 and 5). There were no significant changes in mRNA or protein levels of beta-catenin in this group.

3.3 *Cortical cataract group*

Immunolocalization results show that cytoplamic pools of Cx43 were increased with no localization at cell boundaries

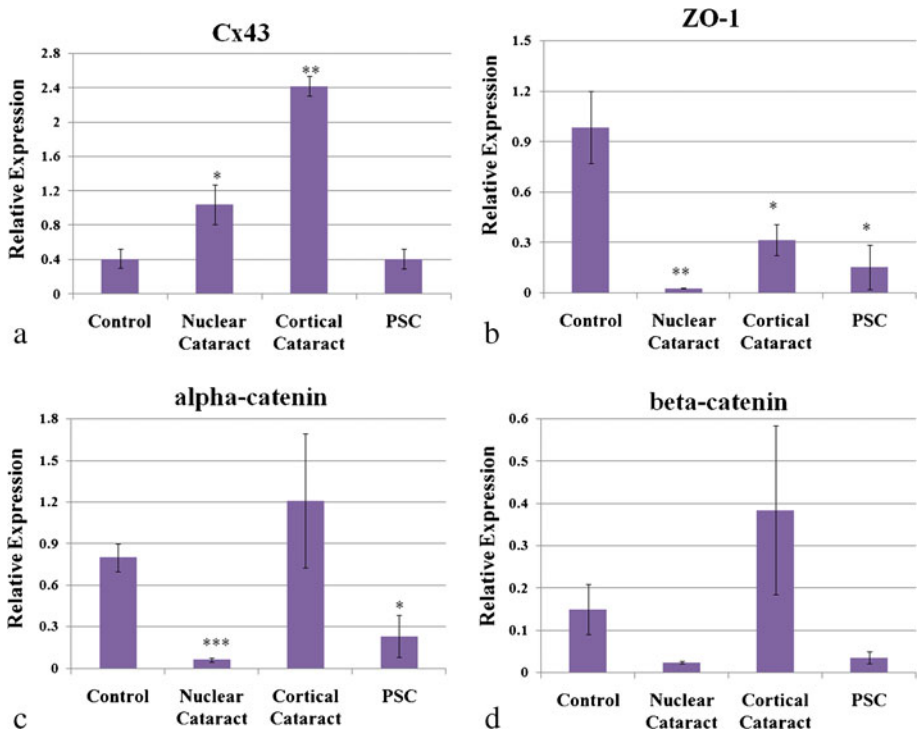

Figure 3. Relative expression of mRNA levels of Cx43, ZO-1, alpha-catenin and beta-catenin in LECs of control, nuclear cataract, cortical cataract and PSC group. Significant up-regulation of Cx43 is observed in nuclear and cortical cataract compared to control. Significant down-regulation of alpha-catenin and ZO-1 is observed in nuclear cataract and PSC. Significant down-regulation of ZO-1 is seen in cortical cataract (*$p<0.05$, **$p<0.01$, ***$p<0.001$). Values are expressed in mean±SE.

(figure 1e). ZO-1 and alpha-catenin distribution was disturbed in the LECs of this group (figure 1e and 2e). No changes were observed in distribution of beta-catenin. Real-time PCR showed significant ($p<0.01$) up-regulation of Cx43 and significant ($p<0.05$) down-regulation of ZO-1 in this group when compared to control. There were no significant changes in mRNA alpha-catenin and beta-catenin in this group when compared to control. Protein levels of Cx43 were significantly ($p<0.001$) increased by 3.0-fold and those of ZO-1 and alpha-catenin were significantly ($p<0.01$) decreased by 2.0- and 1.5-fold in cortical cataract group (figures 4 and 5). There were no significant changes in protein levels of beta-catenin in this group when compared to control.

3.4 *Posterior subcapsular cataract group*

Immunolocalization results show that the distribution of Cx43, ZO-1, alpha-catenin and beta-catenin was similar to controls in this group (figures 1g and h and 2g and h). Real-time PCR showed ZO-1 and alpha-catenin were significantly ($p<0.05$) down-regulated while Cx43 and beta-catenin showed no significant changes in mRNA expression. Protein levels of Cx43 were significantly ($p<0.001$) increased by 2.2-fold and those of ZO-1 were significantly ($p<0.01$) decreased by 1.8-fold when compared to control (figures 4 and 5). The protein levels of alpha-catenin and beta-catenin remained unaltered in this group (figures 4 and 5).

4. Discussion

The present study was designed to study gap junction protein, Cx43, and its regulatory proteins ZO-1, alpha-catenin and beta-catenin, in human LECs obtained from different types of cataract. Previous studies have indicated role of these proteins in cataract; however, no attempt was made to associate expression of these proteins with clinically observed different types of cataract. The metabolic centre of the entire lens is the epithelial cell layer on the anterior capsule. In this study, the anterior capsule specimens obtained by anterior capsulorhexis from human subjects were used. The techniques used to study the expression of cell junction proteins in the present study were immunolocalization, quantitative real-time PCR and Western blot. Immunolocalization enabled us to study the distribution pattern of the protein of interest in the cells. Quantitative real-time PCR facilitated relative quantification of mRNA levels of multiple genes from the same specimen. As the amount of

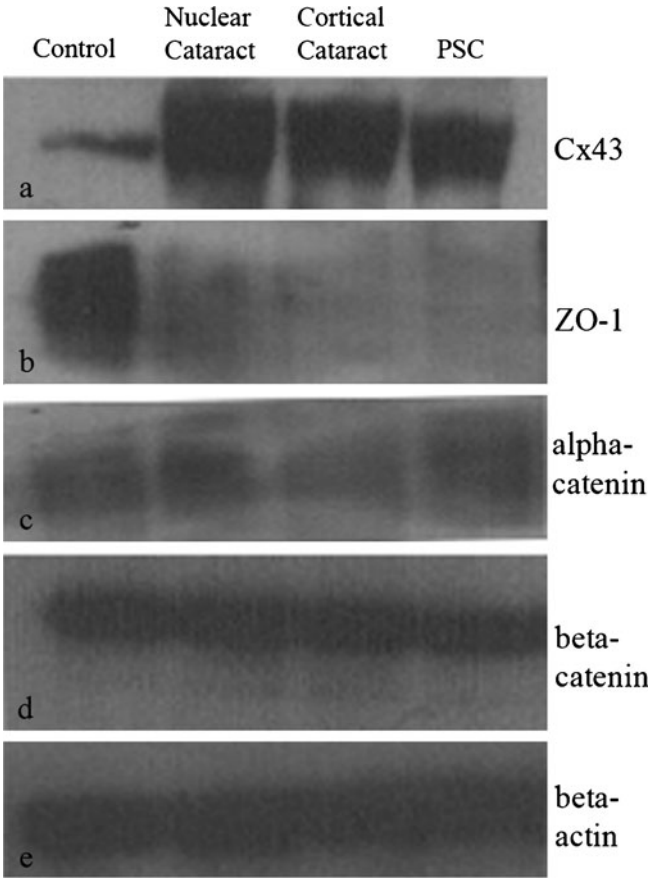

Figure 4. Expression of Cx43, ZO-1, alpha-catenin and beta-catenin by Western blot analysis. Cx43 expression is increased and ZO-1 is decreased in all the cataracts. Alpha-catenin levels are decreased in nuclear and cortical cataract. Similar protein levels of beta-catenin are seen in all the groups. Beta-actin is used as internal control.

protein from single specimen was very low, seven specimens of same cataract types were pooled for western blot analysis.

The exact mechanism that causes the formation of cortical cataract is unknown. However, epidemiologic studies suggest that prolonged exposure to sunlight (Schein *et al.* 1994), UV radiation (Zigman *et al.* 1979), diabetes and vascular diseases (Rochtchina *et al.* 2001) play a vital role in this process. Based on these studies, it can be proposed that cortical cataractogenesis is an intricate cascade mechanism. In the present study, in the cortical cataract group, it was observed that the cytoplasmic pools of Cx43 had increased. It has been shown by an *in vitro* study that exposure of human LECs to ultraviolet-A (UVA) can cause disruption of Cx43 GJ (Wu *et al.* 2011). A similar type of disruption of Cx43 GJ by protein kinase C (PKC) activation has also been shown in canine LECs by 12-*O*-tetradecanoyl-phorbol-acetate (Long *et al.* 2007), under hypoxic conditions in mice (Akoyev *et al.* 2009) and in rabbit LECs using the insulin-like growth factor-1 (Lin *et al.* 2003). Phosphorylation of Cx43 is responsible for disassembly of Cx43 plaques leading to disruption of cell–cell communication (Wagner *et al.* 2002). In rabbit LECs, it has been reported that ZO-1 varies the phosphorylation of Cx43 by modulating the interaction of PKC-γ with Cx43 (Akoyev and Takemoto 2007). Immunostaining showed nonuniform localization of ZO-1 in the cortical cataract group. It is possible that the altered distribution of ZO-1 could cause disassembly of Cx43 and increase its cytoplasmic pools. The limitation of the present study is that the phosphorylated forms of Cx43 were not evaluated.

Catenins are proteins associated with the adheren junctions. In lens, the catenins, alpha, beta and gamma are present in epithelial and fiber cells with varying distribution pattern (Bagchi *et al.* 2002). To the best of our knowledge, there are no reports on the association between alpha-catenin and the cataractous epithelium. The role of catenins in the assembly of gap junctions is established in cardiomyocytes using colocalization and the close association of catenins with ZO-1 and Cx43 during gap junction assembly was shown (Wu *et al.* 2003). In cancer cells, transient expression of alpha-catenin could trigger assembly of Cx43 in the gap junctions and also recruitment of ZO-1 to the cell surface (Govindarajan *et al.* 2002). Interestingly, alpha-catenin staining was disturbed in the cortical cataract group. There was also significant down-regulation of alpha-catenin proteins in the cortical cataract group. Based on our results, we speculate that alpha-catenin plays an important role in regulating the formation of Cx43 gap junction in LECs; but the mechanism responsible for alterations of alpha-catenin remains unclear.

Beta-catenin was another regulatory protein studied. Beta-catenin plays a crucial role both as a signaling molecule as well as cell adhesion molecule (Smith *et al.* 2005). As a cell junction protein, it forms a link between the adheren junction proteins and the actin cytoskeleton. Under the influence of growth factors it acts as a signaling molecule and initiates the canonical Wnt pathway. The unphosphorylated form of beta-catenin translocates to the nucleus, and acts as a central transcriptional regulator of the canonical Wnt signaling pathway. Activation of this pathway in the lens leads to lens pathologies like posterior capsule opacification (PCO), anterior subcapsular cataract and subcapsular fibrosis (Chong *et al.* 2009). Nuclear translocation of beta-catenin has been shown in *in vitro* models of TGF-beta induced, epithelial mesenchymal transition (EMT) and also in clinical samples of anterior subcapsular fibrosis (Chong *et al.* 2009). However, nuclear translocation of beta-catenin was not found in the epithelium of either donor eyes, cataractous eyes (Rungger-Brandle *et al.* 2005) or in the *ex vivo* model of cortical cataract. The diminished association between beta-catenin and cell–cell junctions was observed in the stress-induced *ex vivo* model of cortical cataract (Zhou *et al.* 2007). Our findings show no significant changes in beta-catenin expression between different cataract groups and control.

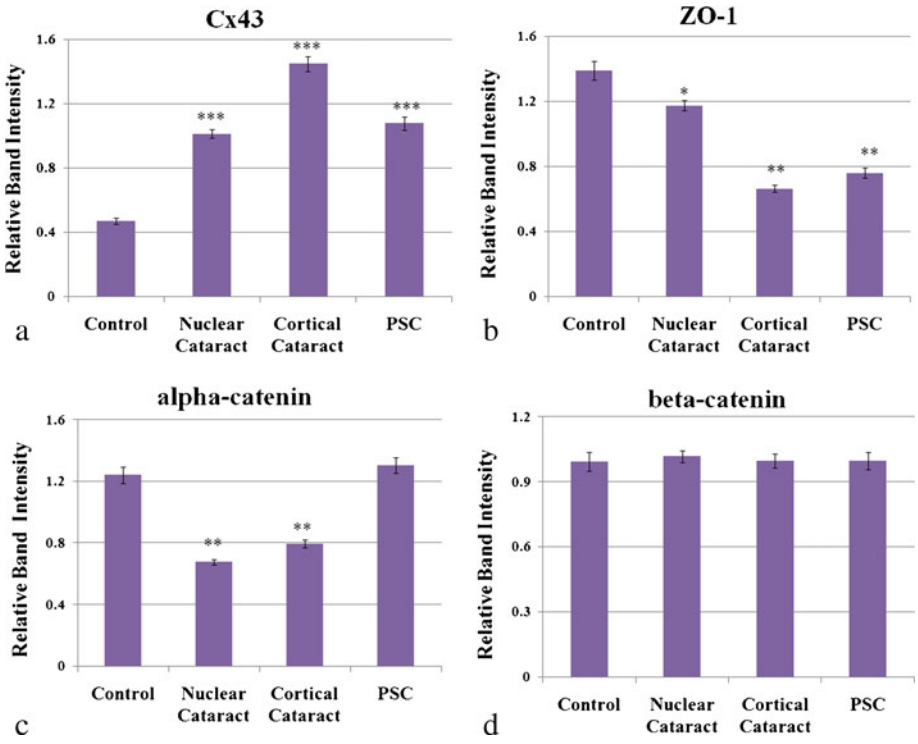

Figure 5. Relative band intensity of Western blot of Cx43, ZO-1, alpha-catenin and beta-catenin in LECs of control, nuclear, cortical and PSC group. Protein levels of Cx43 are increased and that of ZO-1 are decreased in all the cataractous groups. Protein levels of alpha-catenin are decreased in nuclear and cortical cataract group. Protein expression of beta-catenin is similar in all the groups (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Nuclear cataract is usually an age-related type of cataract and oxidation, cross-linking of proteins and protein insolubilization are considered to be its major causes (Truscott 2005). Mutations in Cx50 are known to cause hereditary nuclear cataract (He *et al.* 2011). It has been demonstrated that Cx50 mutations can also inhibit Cx43-mediated cell coupling in the lens epithelium (DeRosa *et al.* 2009). In the present study, mRNA and protein levels of Cx43 were up-regulated in the nuclear cataract group; however, there was no significant change in the localization pattern of Cx43 in nuclear cataract. Decreased levels of alpha-catenin and ZO-1 were found in nuclear cataract but it did not cause increase in cytoplasmic pools of Cx43 as observed in cortical cataract. This could be because of unaltered distribution of alpha-catenin in nuclear cataract. These observations further substantiate importance of alpha-catenin in Cx43 distribution in the LECs. ZO-1 also plays an important role in the paracellular barrier function (Tsukita *et al.* 2009). There are no reports of ZO-1 in relation with human cataract. Reports in other tissues show ZO-1 down-regulation resulting in disturbed paracellular permeability (Pizzuti *et al.* 2004). Data from our studies show down-regulation of ZO-1 mRNA and also of protein in the nuclear cataract group. Also, very weak staining of ZO-1 was observed in nuclear cataract group, implicating some modifications in paracellular transportation. However, further studies are required to establish how alterations in ZO-1 mediated paracellular transportation can lead to formation of nuclear cataract.

PSC is a distinct entity of cataract. Several studies propose human PSC may be the result of defect differentiation of the lens epithelial cells in the equatorial region, causing migration of dysplastic cells to the posterior surface of the lens (Streeten and Eshaghian 1978; Eshaghian and Streeten 1980). Further, it has been explained that low caspase-3 activity in the LECs had resulted in insufficient apoptosis which leads to disturbed differentiation of the LECs in PSC patients (Andersson *et al.* 2003). It has been reported that a high percentage of the LECs of PSC patients exhibited the normal cobblestone morphology with beta-catenin expressed at the cell margin (Rungger-Brandle *et al.* 2005). Our findings illustrate similar results showing that the distribution of beta-catenin and other cell junction proteins in the LECs of PSC patients was not altered despite the changes in expression of cell junction proteins. The changes in expression pattern of these proteins do not mimic their distribution pattern in the LECs of PSC. These changes for now remain inexplicable.

The cell junction proteins were altered mainly in cortical cataract followed by nuclear cataract, and with least

alterations in PSC. The distribution of alpha-catenin and ZO-1 orchestrate the Cx43 gap junctions in the LECs. From this study we report for the first time that altered distribution of ZO-1 and alpha-catenin can lead to modifications of gap junctions containing Cx43 in the LECs and play a role in cataractogenesis. Alterations in cell junction proteins may thus play a crucial role in formation of different types of cataracts. The mechanism underlying these alterations is yet another area of study to be explored further. Further ongoing studies are being carried out to demonstrate the alterations in the types of cellular transportation in different types of cataract.

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