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ORIGINAL ARTICLE

Immunohistochemical Expression of Alpha (A) A Crystallin in Senile Degenerative and Non-Cataract Lenses

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ABSTRACT

Aim: Comparative immunohistochemical study of expression of α A Crystallin in non-cataract lenses and age-related cataract lenses in humans.

Methodology: This was an observational cross sectional study. There are two groups in this study. Group A comprised of 121 senile degenerative cataract lenses from diagnosed patients. Group B included of 10 non-cataract lenses from patients who underwent surgeries for enucleation due to trauma and retinoblastoma. Lenses were fixed in 10% Buffered Neutral Formalin and processed to make paraffin blocks. Immunohistochemistry (IHC) staining was performed on sections using primary antibody for α A crystallin. Data was analyzed through SPSS software version 24.

Results: Immunohistochemical staining of group A showed 80.2% Strong Positive expression while 19.8% showed Intermediate Positive expression of α A Crystallin. 100% Strong Positive expression of α A Crystallin was seen in group B. Comparison of expression of α A Crystallin in two groups showed significant decrease ($p<0.001$) in expression.

Conclusion: Decreased expression of α A Crystallin in IHC stained senile cataract lens indicates the role of structural alterations of lens fibers in pathogenesis of senile cataract. If mechanism involved in causing these alterations can be identified and targeted so that progression of senile cataract may be delayed.

Keywords: Immunohistochemistry, α A crystallin expression, senile cataract, Human eye Lens, Lens Fiber.

INTRODUCTION

The vertebrate lens is a transparent biconvex disc located directly behind the pupil in the eye. Its key function is refraction of light onto the retina[1,2]. It is enclosed completely in a capsule. Underneath the capsule, the subcapsular epithelium is present is composed of a single layer of cuboidal cells[3]. These epithelial cells produce lens fibers, which form the bulk of the lens. Lens fibers are made up of special water-soluble lens proteins, 90% of which are crystallin[1,2]. The α, β and γ Crystallin are found in the vertebrate eye lens of which the α A crystalline accounts for up to 40%[1,2,4]. The primary function of the crystallin is to maintain lens transparency and refractive properties[1,4].

When the lens ages, it loses its transparency and becomes cloudy, a condition known as cataract[5]. Cataract affects the refractive property of lens leading to decreased vision[5]. An estimated 51% blindness globally is attributed to cataract, 32.65% of these cases belong to South Asia[6,7]. Senile cataract is the most common type and occurs after 50 years of age[8].

Fujii et al studied human eye lens but they used analytical ultracentrifuged high molecular weight proteins and water insoluble fractions not the lens as a whole or lens fiber[9]. Upadhyay M. et al studied cataract lens of human specially lens fiber, but they did not use controls for comparison and only used H & E and other stains to study lens architecture, which lack the specificity for crystallin[10]. Zhu et al showed IHC expression of α A crystallin in human cataractous lens and used controls as well but they did it on lens epithelium only[11].

To fill the Paucity in data on specific expression of α A crystallin in lens fiber the objective of the current study was to study immunohistochemical expression of α A crystallin in lens fibers. For this study we used senile degenerative cataract and compared them with non-cataract control lens. Immunohistochemical staining technique was used to observe the expression of α A crystallin in lens fiber in human population.

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MATERIAL AND METHODS

This observational study was conducted at Multidisciplinary Research Laboratory (MDRL), Ziauddin University, Karachi, from August 2020 to March 2021 after the approval by the Ethics Review Committee (1561019SKANA). Group A included 121 senile cataract lenses of the patients above 50 years of age who underwent extracapsular cataract extraction (ECCE) surgeries. Group B included 8 clear lenses from the patients who underwent enucleation surgeries for trauma and retinoblastoma. Samples were collected through non probability, purposive sampling technique. Senile cataract patients were included after the diagnosis of cataract was made by thorough clinical and slit lamp ophthalmological examination, by the ophthalmologist.

The retrieved lens tissues were stored at 4C in 10% Neutral Buffered Formalin (NBF). Patients suffering from Congenital cataract, metabolic disorders like Diabetes, galactosemia, Wilson's disease, history of trauma in eye, eye surgery, history of exposure to radiation, history of thyroidectomy and steroid therapy were excluded.

Processing Of Tissue: Lens tissues after fixation in NBF were dehydrated by passing through ascending concentrations of alcohol, cleared by xylene and infiltrated by paraffin to make tissue blocks.

Sectioning Of Specimen: Transverse sections from the paraffin tissue blocks using rotatory microtome were cut. Sections were 4 microns thick and were allowed to float on hot water bath at 42ºC. The floating sections were then taken on poly-L-lysin coated glass slides for immunohistochemistry. The slides were kept in the hot oven for 2 hours for the purpose of fixing the sections on the slides. They were numbered appropriately for identification. All the slides were routinely de-waxed in xylene and hydrated through decreasing graded alcohol and kept directly in a water bath till the antigen retrieval procedure.

Immunohistochemistry (IHC): Heat-induced epitope retrieval (HIER) method was used for antigen retrieval. The slides were placed in microwaveable vessel filled with 10mM citrate buffer (pH 6.0). The vessel was placed in a microwave resistant box filled with distal water, lid was pierced with holes and microwaved for 6 minutes at 750W. It was ensured that slides remained completely covered with buffer. After a short break of 2 minutes, the dish was

microwaved for another 6 minutes at 750W. Later slides were allowed to stand for 20 minutes before they were removed and rinsed in PBS (pH 7.4) for 5 minutes to remove all traces of retrieval solution.

Slides were then placed in humidity chamber and sections were exposed to hydrogen per oxide solution for 2 minutes to block endogenous peroxidase activity. Sections were then washed by three changes of phosphate buffer saline (PBS) and then nonspecific antibody binding was prevented by applying protein blocking agent (Elabscience, USA) for 2 minutes. Later slides were incubated with primary antibody α A Crystallin (Elabscience, USA) in 1:600 dilution, diluted in standard primary antibody diluent (Elabscience, USA) for one hour in a humidity chamber. All the unbound antibody was removed by washing the sections with 3 changes of PBS. Slides were then incubated with biotinylated secondary antibody (Elabscience, USA) for 30 minutes and streptavidin peroxidase (Elabscience, USA) for 45 minutes. Alpha A Crystalline were visualized by incubating sections with Di-amino Benzidine (DAB) substrate solution (Elabscience, USA) for 10-20 minutes in a dark room. Slides were then washed in water for 2 minutes and were counter stained with hematoxylin. The slides were then mounted with resin based mounting medium after dehydration and clearing sequences.

To validate and differentiate false positive staining of α A Crystallin in IHC, Negative and Positive controls were prepared [12]. All the steps of IHC were followed in preparing positive control but with the controlled liver and kidney tissue. All the steps of IHC were followed in preparing negative control except for application of primary antibody. IHC staining in negative control showed no expression of α A Crystallin, that is no brown stain. (Photomicrograph 1). Evaluation of IHC stained slides was done for stain intensity; for which 10 fields were chosen randomly at 400X magnification. IHC staining was scored as 0, 1, 2, or 3 corresponding to the presence of negative, weak, intermediate, and strong brown staining, respectively[13].

Statistical Analysis: Data was analyzed using SPSS software version 24. Difference in expression was analyzed by using chi square test. Mean and SD values were calculated for quantitative variables, Frequency and percentage for categorical variables. P-value <0.05 was considered statistically significant at 95% confidence interval.

RESULTS

Mean age of group A containing 121 senile cataract lens was 61.82 ± 7.6 years. 63.6% of samples were from male patients and 36.4% from female patients (Table 1). IHC staining in group B containing non-cataract lens showed 100% Brown stain, that is, Strong Positive expression of α A Crystallin. (Table 2, Photomicrograph 2) IHC staining of group A Senile cataract lens showed 97(80.2%) Strong Positive expression while 24(19.8%) showed Intermediate Positive expression of α A Crystallin. (Table 2, Photomicrograph 3 and 4). IHC stained group A senile cataract lens also showed difference in pattern of expression of α A Crystallin. 72(59.5%) showed diffuse expression (Photomicrograph 5), while 49(40.5%) samples had shown focal/patchy expression (Photomicrograph No. 6). The expression of α A Crystallin was significantly decreased (p<0.001) in group A when compared with group B non-cataract lens.

Figure 1: IHC stained negative control of senile cataract lens showing no expression of α A Crystallin shows no brown stain.((100X)

Figure 2: IHC stained non cataract lens showing positive expression of α A Crystallin. (100X)

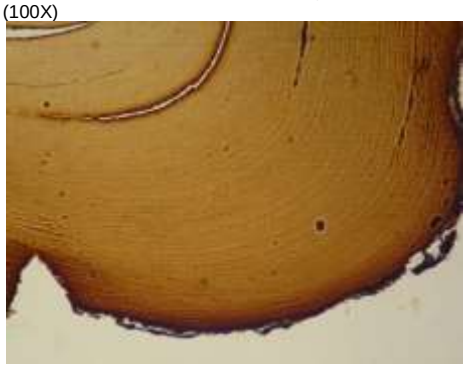


Figure 3: IHC stained senile cataract lens showing strong positive expression of α A Crystallin (100X)

Figure 4: IHC stained senile cataract lens showing intermediate positive expression of α A Crystallin (100X)

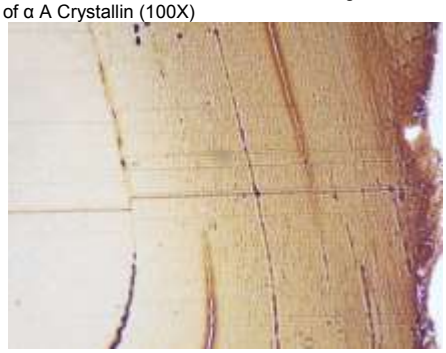


Figure 5: IHC stained senile cataract lens showing focal pattern of expression of α A Crystallin (100X)

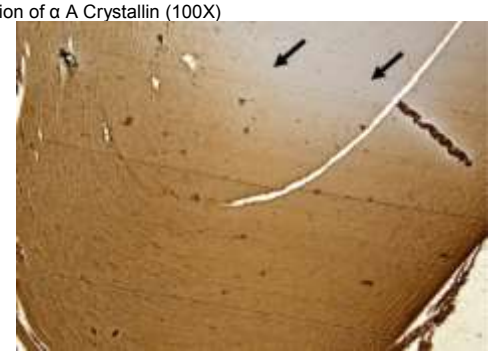


Figure 6: IHC stained senile cataract lens showing diffuse pattern of expression of α A Crystallin (100X)

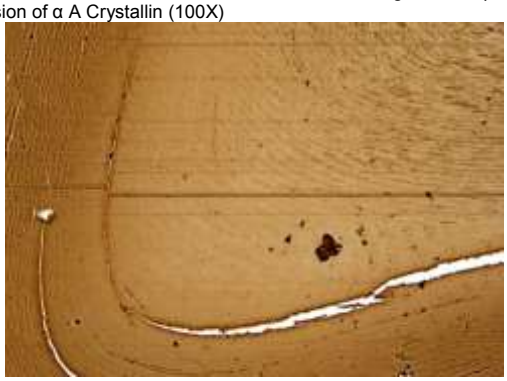


Table 1: Sample distribution in Group A Senile cataract lens (n=121) according to Gender

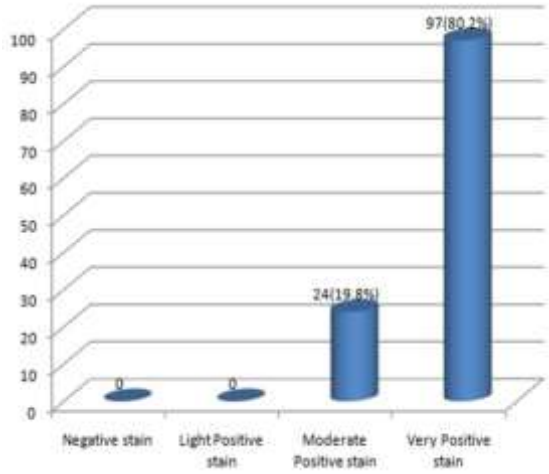
Gender	Frequency	%age
Male	77	63.6
Female	44	36.4
Total	121	100

Table 2: Difference in IHC expression of α A Crystallin in Group A senile cataract and Group B non-cataract lens.

Group of lenses	IHC expression & score			
	Negative (0)	Weak +ve (1)	Intermediate +ve (2)	Strong +ve (3)
Group A Cataract lens (n=121)				
Frequency	00	00	24	97
%age	00	00	19.8	80.2
Group B Non-cataract lens (n=10)				
Frequency	00	00	00	10
%age	00	00	00	100

*p value < 0.05 = Significant

Figure 1: IHC expression of α A Crystallin in Group A senile cataract lens.



DISCUSSION

Opacification of the lens substance is known as cataract. If located on the visual axis, it leads to visual impairment and blindness[5]. 51% of cataract is attributed as a cause of blindness globally[6]. Age related cataract is the main type followed by congenital, traumatic and occupational[8].

The mean age of senile cataract lens of our study matches with the mean age of Michal Szymon Nowak 60.4 ± 7.1 years[14]. Also 42% of cataract cases in the study conducted by Upadhyay M. et al. belonged to age group of 60 – 70 years [(10)]. Kant et al. described the beginning of senile cataractogenesis around the fourth decade of life, pathogenesis of which was attributed to the oxidative stress damage caused by a barrier to the transport of glutathione around the lens nucleus[8,15].

We observed increased male frequency as compared to females in population of Karachi, Pakistan, in contrast to other studies conducted in other regions. Uzma Ali Kant reported 56.37% female and 43.63% male in Rawalpindi, Pakistan population[15]. Nirmalan et al also observed higher female (55.19%) to male (44.77%) incidence of cataract in Indian Population[16]. A Polish study conducted in 2010 also showed parallel higher ratio of female over male[14].

The contrasting higher rate of cataract in male in our study can be correlated with the increased length of environmental insults especially in the occupational type[17]. This has been proven by Bragin et al. in their study which they conducted among industry workers. Their sample likewise showed higher number of males 75%[17,18]. Environmental insult also includes Ultraviolet B radiations, main source of which is long hours of sun exposure. Hui et al. studied the Ultraviolet B induced oxidative damage causing crystallin denaturation in lens epithelium[19].

Our study demonstrated decreased IHC expression of α A Crystallin in senile cataract lens than in non-cataract lens. These findings correspond with the findings of Zhu et al., who proved decreased expression of α A Crystallin in lens epithelium in age related cataract and high myopic cataract compared to controls using IHC[11]. The decreased expression of α A crystallin in lens epithelial cells is also observed by Hua et al., due to ultraviolet B radiation insults in rat eye lens[19].

The difference in pattern of IHC expression that is diffuse and focal, of α A Crystallin is same to that reported by Upadhyay et al., in cataract lenses using H & E and other stains[10].The diffuse and focal pattern of expression showing structural changes in fibers in cataract lens from increasing age group proves the fact that lens fiber changes are related to ageing, priming the formation of cataract in human lens[10,20].

CONCLUSION

This study increases our understanding in studying the role of lens fiber modifications in pathophysiology and progression of senile cataract and correlates the decreased expression of α A Crystallin through IHC. These modifications can lead us towards the understanding of the lens fiber membrane interactions, role of α A Crystallin within and with the cell fiber membranes. Weekly consumption of fish, green leafy vegetables, citrus fruits and pure cooking oil is of high importance as impact of type of food is focal in cataract formation. Daily vitamin supplements and water intake is recommended. Protective eyewear is advisable against sun's harmful rays as an effective method of UVR protection for the eyes. Future research studies should be directed towards better understanding of molecular process involved in cataractogenesis. This may lead to better and early treatment strategies, which will ultimately help in decreasing the burden of this disease.

Recommendation: Research aimed at identifying structural disturbances at molecular level which may decrease the chaperone function of α A Crystallin with age is required. This will help in identifying molecular or genetic targets involved in cataract formation.

Conflict of interest: Nil

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