

Summary and Conclusion

Cataract is the opacification of lens which accounts for majority of the blindness caused in India. It generally occurs with the advancement of age and results in blurred vision. The only treatment prevailing today is the removal of this cataractous lens by surgery and replacing it with intraocular lens. Lens crystalline proteins are found in major proportion in the lens that may become susceptible to post translational modifications with increase in age. There are many confounding factors and clinical complications behind the cause of cataract. Diabetes mellitus and hypertension are two among these major clinical complications that are associated with the development of cataract. Oxidative stress in lens is thought to play a crucial role in the progression of cataract. The present study was designed to compare the protein changes and biochemical profiling among cataractous subjects with and without clinical complications (diabetes mellitus and hypertension). This might reveal the group of cataractous subjects who are more liable to cataract progression. The objectives of the present research work designed with the aforementioned facts were:

- To analyse the changes in soluble protein and insoluble protein in the eye lens of the cataractous subjects
- To analyse any correlation between selected biochemical parameters and insoluble protein content in eye lens
- To study the eye lens architecture in cataractous subjects
- To study post translational changes in eye lens proteins leading to cataract

The cataractous eye lens samples for the research work were obtained from Aravind Eye Hospital, Coimbatore, Tamil Nadu and Eye

Foundation, Coimbatore, Tamil Nadu. Approval from Institutional Human Ethical Committee (HEC.2011.26) was obtained for the study. Questionnaires were administered to the subjects under the study and informed consent was obtained from them. The study was carried out in 280 cataractous subjects who satisfied the desired criteria. They were categorised into six groups namely:

- Apparently Normal Cataract Men (ACM) with n= 46
- Apparently Normal Cataract Women(ACW) with n= 47
- Diabetic Cataract Men (DCM) with n= 50
- Diabetic Cataract Women (DCW) with n= 50
- Hypertensive Cataract Men (HCM) with n= 43
- Hypertensive Cataract Women (HCW) with n= 44

The cataractous lenses from conventional surgery were obtained and preserved in saline at -40°C until further analysis. Lens homogenate was prepared in 0.1M sodium phosphate buffer for the biochemical profiling. The study was carried out in five phases.

In Phase I, the subjects were selected and grouped as mentioned above. From the questionnaires, the demographic characteristics and life style of the selected cataractous subjects were recorded. The results seemed to have no influence of marital status on cataract development. Status of unemployment was found to be more among apparently normal cataract women. Lower economic status was observed in almost all the groups which suggested that economic status may have an influence on the development of cataract like cases of malnourishment. Majority of the cataractous subjects were non vegetarians. Subjects with clinical complications showed higher proportion of consumption of non vegetarian diet. Consumption of fruits and raw vegetables were also found to be less in almost all the cataractous subjects selected for the study. Both diabetic cataract men and women were observed to consume very low quantity of fruits and raw vegetables. Apparently normal cataract men were observed to do

exercise like walking for 3 days/week. Hypertensive cataractous subjects were observed to handle stress very poorly when compared to diabetic and apparently normal cataractous subjects.

In Phase II, the profile of the cataractous subjects were documented which included age, height, weight, body mass index, systolic pressure and diastolic pressure. Subjects with clinical complications were liable to develop cataract at much earlier age as compared to apparently normal cataract group. Height, weight and BMI differed significantly among ACM and ACW groups and also among DCM and DCW groups. Hypertensive cataractous subjects showed a significant increase in systolic and diastolic pressure as compared to other groups.

Biochemical assessment and their correlation with cataract development was attempted in Phase III. The lenses extracted after surgery from subjects with and without clinical complications were preserved in saline. The lens dimensions namely diameter, thickness and weight were recorded. The results suggested that these dimensions differed significantly among cataractous subjects with and without clinical complications. There was no significant difference observed in lens dimensions among men and women of apparently normal and clinically complicated cataract groups.

Lens is comprised of mainly crystalline proteins and they may undergo some changes during cataract development. In order to analyse these changes, the levels of soluble protein, insoluble protein and total protein were estimated in the eye lens extracted from selected subjects. The results indicated that soluble protein and total protein levels were decreased in cataractous subjects whereas insoluble protein levels were increased in cataractous subjects. A significant increase ($p < 0.05$) in the levels of insoluble protein was observed in DCW group as compared to men and women of apparently normal cataract groups.

As the lens is exposed to oxidative stress, the enzymatic antioxidant activity, activities of glutathione system, non enzymatic antioxidant levels, lipid peroxidation status, nitrite levels and protein oxidation parameters were

analysed. The activities of SOD and CAT were found to be significantly decreased ($p < 0.05$) in all other five groups when compared to ACM group. The components of glutathione system comprised of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and reduced glutathione (GSH). A significant variation ($p < 0.05$) in the status of glutathione system was observed between diabetic cataractous groups and hypertensive cataractous groups. On the whole, cataractous lenses operated from diabetic groups exhibited decreased activities of GST and GR.

The levels of vitamin C and E were found to be decreased in all five groups when compared to ACM group whereas vitamin A levels seemed to show no significant variations among the cataractous groups.

A significant increase ($p < 0.05$) in the levels of lipid peroxidation was observed in the lenses operated from diabetic cataract women when compared to ACM group. Nitrite is a metabolite of nitric oxide that may cause changes in lens protein. Higher concentration of nitrite was observed in diabetic cataract men and women when compared to other groups.

A significant increase ($p < 0.05$) and a significant decrease ($p < 0.05$) were noted in the levels of protein carbonyl and protein sulphydryl respectively which were the markers of protein oxidation, in cataractous lens extracted from diabetic cataract groups in comparison to ACM group and hypertensive cataract groups.

Polyol pathway enzymes included aldose reductase and sorbitol dehydrogenase which have a major role in the cataract development. Diabetic cataractous subjects showed major significant changes in the activities of polyol pathway marker enzymes. Membrane bound enzymes that included $\text{Na}^+\text{K}^+\text{ATPases}$, $\text{Ca}^{2+}\text{ATPases}$ and $\text{Mg}^{2+}\text{ATPases}$ play a major role in the membrane fluidity. Their altered activities might result in the opacification of lens. The activities of membrane bound enzymes in cataractous eye lens were found

to be significantly decreased ($p < 0.05$) in diabetic cataract and hypertensive cataract groups in comparison to ACW group.

Levels of all the four glycoproteins hexose, hexosamine, fucose and sialic acid in the lens tissue extracted from diabetic cataract subjects and hypertensive cataract subjects were found to be significantly increased ($p < 0.05$) in comparison to ACM group, suggesting that diabetic cataract subjects showed an increased concentration of glycoproteins when compared to hypertensive cataract subjects and apparently normal cataract subjects.

Among the basic biomolecules, glucose levels in cataractous eye lens from diabetic cataract patients indicated a significant increase ($p < 0.05$) when compared to other groups. Levels of DNA and RNA in cataractous eye lens from DCM and DCW were observed to be significantly decreased ($p < 0.05$) as compared to ACM group. A significant decrease in the levels of total cholesterol was observed in lenses from DCW group as compared to ACW group and a similar trend was also observed between ACW group and ACM group.

Correlation analysis was performed to identify the association of biochemical parameters with cataract development. The results suggested a positive correlation between age and levels of insoluble protein. Lens dimensions were found to have a negative correlation with age and lens diameter alone showed a significant correlation with age. A strong inverse correlation was observed between soluble protein and insoluble protein of the eye lens tissue.

A positive correlation was observed between lipid peroxidation and insoluble protein in eye lens extracted from cataract subjects with and without clinical complications. A significant negative correlation was observed with insoluble protein between enzymatic antioxidants and glutathione system components whereas non enzymatic antioxidants showed a non significant negative correlation. All the antioxidants showed a negative correlation with insoluble protein suggesting the role of antioxidants in cataract development. Protein carbonyl levels showed a significant positive correlation with insoluble

protein levels in eye lens tissue whereas protein sulphhydryl levels were negatively correlated with insoluble protein concentration in the eye lens of the cataractous subjects. Activity of aldose reductase showed a significant positive correlation with insoluble protein concentration and lipid peroxidation levels in the eye lens from cataractous subjects whereas a significant negative correlation was observed between the activities of aldose reductase and sorbitol dehydrogenase in cataractous eye lens.

Glycoprotein staining proved that glycation would have occurred to a larger extent in DCM and DCW as compared to ACM, ACW, HCM and HCW groups. Gamma crystalline protein was isolated by gel filtration chromatography and subjected to SDS PAGE to determine the molecular weight of the protein. These fractions were then quantified by HPLC which revealed decreased amount of the protein in eye lens extracted from diabetic cataractous subjects when compared to other groups.

Phase IV involved the morphological and histopathological analysis of the cataractous lenses from selected subjects. Lenses were viewed through Environmental Scanning Electron Microscopy (SEM) that was coupled with Energy Dispersive Spectroscopic (EDAX). The SEM images showed the morphological appearance of the eye lens tissue of cataractous subjects with and without clinical complications. The cataractous lenses from all the groups showed irregularity in the arrangement of fibres with a wide gap between them. The mineral composition from cataractous lenses was revealed from EDAX data. Histopathological analysis revealed the damage caused to the cataractous lens tissue from subjects with and without clinical complications.

In Phase V, Fourier Transform Infrared (FTIR) Spectroscopy and Raman Spectroscopy were performed to identify the functional groups. The change in peaks obtained from FTIR and Raman Spectroscopy in lens extracted from cataract subjects with and without clinical complications suggested that the proteins might undergo post translational modifications which might change the

secondary structures of proteins. With these spectral analyses, it was difficult to identify post translational modifications.

To conclude, diabetic and hypertensive subjects are suggested to develop cataract at an early age compared to apparently normal cataractous subjects. From this study, it may be said that diabetic cataractous subjects showed more variation and were more affected during cataract development. It may become necessary that if a subject is diagnosed with diabetes or hypertension, then an ophthalmic examination and timely treatment may prevent the development of cataract.

Scope for Future Research

- Post translational modifications of the eye lens proteins can be studied by two dimensional electrophoresis
- Molecular docking studies for aldose reductase inhibitors can be investigated
- Plants can be explored for anti cataract properties
- The lens crystalline protein changes can be studied by LCMS and NMR studies