ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIO CHEMISTRY

OXIDATIVE STRESS AND CALCIUM LEVELS IN SENILE AND TYPE 2 DIABETIC CATARACT PATIENTS

Corresponding Author

DEEPA. K

Department of Biochemistry, JSS Medical College, Mysore, India.

Co Authors

MANJUNATHA GOUD B.K[2], NANDINI M[3], ASHA KAMATH[4], and SUDHIR[5], BHAVNA NAYAL[6].

[2]Department of Biochemistry, MMMC, Manipal University, Manipal, Karnataka, India.
[3]Department of Biochemistry, KMC, Manipal University, Mangalore, India.
[4]Department of Community Medicine, KMC, Manipal University, Manipal, India.
[5]Department of Community Medicine, JSS Medical college, Mysore, India.
[6]Department of Pathology, MMMC, Manipal University, Manipal, Karnataka, India.

ABSTRACT

Diabetes mellitus is a major risk factor for the development of cataract and oxidative stress is thought to be a major factor to initiate this process. The transparency of lens depends on optimum concentration of intracellular calcium, which is altered by the process of oxidative stress. The main objective of the present study is to compare level of oxidative stress markers in both senile and diabetic cataract patients. Serum was analyzed for MDA, total antioxidant activity [AOA], and total calcium, while lens was analyzed for AOA and total calcium. There was a significant increase in serum MDA ($p<0.001$) in patients with diabetic cataract. Antioxidant activity was significantly reduced in serum ($p<0.05$) as well as lens ($p<0.01$) in these patients. However there was no significant change in the serum and lens calcium levels. Comparison between mature and immature cataractous lenses showed a significant increase in the calcium and decrease in antioxidant activity. A positive association of lens calcium with serum MDA ($r=0.714$) was observed. Thus, it is inferred that diabetic patients are subjected to oxidative stress at much earlier age than compared to senile cataract patients.

INTRODUCTION

Cataract is the major cause of curable blindness, accounting for more than half of the total blindness and its prevalence in developing countries is more than in developed once (1,2). One of the often seen consequences of aging process is development of cataract and diabetes mellitus is a major risk factor for the development of cataract at an early age compared to senile cataract (3-5).
Causative factors of cataract are many and its etiology is unclear. However imbalance between the oxidant and antioxidant levels is thought to be the major factor which initiates the process of cataractogenesis (6). The transparency of the lens depends on the unique arrangement of tightly packed fibers which in turn rely on protein structure and optimum concentration of calcium. This transparency is altered by the process of oxidative stress Thus uncontrolled peroxidation of biomembrane, a direct effect of oxidative stress can lead to profound effects on membrane structure and functions thereby, affecting the calcium homeostasis in lens (7). Altered calcium homeostasis contributes to lens opacity in number of way (8)
An antioxidant is a molecule that is able to combat reactive oxygen species (ROS) and there by plays an important role against the oxidative stress induced lens opacity (9). Hyperglycemia, seen in diabetic cataract can stimulate oxidative stress by the auto oxidation of glucose in the presence of transition metals Hence malondialdehyde (MDA) and total antioxidant activity is estimated to assess the level of oxidative and antioxidant status in senile and diabetic cataract patients and total calcium is estimated as effect of disturbed balance between these statuses. Thus the main objective of the present study is to compare the level of oxidative stress in both senile and diabetic cataract patients. The result of this study may be useful in deciding whether oxidative stress has any role in the development of diabetic/ senile cataract. Supplementation of antioxidant to the patients before the threshold age group could be able to prevent the occurrence of lens opacity.

MATERIAL AND METHODS

The study included two groups of 25 subjects in each, aged between 45 -70 years GROUP 1- senile cataract patients and GROUP 2- Type 2 diabetic cataract patients. History of steroid intake, ophthalmic disease, and other systemic disorders are excluded from study. The study was approved by the Institutional Time Bound Research Ethics Committee. A written informed consent was taken from the subjects.
4ml of venous blood was collected during pre-operative peroid in plain vacutainers under aseptic precaution. Serum was separated and analyzed on same day for the following parameters: Malondialdehyde [MDA], total antioxidant activity and total calcium.
Cataractous lens was obtained after surgery. Lens were collected in ice cold 0.9% normal saline container and they are separated as mature cataractous lens and immature accordingly(4) and they were transferred in ice box to the place of analysis and analyzed on the same day immediately for the following parameters: Antioxidant activity, calcium and proteins.

Biochemical estimations
Serum MDA was estimated by thiobarbituric acid (TBA) reaction (10,11). TBA reacts with MDA to give a pink colour which is measured spectrophotometrically at 535nm. The total antioxidant activity was determined by Koracevics method (12). The assay measures the capacity of serum to inhibit the production of thioabarbituric acid reactive substances from sodium benzoate under the influence of the

oxygen free radicals derived from Fenton reaction. The reaction was measured spectrophotometrically at 535nm.Calcium in the serum and lens was determined by Cresolphthalein complexone method (13) using calcium kits from Aspen Lab Pvt Ltd. Cresolphthalein complexone a metal complexing dye reacts with calcium ions in alkaline medium forming a purple color. Intensity of the color formed is directly proportional to the calcium concentration and was measured photometrically at 570nm. For determining the lens calcium, the lens was homogenized with 1.2ml ice cold 0.01M phosphate buffer pH 7.4. Mixture was centrifuged at 2500rpm for 10mins. 100µl supernatant was used for analysis. Lens antioxidant activity was also estimated by Koracevics procedure using 10µl of the supernatant. Protein content was determined by the Lowry's method (13). Blue colour obtained was measured spectrophotometrically.

Statistical analysis

The data was subjected to statistical analysis by using Analysis of Variance [ANOVA]. Since two way ANOVA showed that the interaction between cataract and maturity is significant, the comparison between the type of cataract and maturity was done separately using independent 't' test. Pearson's correlation was applied to correlate between parameters. The results are expressed as mean ± standard deviation (SD). $p<0.005$ was considered statistically significant.

RESULTS

The values of serum MDA, antioxidant activity and total calcium is presented in table1 and the values of antioxidant activity and calcium in the lens are presented in table 2.

Significant increase in the serum MDA and decrease in the antioxidant activity is observed in diabetic cataract patients. A significant reduction in antioxidant activity is also seen in diabetic cataractous lens. However there is no significant change in serum calcium levels. Comparison of serum and lens parameters in patient with immature and mature cataract is shown in table-3. A significant increase in serum MDA and a decrease in antioxidant activity were observed in mature cataractous patients .A increase in the lens calcium was also observed in mature cataract. These changes were seen in both senile and diabetic cataractous lens

A negative correlation of AOA with MDA was seen in serum and lens of patients with diabetic and senile cataract and this is shown in fig 1 and 2 (r= -0.805 and -0.869). Similarly a positive correlation of lens calcium with serum MDA was observed in fig-3 and the r value is 0.714.

Table - 1

Comparison of serum values between Senile and Diabetic cataract patients

	n	Senile Cataract (Mean ± SD)	n	Diabetic Cataract (Mean ± SD)
Age (Years)	25	66.72 ±7.329	25	60.36 ±7.46**
MDA (µmol/L)	25	5.586 ±1.117	25	6.652 ±0.587***
Antioxidant Activity(mmol/L)	25	0.853±0.092	25	0.793±0.073*
Calcium (mg/dl)	25	9.046± 0.431	25	8.945± 0.407

n = number of subjects
**=significant (p < 0.05)*
***=Highly significant (p < 0.01)*
****= Very highly significant (p < 0.001)*

Table - 2

Comparison of lens values between senile and diabetic cataract patients

Lens	n	Senile Cataract	n	Diabetic Cataract
Antioxidant Activity [mg/mg of protein]	25	1.171± 0.329	25	0.942 ±0.207**
Calcium (mg/mg of protein)	25	0.899 ±0.121	25	0.938 ±0.116

p- value < 0.01

Table - 3

Comparison of serum and lens parameters between Immature and Mature cataract

	n	Immature	n	Mature
Age (years)	24	62.25±7.49	26	64.73±8.398
MDA (µmol/l)	24	5.35±0.939	26	6.830±0.451***
Antioxidant Activity(mmol/l)	24	0.886±0.069	26	0.765±0.059***
Calcium(mg/dl)	24	8.932 ±0.438	26	9.055±0.397
Antioxidant Activity (mg/mg of lens protein)	24	1.320± 0.208	26	0.820± 0.085***
Calcium (mg/mg of lens protein)	24	0.805± 0.416	26	1.024± 0.056***

p value < 0.001

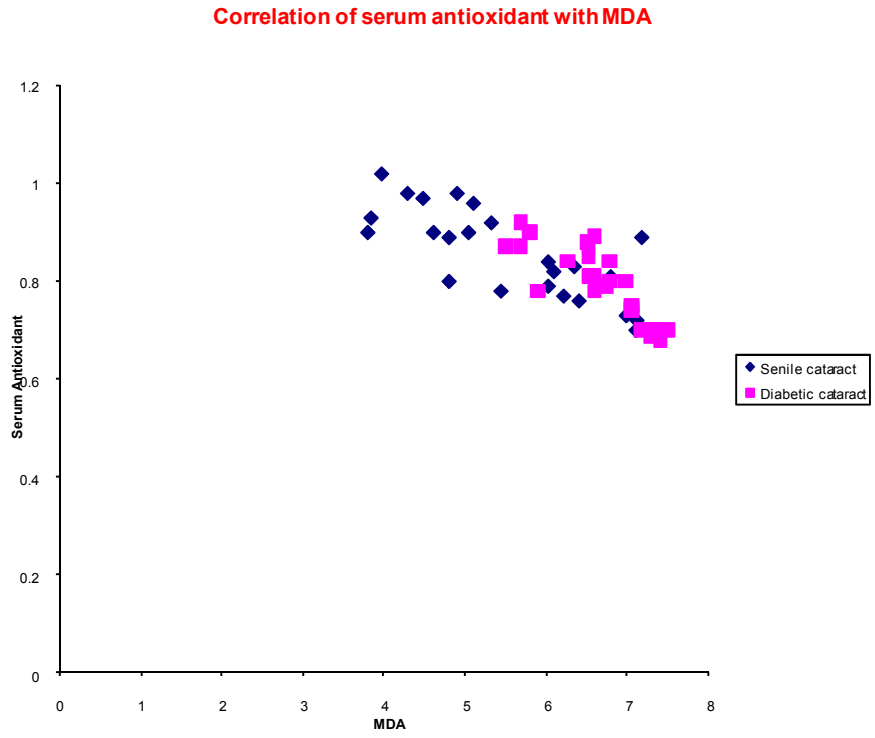
Correlation of serum antioxidant with MDA
Serum Antioxidant
MDA
Senile cataract
Diabetic cataract

Fig-1

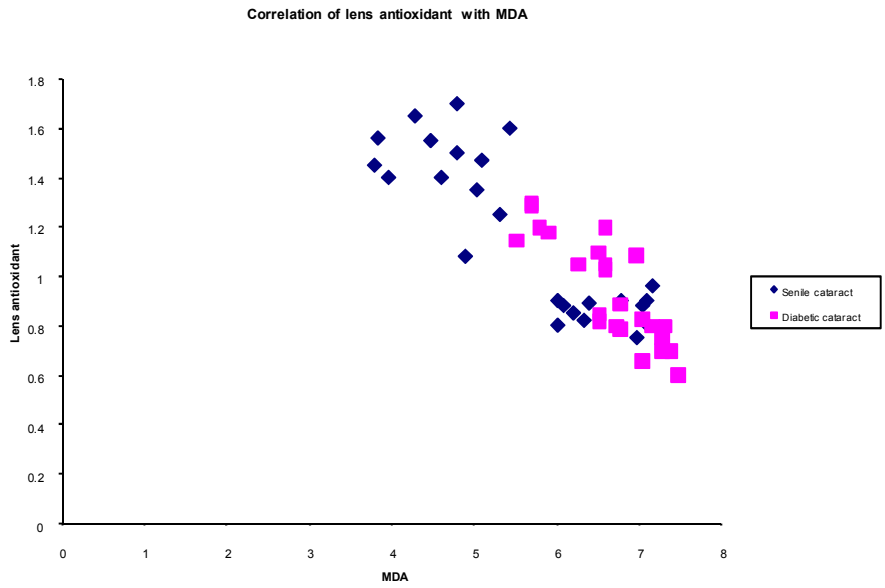
Correlation of lens antioxidant with MDA
Lens antioxidant
MDA
Senile cataract
Diabetic cataract

Fig-2

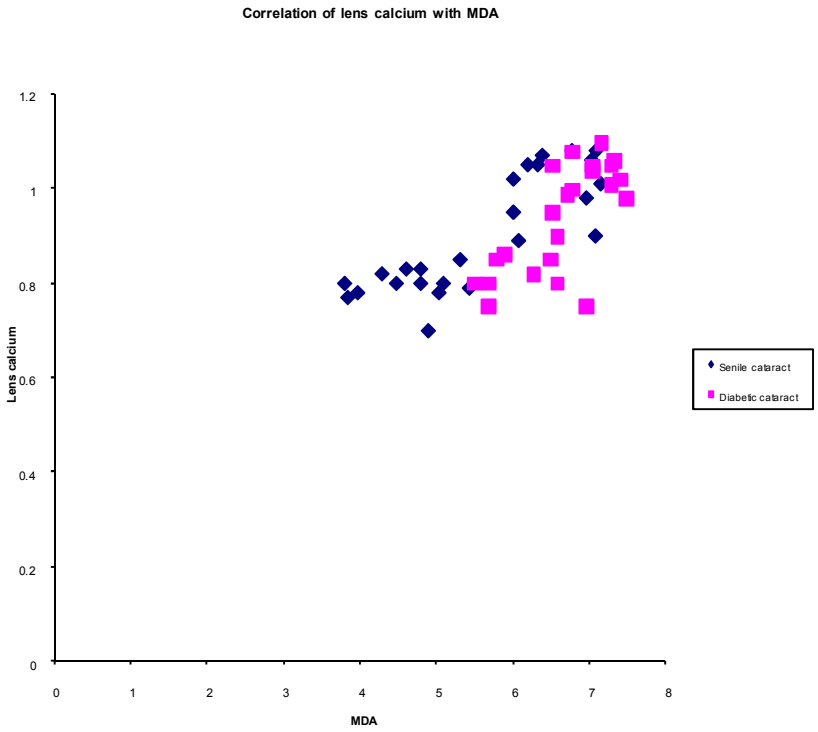

Fig- 3

DISCUSSION

Oxidative stress plays an important role in pathogenesis of cataract, the most important cause of visual impairment associated with advanced age and diabetes. In our study we have observed high levels of MDA in diabetic cataracts compared with senile cataract patients. A 19% increase in the serum MDA was observed in diabetic cataract in comparison with the senile cataract in our study. A 27% increase in the serum MDA values were observed in mature diabetic cataractous groups (15).

The increased levels of lipid peroxidation product (MDA) in diabetes are due to increased production of reactive oxygen species caused by hyperglycemic status, hyperinsulemia and hyperlipidemia which are commonly associated with diabetes (16, 17, 18). The total antioxidant activity in serum as well as in the lens was reduced significantly in diabetic cataracts. It is about 7% and 19% respectively. Previous studies have reported a reduced total antioxidant status in aqueous humor in patients with diabetic cataract than those with senile cataract (19). The reduced antioxidant activity in diabetics could be explained by hyperglycemia induced complication such as reduced gene expression of antioxidant enzymes and inactivity of enzymes by glycation (20, 21).

In our present study, the serum and lens calcium levels between senile and diabetic cataract has not shown much significant differences. This is in agreement with the studies done by Daxin (22). In the present study, the serum calcium levels at different stages of cataract i.e. immature and mature

has not shown any significant difference, but the lens calcium levels in mature stage of cataract is increased than in immature stage. The increased level of calcium in mature cataract lens is due to increase lipid peroxidation which has altered the membrane permeability of lens leading increased intracellular calcium levels and in turn leads to cataract formation (23, 24, 25)

Thereby, the present study proves that the total antioxidative activity in serum and lens is a dynamic equilibrium that is influenced by the interactions between each antioxidant constituents, is reduced significantly in diabetic cataract compared to that of senile. Hence, estimation of total antioxidant activity can serve as good predictive marker in assessing the disturbed redox status which is responsible for opacification of lens.

Thus from our studies, it is inferred that diabetic patients are subjected more to oxidative stress at much earlier age than compared to senile cataract patients. Hence supplementation of an adequate dose of antioxidant to these diabetics at much earlier age may be beneficial in delaying the diabetic complications.

REFERENCES

1. Javitt JC, Wang F, West SK. Blindness due to cataract: Epidemiology and prevention.*Annu Rev Public Health,* 17:159-77, (1996)
2. Nirmala PK, Krishnadas R, Ramakrishna R, Thulasiraj RD, Katz J, Tielsch JM, Robin AL, Lens Opacities in a rural population of Southern India: the Aravind Comprehensive Eye study. *Invest opthalmol Vis Sci,* 44:4639-43, (2003)
3. West SK, Valmadrid CT, Epidemiology of risk factor for age related cataract. *Survey of ophthalmology,* 39:323-327, (1995)
4. Ramanjit Sihata, Radhika Tandon.The Lens Parson's disease of eye, 20th edition Elsevier's, 2007; 247-249.
5. Micelli ferari T, G Vendmiale. Role of lipid peroxidation in the pathogenesis of myopic and senile cataract. *British journal of ophth*, 80:840-43, (1996)
6. Ughade SN, Zodpey SP, Khandkar VA. Risk factors for cataract; a case control study. *Indian journal ophthalmology*, 46:221-27, (1998)
7. Goosay JD, Tuan WM, Garcia CH. A Lipid peroxidation mechanism for cataract formation in rabbit. *Inv ophth Vis sc,* 25: 608-12, (1984)
8. Biju PG, Rooban BN, Lija, Drevagenim D, Gayathridevi. Prevents selenite induced oxidative stress and calplain activation in cultured rat lens. *Molecular vision,* 13: 1121-29, (2007)
9. Paul L. Kaufman. Albert Alon. The Lens Alders Physiology of eye clinical application Harcourt. Brace and company Ltd., 10th edition page 120-21.
10. Esterbaeur H, chessman KH. Determination of aldehydic lipid peroxidation products, MDA and 4-hydroxynanenal'. Methods in enzymolmgy. *Academic pres*, 186:407-21, (1990)
11. Poornima K, Nandini M, Asha K. Oxidant and antioxidant status in vegetarian and fish eaters. *Indian .J. Clin. Biochem,* 18:197-205, (2003)
12. D. Koracevic.g Koracevic V Djordjevic S Andrejevic, V Cosic. Methods of the measurement of antioxidant activity in human fluids. *J.ClinPathology,* 54:356-361, (2001)
13. Stern J and WHP Lewis. The colorimetric estimation of calcium in serum with o-cresolphthalein complexone. *Clinica chemical Acta*, 6:576-80, (1957)

14. Lowry, Harnold Varlay, Alan H, Gawenlock, Maurice bell. Practical Clinical Biochemistry volume 1, 5th edition 555-556.
15. Orkida Donma, Eda Ozkanat, Yorulmaz . Blood and lens lipid peroxidation and antioxidant status in normal, senile and diabetic cataractous patients. *Current eye res,* 25:9-16, (2002)
16. Evans JL, Gold fine. Oxidative stress and stress activated signaling pathways a unifying hypothesis of type 2DM. *Endocrine review*, 23:599-622, (2002)
17. Jacob Vaya, Michael Aviram. Nutritional antioxidant mechanism of action, analysis of activities and medical applications. *Free Rad Biol Med*, 28: 141-164, (2000)
18. Gort A, Imlay JA. Balance between endogenous super oxide stress and antioxidant defenses. *Journal Bacterial*, 180: 1402-10, (1998)
19. Hulya Aksoy. Diabetic cataract and the total antioxidant status in aqueous humor. *Clinca chemi and laboratory medicine,* 2:143-145, (2001)
20. King GI, Biochemical and molecular mechanisms in the development of diabetic vascular complication. *Journal of Diabetes,* 45-50, (1996)
21. Nishikawa, Normalizing mitochondrial super oxide production blocks, three pathways of hyperglycemic damage. *Nature*, 404:787, (2000)
22. Daxin Tang, Douglas Borchman, Mart C, Yappert, Influence of age, diabetic and cataract on calcium, lipid calcium and protein calcium relationships in human lenses. *Inv ophth vis.sci,* 44: 2059-66, (2003)
23. Tomlinson J, Bannister SC. Analysis of rat lens calcium fluxes, evidence for sodium-calcium exchange. *Exp eye Res,* 52: 619-27, (1991)
24. Borchman D, Lamba OP, Yappert MC, Structural characterization of lipid membrane from clear and cataractous human lenses. *Exp eye res,*57:199-208, (1993)
25. Galvan A, Levis CF. Calcium regulation by lens plasma membrane vesicles. *Arch Biochem and Biophy*, 264:472-78, (1988)