ASSESSMENT OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN AGE RELATED CATARACT IN A RURAL POPULATION

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ABSTRACT

Oxidative stress was assessed by estimating lipid peroxidation product (LPO) in the form of thiobarbituric acid reactive substances (TBARS), enzymatic antioxidants in the form of superoxide dismutase (SOD), catalase and nonenzymatic antioxidant vitamins e.g.vitamin C, β carotene and vitamin E in either serum or plasma or erythrocytes in 190 cases of age related cataract in the age group of 50-80 years. 190 cases were grouped into three morphological types namely, 73 cases of cortical, 77 cases of posterior subcapsular and 40 cases of nuclear cataract and values of LPO and antioxidants were compared with 78 cases of age matched healthy control groups. Plasma TBARS levels were significantly high but serum SOD, plasma vit C and β carotene levels were significantly low in cataract cases when compared with control groups. There were no significant differences in the erythrocyte levels of catalase and plasma levels of Vit E between cataract cases and control groups. No significant changes of parameters were seen among three different morphological types of age related cataract. The present study shows that the oxidative stress may play an important role in the age related cataract.

KEY WORDS

Oxidative stress, Lipid peroxidation product, Antioxidant, Age related cataract.

INTRODUCTION

Oxidative stress is a term used to describe any challenge in which pro-oxidants predominate over antioxidants (1); it may be due to either increased production of reactive oxygen species (ROS) or decreased levels of antioxidants (enzymatic and nonenzymatic) or both. Oxidative stress is implicated in the etiopathogenesis of a variety of diseases like Cancer, Atherosclerosis, Cataract, Diabetes mellitus, Alzheimer's disease, Parkinson's disease etc.(2). Although the precise etiopathogenesis is not clear, oxidative stress is thought to play a crucial role in the development of age related cataract (3). Cataract is a complete or partial opacification of sufficient severity on or in the human lens or capsule to impair vision. Cataract is one of the major causes of visual impairment leading eventually to blindness. In developing countries like India, the magnitude of the problem is overwhelming. Age related cataract is the most common variety of cataract. Usually some degree of cataract is present after the age of 50 years. One

difficulty in studying the role of antioxidants in human cataract formation is the inability to measure directly these antioxidant components in the lens in vivo (4,5). Another difficulty in studying the role of pro-oxidant and antioxidants in the lens is the difficulty in getting the normal human lenses, which should be used as controls.

Therefore, several investigators (4-10) used plasma or serum or erythrocyte to characterize pro-oxidant and antioxidant status in patients with cataract and the control groups. In the present study, we have estimated TBARS, enzymatic antioxidants (SOD, Catalase) and non-enzymatic antioxidant vitamins (Vit C, β -carotene, Vit E) in serum or plasma or erythrocytes in age related cataract cases (50-80 years) specially in different morphological types, namely cortical, posterior subcapsular & nuclear cataract and compared these parameters with the age matched control groups and that too in a rural population.

MATERIALS AND METHODS

190 cases of cataract aged 50-80 years who were admitted in the ophthalmology ward of MGIMS, Sevagram which is a medical college and hospital situated in the rural area of Wardha District of Maharashtra were screened. There were basically three types of age related cataract cases namely cortical, posterior subcapsular (PSC) and nuclear. Only pure cortical, pure PSC and pure nuclear cases were

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taken in the study excluding the mixed varieties of cataracts on the basis of the examination and diagnosis of a senior ophthalmologist. Only those cases that had a single type of opacity in both eyes were placed in the pure category. There were 73 patients in pure cortical group, 77 patients in pure PSC group and 40 patients in pure nuclear group of cataracts. Control group comprised of 78 persons aged 50-80 years with a visual acuity of 6/6 or better in both eyes and no lens opacities in either eye on slit lamp or ophthalmoscopic examination. Patients with ocular surgery, trauma, infection, inflammation of the eye, diabetes mellitus were excluded from the study. Known cases of cardiovascular disorders, rheumatoid arthritis and carcinomas where free radical damages has been commonly incriminated were also excluded from the study. Other known and unknown diseases and disorders which cannot be excluded, will affect the cataract cases and control groups similarly.

Chemicals

SOD and malondialdehyde (MDA) standards were taken from Sigma chemicals USA, Catalase from BDH biochemicals U.K., β carotene from Merck India, Vitamin C as L- Ascorbic acid from SRL India, Vitamin E as DL - α - tocopherol acetate from S.d. fine chemicals Ltd. India. All other chemicals that were locally purchased were of highest purity.

Blood Collection

About 10ml fasting blood sample was collected under sterile condition from each individual. 8ml blood was taken in EDTA vials; plasma was separated for the estimations of TBARS, β carotene, Vitamin C & Vitamin E. Erythrocytes were washed with cold isotonic saline and used for estimation of catalase. 2ml blood was taken in plain vial; serum separated and used for estimation of SOD.

Estimations

The following assays were done –

Thiobarbituric acid reactive substances (TBARS): TBARS were estimated in plasma as described by K. Satoh (11) employing MDA as a reference standard. TBARS levels were expressed as n mole/ml of plasma.

Superoxide dismutase (SOD): SOD was assayed in the serum according to the method of Marklund and Marklund (12). SOD levels were expressed as units/ml. 1 unit of SOD activity being defined as amount of enzyme required to cause 50 % inhibition of pyrogallol autooxidation .

Catalase: It was assayed in erythrocyte haemolysate according to the method of Sinha (13). It's activity was expressed as units / mg protein.

Vitamin C: Vitamin C was estimated in plasma by

using the property of its oxidation into dehydroascorbic acid (14). Values were expressed as mg/dl of plasma.

β Carotene: β Carotene was estimated in plasma according to the method of Bradley and Hornbeck (15). Values were expressed as μ g/dl of plasma.

Vitamin E: Vitamin E was estimated in plasma according to the method of Bieri *et al* (16). Values were expressed as mg/dl of plasma.

Statistical analysis:

All results were expressed in mean $\pm$ SD. One-way analysis of variance (ANOVA) was used to test the significance of difference and Student's Newman Keul test to test the significance of difference between two groups. The rejection criteria for the null hypothesis were fixed at probability level of 0.05.

RESULTS

Mean plasma levels of TBARS (table 1) were high in nuclear (2.81 ± 0.638 nmole /ml), considerably higher in posterior subcapsular (2.94 ± 0.685 nmole/ml), highest in cortical (3.08 ± 0.594 nmole/ml) cataracts and all the values were statistically significant when compared with control groups (2.22 ± 0.640 nmole/ml). Serum SOD (Table 1) levels were lowered in nuclear (2.32 ± 0.605 units/ml) and posterior subcapsular cataract (2.28 ± 0.730 units/ml) and showed lowest levels in cortical (2.20 ± 0.718 units/ml) cataracts and all these values were statistically significant when compared with control group (3.04 ± 0.837 units/ml). Erythrocyte catalase (Table1) levels were slightly lowered in nuclear (63.86 ± 9.152 units / mg protein), posterior subcapsular (64.66 ± 8.939 units /mg protein) and cortical (65.02 ± 9.223 units /mg protein) cataracts as compared with control group (66.66 ± 8.867 units /mg protein); but the values were not statistically significant. Vitamin C levels in plasma (Table 1) were also seen to be significantly lowered in nuclear (0.73 ± 0.095 mg/dl) posterior subcapsular (0.73 ± 0.096 mg/dl) and cortical (0.72 ± 0.102 mg/dl) cataract when compared with control group (0.86 ± 0.127 mg/dl).

β carotene (Table 1) levels in plasma were seen to be significantly low in nuclear (54.03 ± 9.792 μ g/dl) posterior subcapsular (54.65 ± 10.761 μ g/dl) and cortical (55.00 ± 9.549 μ g/dl) cataract cases when compared with control group (65.46 ± 9.748 μ g/dl). Vitamin E levels in plasma (Table 1) in nuclear (0.78 ± 0.089 mg/dl), posterior subcapsular (0.79 ± 0.104 mg/dl) and cortical (0.79 ± 0.105 mg/dl) cataract cases were marginally lowered than control value (0.81 ± 0.094 mg/dl), however the differences were not statistically significant.

The values of parameters among three morphological types of cataracts were not statistically significant (Table 1)

Table 1: Levels of lipid peroxidation product and antioxidants in different components of blood in age related cataract cases and control group (50-80 yrs.)

Parameters	Pure Cortical (n=73)	Pure PSC (n=77)	Pure Nuclear (n=40)	Control (n=78)
TBARS (n mole/ml)	3.08 ± 0.594 *	2.94 ± 0.685 *	2.81 ± 0.638 *	2.22 ± 0.640
SOD (units/ml)	2.20 ± 0.718 *	2.28 ± 0.730 *	2.32 ± 0.605 *	3.04 ± 0.837
Catalase (units / mg protein)	65.02 ± 9.223 ^{NS}	64.66 ± 8.939 ^{NS}	63.86 ± 9.152 ^{NS}	66.66 ± 8.867
Vit C (mg/dl)	0.72 ± 0.102 *	0.73 ± 0.096 *	0.73 ± 0.095 *	0.86 ± 0.127
β carotene(μg/dl)	55.00 ± 9.549 *	54.65 ± 10.761 *	54.03 ± 9.792 *	65.46 ± 9.748
Vit E (mg/dl)	0.79 ± 0.105 ^{NS}	0.79 ± 0.104 ^{NS}	0.78 ± 0.089 ^{NS}	0.81 ± 0.094

n =number of cases or control groups

All values are expressed in Mean ± SD

* =Significant, when compared with control groups (P<0.001)

NS= Non –Significant when compared with control groups (P>0.05)

Values of parameters among three morphological types of cataract are not statistically significant (P> 0.05).

DISCUSSION

The study had shown certain significant findings. Thiobarbituric acid reactive substances (TBARS), the indicator of lipid peroxidation (LPO) were significantly elevated in cortical, PSC and nuclear types of cataract when compared with control group suggesting that increased TBARS and LPO may be considered as indicators of cataract formation. Garg *et al* (6) reported significant increase in serum levels of lipid peroxidation products in the form of malondialdehyde both in early and advanced senile cataracts with respect to control groups. Donma *et al* (9) also reported higher plasma TBARS in senile cataract when compared to the control group. In cases of development of age related cataract, LPO may also be the real cause of the destruction of the plasma membrane of the lenticular fibers and the subsequent oligomerization of the crystalline lenses (17). Our findings in plasma TBARS follow similar trend as observed by Garg *et al* (6) and Donma *et al* (9). Increased LPO was also observed in lens of cataract patients by Babizhayer *et al* (17).

Enzymatic antioxidant status was studied by estimating serum SOD and erythrocyte catalase activity. SOD levels were decreased in cortical, PSC, nuclear

cataracts when compared with control (P<0.001). SOD is enzymatic antioxidant which provides first line of the defense that acts by quenching O_2^- and converting it into H_2O_2 (2). There may be two reasons for lowering of SOD in our study; first, as more and more ROS like O_2^- are produced, SOD is being used up in the process when it converts O_2^- to H_2O_2 and second, H_2O_2 also causes inhibition of SOD activity (18). Garg *et al* (6) and Donma *et al* (9) reported decreased blood levels of SOD in senile cataract cases with respect to control. There are also reports where there is no significant change (4) or even increased (19) SOD levels in the blood of cataract cases when compared with controls. Fecondo and Augusteyn (20) observed precipitous decrease in SOD in the human cataractous lenses. Our study in serum SOD also follows similar trends like that of Garg *et al* (6) and Donma *et al* (9). Erythrocyte catalase levels were slightly decreased in cortical, PSC and nuclear cataracts when compared with control groups but the difference was not statistically significant. Catalase is an enzyme, which is present in most cells and catalyses the decomposition of H_2O_2 to water and oxygen and is found to act 10^4 times faster than peroxidase (21). Casado *et al* (19) reported no significant change in erythrocyte catalase activity between cataract cases and controls. Bhuyan and

Bhuyan (18) found decrease in catalase activity along with SOD but another study (20) observed no change in catalase activity in human cataractous lenses. Our study also supports the finding of Casado *et al.*

Vitamin C levels in plasma were significantly decreased in cortical, PSC and nuclear cataract when compared with control groups ($P < 0.001$). Vitamin C is considered the most important antioxidant in extracellular fluids and the only endogenous antioxidant that can completely protect the lipids from detectable peroxidative damage induced by aqueous peroxyl radical. Vitamin C acts as a co-antioxidant by regenerating α -tocopherol from α -tocopheroxyl radical produced during scavenging of ROS (21). Jacques *et al* (22) reported decreased incidence of PSC with elevated Vitamin C level in blood. No significant association between serum or plasma levels of ascorbic acid and age related cataract was found in other case control studies (6,8). Our study shows significantly decreased levels of Vitamin C in all three morphological types of cataract which may be due to its utilization by counteracting ROS or due to its oxidation by O_2^- which has been reported earlier (18). β carotene levels in plasma were significantly decreased in all three morphological types of age related cataracts when compared with control group ($p < 0.001$). β carotene protects the biological system by deactivating singlet oxygen (1O_2) which largely depends on physical quenching. The study of Jacques *et al* (22) seems to indicate some correlation between lower plasma carotenoid levels and higher incidence of age related cataract. Knekt *et al* (7) predicted the low serum concentration of β carotene as an increased risk of cataract in case control cohort study. Gale *et al* (8) reported lowest risk of nuclear cataract with the highest plasma concentration of β carotene. Garg *et al* (6) and Olmedilla *et al* (10) found no significant correlation between cataract and serum concentration of β carotene. β carotene exhibits good free radical trapping antioxidant behavior under lower partial pressure of oxygen prevailing in lens (6).

Plasma Vitamin E levels were marginally lowered in cortical, PSC and nuclear cataracts but the changes were not statistically significant. Vitamin E is thought to be an important chain breaking antioxidant and can directly scavenge ROS. It is a major lipid soluble antioxidant present in cellular membranes, which protects against LPO. Low serum concentration of vit E was associated with increased risk of cataract (6,7). Olmedilla *et al* (10) and Gale *et al* (8) reported no significant correlation between serum or plasma levels of Vitamin E and age related cataract.

There were also no statistically significant changes of the parameters among three different morphological types of age related cataract. The changes may be in the lens, which is not reflected in the plasma or serum

or erythrocytes in our study or due to other factors which may be responsible for the morphological variations in age related cataract development.

In conclusion, the present data indicate that oxidative stress as measured by pro-oxidant and antioxidant levels in blood may play an important role in age related cataract development. Antioxidant therapy may have a role to play in delaying the onset and progression of age related cataract.

ACKNOWLEDGEMENTS

The authors are grateful to Shri. Dhirubhai Mehta, President of Kasturba Health Society and Dr. (Mrs.) P. Narang, Dean, MGIMS for their keen interest and encouragement. Technical assistance of Mr. Pramod Deshmukh is appreciated.

REFERENCES

1. Venkatesh, P., Garg, S., Verma, L., Tewari, and Garg, S. (2001) Antioxidants: Basic Concepts in relation to the eye. *Ind. J. Clin. Biochem.* 16(1), 9-14
2. Irashad, M. and Chaudhuri, P.S. (2002) Oxidant – antioxidant System: Role and significance in human body. *Ind. J. Exp. Biol.* 40, 1233-1239
3. Spector, A. (1995) Oxidative stress – induced cataract: mechanism of action. *FASEB. J.* 9, 1173-1182
4. Jacques, P.F., Chylack, Jr. Leo, T., McGandy, R.B. and Hartz, S.C. (1988) Antioxidant status in persons with and without senile cataract. *Arch. Ophthalmol.* 106, 337-340.
5. Mohan, M., Sperduto, R.D., Angra, S.K., Milton, R.C., Mathur, R.L., Underwood, B.A., Jaffery, N., Pandya, C.B., Chhabra, V.K., Vajpayee, R.B., Kalra, V.K. and Sharma, Y.R. (1989) India – US case control study of age related cataracts. *Arch. Ophthalmol.* 107, 670-676.
6. Garg, R., Verma, M. Mathur, S.P. and Murthy, P.S. (1996) Blood lipid peroxidation products and antioxidants in senile cataract. *Ind. J. Clin. Biochem.* 11(2), 182-186.
7. Knekt, P., Heliovaara, M., Rissanen, A., Aromaa, A., and Aaran, R.K. (1992) Serum antioxidant vitamins and risks of cataract. *B.M.J.* 305, 1392-4.
8. Gale, C.R., Hall, N.F., Phillips, D.I.W. and Martyn, C.N. (2001) Plasma antioxidant vitamins and carotenoids and age related cataract. *Ophthalmology* 108, 1992-1998
9. Donma, O., Yorulmaz, E., Pekel, H. and Syugul,

- N. (2002) Blood and lens lipid peroxidation and antioxidant status in normal individuals, senile and diabetic cataractous patients. *Curr. Eye Res.* 25 (1), 9-16.
10. Olmedilla, B., Granado, F., Blanco, I., Herrero, C., Vaquero, M. and Millan, I. (2002) Serum status of carotenoids and tocopherols in patients with age related cataracts: a case control study. *J. Nutr. Health Aging* 6(1), 66-68.
11. Satoh, K. (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chim. Acta* 90, 37-43.
12. Marklund, S. and Marklund, G. (1974) Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* 47, 469-474.
13. Sinha, A.K. (1972) Colorimetric assay of catalase. *Analytical Biochemistry* 47, 389-394.
14. Tietz, N.W. (1994) Textbook of clinical chemistry, 2nd edn, Eds. Carl A. Burtis and Edward R. Ashwood. W.B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo p. 1313-1314.
15. Bradley, D.W. and Hornbeck, C.L. (1973) A Clinical evaluation of an improved TFA micromethod for plasma and serum Vitamin A. *Biochem. Med.* 7, 78-86.
16. Bieri, J.G., Teets, L., Belavady, B. and Andrews, E.L. (1964) Serum vitamin E levels in a normal adult population in the Washington DC area. *Proc. Soc. Exptl. Biol. Med.* 117, 131-133.
17. Babizhayev, M.A. (1989) Accumulation of lipid peroxidation products in human cataracts. *Acta Ophthalmol.* 67, 281-287.
18. Bhuyan, K.C. and Bhuyan, D.K. (1978) Superoxide dismutase of the eye. Relative functions of superoxide dismutase and catalase in protecting the ocular lens from oxidative damage. *B.B.A.* 542, 28-38.
19. Casado, A., De La Torre, R. and Lopez-Fernandez, E. (2001) Antioxidant enzyme levels in red blood cells from cataract patients. *Gerontology* 47(4), 186-188.
20. Fecondo, J.V. and Augusteyn, R.C. (1983) Superoxide dismutase, Catalase and Glutathione peroxidase in the human cataractous lens. *Exp. Eye Res.* 36, 15-23.
21. Ray, G. and Husain, S.A. (2002) Oxidants, antioxidants and carcinogenesis. *Ind. J. Exp. Biol.* 40, 1213-1232.
22. Jacques, P.F., Hartz, S.C., Chylack Jr. L.T., McGand, R.B. and Sadowski, J.A. (1988) Nutritional status in persons with and without senile cataract : blood vitamin and mineral levels. *Am. J. Clin. Nutr.* 48, 152-158.