Indian Journal of Clinical Biochemistry, 2007 / 22 (2) 109-113

EVALUATION OF SERUM ZINC LEVEL AND PLASMA SOD ACTIVITY IN SENILE CATARACT PATIENTS UNDER OXIDATIVE STRESS

Indranil Chakraborty, Sanjoy Kunti, *Mousumi Bandyopadhyay, Anindya Dasgupta
****Gopal Deb Chattopadhyay and Sandip Chakraborty**

*Departments of Biochemistry and *Ophthalmology, Burdwan Medical College & Hospital, Burdwan-713104.*
*****Bureau of Applied Economics and Statistics, Govt. of West Bengal, Burdwan-713101.*

ABSTRACT

An imbalance in the systemic redox status leading to oxidative stress has been an important factor in development of senile cataracts, which is reflected by an increase in serum TBARS and a decrease in plasma SOD activity. Zinc has been an important cofactor required for structural stability of SOD. In the present study the role of serum zinc level and plasma SOD activity was analyzed in senile cataract patients showing significant oxidative stress. Serum TBARS, plasma SOD and serum zinc level was measured in thirty randomly selected senile cataract patients against properly matched controls. Although, the analysis of means showed a significant increase in serum TBARS and decrease in plasma SOD and serum zinc level in cases, but plasma SOD was found to be just significantly correlated (p = 0.05) with the serum zinc only in the cases. The results of partial correlation studies and multiple regression analysis, also, showed only a significant correlation and predictable dependence between serum TBARS and plasma SOD, excluding any role of serum zinc level. The present study concludes that it is chiefly the plasma SOD activity, but not the serum zinc level, that determines the proneness of the patients for development of senile cataract.

KEY WORDS

Senile cataract, Oxidative stress, TBARS, SOD, Zinc.

INTRODUCTION

Development of cataract in senile age group is supposed to be due to multiple factors. Increased lipid peroxidation due to oxidative stress has been proved to be an important factor of those (1). Oxidative stress generally causes damage to the membrane polyunsaturated fatty acids (PUFA) leading to generation of malondialdehyde (MDA), a thiobarbituric acid reacting substance(TBARS). Serum TBARS has been well correlated with oxidative stress in tissues in several earlier and recent studies (2-5). The superoxide dismutase (SOD), on the other hand, functions mainly as a first order antioxidant enzyme, mainly by neutralizing the effect of superoxide anion which is an important precursor for oxidative stress in the tissues (6, 7). Human SOD is mainly dependent on the metal ions copper and zinc for its catalytic activity (8) and structural stability (9) respectively, and is distributed both in the intracellular and extracellular compartments. Mn- SOD, a manganese dependent form of SOD, is also found but is sequestered within the mitochondria. Although, SOD functions chiefly as an intracellular antioxidant the plasma extracellular SOD levels may be an important sensitive biomarker for age related changes in the antioxidant capacity(10).

Several studies have indicated an increase in serum TBARS and a decrease in plasma SOD activity signifying an imbalance between the pro-oxidant and anti-oxidant states in the body leading to an imbalance in systemic redox status(5,11). On the other hand zinc, which is one of the important cofactors in the human SOD(9), is also widely distributed for several other functions in the body. The distribution of zinc within the body is chiefly confined in the intracellular sites, where it is utilized for its versatile biological role e.g. in the maintenance of RNA and DNA polymerase activity, maintenance of the cell structure, stabilization of the nucleic acid structure, maintenance of the

Address for Correspondence :

Dr. Indranil Chakraborty
Professor & Head,
Dept. of Biochemistry, Medical College, Kolkata
E-mail : anindya_dg@rediffmail.com

transport process, immunological processes and wound healing etc.(12). Hence, the amount of zinc present in SOD only, may not reflect the true zinc status of the body. So, although zinc deficiency is related to increased sensitivity to oxidative stress(13), the zinc concentration in the extracellular compartment e.g. in the plasma or serum may not correlate with the SOD activity, particularly during oxidative stress. Keeping these factors in mind, an effort was made in the present study to assess and analyze the role of serum zinc in the activity of SOD in plasma in the senile cataract patients showing a significant amount of oxidative stress. Serum TBARS concentration and plasma SOD activity were selected as the markers for the oxidative damage and the antioxidant activity respectively. Serum zinc concentration was estimated to analyze its role in the plasma SOD activity as mentioned above.

MATERIALS AND METHODS

Selection of Cases and Controls : The present study was undertaken as a hospital based case control study carried out in the patients attending as the out door patients in the Department of Ophthalmology of Burdwan Medical College, Burdwan. 30 patients of more than 50 years of age having cataracts were selected in a simple random manner during the period of one year i.e. from November, 2004 to November, 2005. Their mean age $\pm$ SD was 55 $\pm$ 5 years. During selection of the cases it was made sure that they were free from any chronic disease or metabolic disorder. 30 controls were selected from the age and sex matched people attending the same OPD with refractive errors, but without any cataract during the same period. The controls were also selected in a simple random manner and were free from any chronic or metabolic diseases at the time of presentation. Both cases and controls were explained fully about the study and written consents were obtained from them.

Sample collection : From each case and control about 5 ml. of venous blood was collected and separated in two aliquots: 3 ml. in a dry vial without any anticoagulant for obtaining the serum; and 2 ml in a heparin vial for obtaining the plasma.

Serum TBARS was measured by its reaction with thiobarbituric acid (14). Estimation of plasma SOD was done by the method of Kakkar et al(15). Serum zinc was measured by the method based on the Nitro-PAPS method developed by Akita Abe and Sumiko Yiamashita(16).

The results obtained were statistically analyzed with the help of SPSS software. Mean values for each of the parameters were compared between cases and controls by Student's 't' test and the level of significance (p value) was obtained. The correlations between different parameters were analyzed by computing the correlation coefficients and the corresponding level of significance. Multiple regression analysis and partial correlation studies were performed to analyze the relative and predictive effect of serum zinc and the plasma SOD activity in development of senile cataract in presence of the oxidative stress.

RESULTS

The results shown in the Table-1 indicated a significant difference between the means of all parameters between cases and controls. Serum TBARS (X_3), the marker for the oxidative stress was found to be significantly high in the cases, whereas, the levels of plasma SOD (X_1), the marker of the antioxidant activity, with its cofactor zinc (X_2), were found to be significantly ($p < 0.05$) lower in them.

Table 1 : Level of blood biochemical parameters in experimental and control groups

Parameters	Non cataractous (n=30)	Cataractous (n=30)	Level of significance
SOD (U/ml) (Mean$\pm$ SD)	4.06 ± 0.26	3.28 ± 0.32	P <0.001
TBARS (nmol/ml) (Mean$\pm$ SD)	7.14 ± 1.36	8.27 ± 1.88	P <0.05
Zinc (µg/dl) (Mean$\pm$ SD)	96.96 ± 24.27	64.37 ± 27.17	P <0.05

Statistical analysis done by Student's 't' test.

The results of Table-2 revealed a positive correlation between serum zinc and plasma SOD level significant at 5 percent level ($p = 0.05$) in cases, but no such significant correlation was evident in the control population. On the other hand, a significant negative correlation was found between the serum TBARS and plasma SOD in both cases and controls. Partial correlation studies, as provided in the same Table, indicated further that the correlation between the serum TBARS and serum zinc was insignificant when the effect of SOD was eliminated. But, when the effect of serum zinc was eliminated, the partial correlation between the serum TBARS and plasma SOD became statistically significant ($p<0.05$).

In the Table-3, the results of multiple regression analysis have been shown. From the F- value and its corresponding p- value in Table-3 it can be seen that there exists significant linear regression (TBARS = $a + b_1$ SOD + b_2 Zinc) relationship of TBARS based on plasma SOD and zinc for all cataract, non

Table 2 : Correlation between different parameters both in cataract and non cataract patients (SOD=X_1, Zinc=X_2, TBARS= X_3)

	Cataract Patients	Significance	Non cataract patients	Significance
Bivariate correlation study	r_{12} = 0.3604	p = 0.05	r_{12} = 0.2244	p > 0.05
	r_{13} = - 0.6298	p < 0.001	$r_{1\ 3}$= - 0.5568	p = 0.001
	r_{23} = - 0.2011	p > 0.05	r_{23} = - 0.2976	p > 0.05.
Partial correlation study	$r_{23.1}$ = 0.0357	p > 0.05	$r_{23.1}$= -0.2133	p > 0.05
	$r_{13.2}$ = -0.6099	p < 0.001	$r_{13.2}$ = -0.5267	p < 0.001

r_{12} : correlation between X_1 and X_2; r_{13} : correlation between X_1 and X_3; r_{23} : correlation between X_2 and X_3;
$r_{23.1}$: partial correlation between X_2 and X_3 eliminating the linear effect of X_1;
$r_{13.2}$: partial correlation between X_1 and X_3 eliminating the linear effect of X_2

cataract and combined group. Further, the p-values of coefficients of different variables depicts the picture that coefficient corresponding to Zinc are not significantly different from zero, but that of plasma SOD are significantly different from zero in cataract, non cataract and combined groups indicating the fact that TBARS is clearly significantly dependent on plasma SOD but not on serum Zinc.

DISCUSSION

Increased generation of the free radicals and decreased antioxidant activity has been proposed to play an important role in cataract formation in senile age group in various earlier and recent studies (17,18). Serum TBARS is a measure of systemic oxidative damage. In the present study, the significantly raised level of serum TBARS in cataract patients supported the systemic imbalance of lipid redox status in senile cataract patients. On the other hand, a significant decline in plasma SOD activity ($p < 0.001$) in them correlated well with the fact that a decrease in antioxidant defense might be responsible for shifting the systemic redox balance towards oxidative stress which might have contributed to formation of senile cataracts. These findings are well supported by some other studies also where a significantly decreased SOD activity was found in senile cataract patients(19). Generally, an age related decrease in the SOD activity affecting both the cytosolic(5), and extracellular type(10) has been found in several studies including both animals and humans. This decrease might be due to several causes like decreased mRNA levels of the corresponding enzymes(20), oxidative modification of the enzyme(21) etc. But during the aging process ROS may also lead to induction of antioxidant enzyme activity as an adaptive phenomenon to oxidative stress(21) as it generally does in both prokaryotes and eukaryotes. The rate of SOD biosynthesis in E. coli was, in fact, shown to be dependent on the level of $O_2^{-\cdot}$ inside the cells; and mutants of E. coli deficient in SOD were found to be exquisitely sensitive to oxygen toxicity. Hence, it can be proposed in the present study that a successfully executed adaptive phenomenon against oxidative stress resulted in higher levels of plasma SOD in the aged control population which might have protected them from the senile cataract. On the contrary, the cases, who suffered from cataract, could not show such an adaptive response to oxidative stress and so, the SOD activity was found to be lowered in them significantly. This could have resulted from either genetic or environmental factors or both. A decreased synthesis of the enzyme itself e.g. due to decreased mRNA levels has been described already in some

Table 3 : Multiple regression of TBARS based on SOD & Zinc : (SOD=X_1, Zinc=X_2, TBARS=X_3)

Groups	Coefficient of Variables (p-value)			Multiple Correlation	F-value
	Intercept (a)	SOD (b_1)	ZINC (b_2)		(p-value)
Cataract	20.43 (p=.00)	-3.74 (p=.0004)	0.002 (p=.8541)	0.6304	0.6304 (p=.0011)
Non cataract	18.95 (p=.00)	-2.66 (p=.0033)	0.0102 (.26660	0.5843	6.99 (p=.0036)
Combined group	15.51 (p=.00)	-2.09 (p=.00)	0.0011 (p=.8764)	0.6052	16.46 (p=.00)

Statistical analysis was done by using SPSS software.

aged patients (20). Oxidative damage of the SOD enzyme itself, leading to its diminished activity due to decreased levels of alpha tocopherols, vitamin A, or ascorbate in the tissues could have been other possible mechanisms for the decreased SOD activity in cataract patients(22,23). Several environmental factors like dietary nutrients, alcohol intake, and exposure to other pollutants, as proposed in some earlier studies, might have played important roles in diminishing the levels of above mentioned antioxidant vitamins (22, 24). Zinc being chiefly an intracellular metal is stored in various intracellular proteins and enzymes, SOD being one of them. Although circulating zinc in plasma and serum often has been shown to indicate human zinc deficiency, it does not always accurately reflect the whole body zinc status (25). Thus, although zinc is an important element for maintaining the stability of SOD(9), the serum zinc level in normal control population may not be correlated with the plasma SOD level and cannot predict the activity of SOD in them, as found in the present study. But in the senile cataract patients as there is a significant depletion of total body SOD including its concentration in plasma under conditions of oxidative stress, the total body zinc is also depleted which is reflected by a correlated decrease in its serum concentration. On the other hand zinc is not found to be a good predictor of senile cataract as evidenced by the partial correlation study (Table-2) and multiple regression analysis (Table-3). The results of the partial correlation study showed clearly that when the effect of plasma SOD was eliminated, serum TBARS failed to show any significant relationship with the serum zinc, but when the effect of the serum zinc was eliminated, plasma SOD became significantly negatively correlated with the serum TBARS. The multiple regression analysis also, clearly demonstrated a significant predictable dependence of serum TBARS on plasma SOD, but ruled out any such dependence of serum TBARS on the serum zinc.

Thus, all results in the present study, when taken together suggested that it was the plasma SOD activity, but not the serum zinc level, which could be a good predictor for the development of senile cataract due to systemic redox imbalance and increased oxidative stress. However, the present study dealt mainly with the concerned parameters in the plasma or serum in a limited number of patients available in the hospital during a period of one year. More detailed studies involving a larger population section in the community including several environmental factors like their nutritional behavior, intake of alcohol and exposure to other pollutants etc. along with quantification of the changes mentioned above at the tissue level are required for a more direct and accurate analysis in future.

REFERENCES

1. Micelli-Ferrari T, Vendemiale G, Grattagliano I, Boscia F, Arnese L, Altomare E, and Cardia L. Role of lipid peroxidation in the pathogenesis of myopic and senile cataract. Br J Ophthalmol 1996; 80: 840-3.
2. Mayes PA, Botham MK. Lipids of Physiologic Significance, In: Harper's Illustrated Biochemistry, Eds. Rodwell, VW. Mayes, PA. Granner, DK. Murray RK. International edition by McGraw Hill(Asia) 26th ed. 2003:120.
3. Clarkson PM. Antioxidants and physical performance. Crit.Rev. Food Sci Nutr 1995; 35:131-141.
4. Sulochana KN, Punithan R, Ramakrishna S. Oral supplementation of zinc promotes erythrocyte superoxide dismutase activity in chronic cigarette smokers-Report of a pilot clinical trial. Ind J Pharmacol 2001; 33: 224.
5. Kashiwagi K, Shinkai T, Kajii E, Kashiwagi A. The effects of reactive oxygen species on amphibian aging. Comp Biochem Physiol C Toxicol Pharmacol 2005 Feb; 140(2):197-205.
6. Klug-Roth D, Fridovich I, Rabani J. Pulse radiolytic investigation of superoxide catalyzed disproportionation. Mechanism for bovine superoxide dismutase. J Am Chem Soc 1973;95:2786-90.
7. Klug D, Rabani J, Fridovich I. A direct demonstration of the catalytic action of superoxide dismutase through the use of pulse radiolysis. J Biol Chem 1972; 247: 4839-42.
8. Fee JA, Briggs RG. Studies on the reconstitution of bovine erythrocyte superoxide dismutase. V. Preparation and properties of derivatives in which both zinc and copper sites contain copper. Biochim Biophys Acta 1975; 400:439-50.
9. Forman HJ, Fridovich I. On the stability of bovine superoxide dismutase. The effect of metals. J Biol Chem 1973; 248: 2645-9.
10. Adachi T, Wang J, Wang XL. Age related change of plasma extracellular-superoxide dismutase. Clin Chim Acta 2000 Jan; 290(2):169-78.
11. Taylor. Relation among Aging, antioxidant status and cataract. Am J Clinical Nutrition 1995 Dec; 62(6 suppl):14395-475.
12. Milne DB. Trace elements. In: Tietz Fundamentals of Clinical Chemistry. Eds. Burtis CA and Ashwood ER. W.B Saunders Company (Philadelphia) 5th ed. 2001: 578.
13. Mezzetti A, Pierdomenico SD, Costantini F. et.al. Copper/ Zinc ratio and systemic oxidant load: Effect of aging and age-related degenerative diseases. Free Rad Biol Med 1998; 25:676-81.
14. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the auto oxidation of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys 1962; 98:253-61.

15. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 1984;21:130–2.

16. Akita Abe, Sumiko Yiamashita. Colorimetric method for the estimation of zinc. Clin Chem 1989; 35/4:552-4.

17. Fuziwara H, Takigawa Y, Suzuki T, Nakata K. Superoxide dismutase activity in cataractous lenses. Jpn J Opthalmol 1992; 36: 273-80.

18. Augasteyn RC. Protein modification by cataract formation. Academic Press, New York 1981: 71-115.

19. Fuziwara H, Takigawa Y, Suzuki T, Nakata K. Superoxide dismutase activity in cataractous lenses. Jpn.J Opthalmol 1992; 36:273-80.

20. Luo L, Chen H, Trush MA, Show MD, Anway MD, Zirkin BR. Aging and the Brown Norway Rat Leydig Cell Antioxidant Defense System. J Androl 2005 Nov 22 [Epub ahead of print].

21. Augustyniak A, Skrzydlewska E. Antioxidant abilities during aging (article in Polish). Postepy Hig Med Dosw (Online) 2004 Mar 30; 58:194-201.

22. Xue AN, Cai QY, Wang SQ, Zhou AS, Li WX, Fu P, Chen XS. Antioxidant status in persons with and without senile lens changes. Biomed Environ Sci 1996 Sep; 9(2-3):144-8.

23. Jacques PF, Chylack LT Jr, McGandy RB, Hartz SC. Antioxidant status in persons with and without senile cataract 1988 Mar; 106(3):337-40.

24. Metelitsyna IP. Metabolic disturbances in blood from patients with senile cataracts accompanied by somatic diseases. Ukr Biokhim Zh 1998 Mar-Apr; 70(2):110-5.

25. Milne, DB. Trace elements. In: Tietz Fundamentals of Clinical Chemistry. Eds. Burtis CA and Ashwood ER. W.B Saunders Company (Philadelphia) 5th ed. 2001:579.