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ASSESSMENT OF OXIDATIVE STRESS MARKERS IN CATARACT

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ABSTRACT: Introduction: In spite of the progress made in surgical techniques for cataract removal all over the world during last ten years; still age related cataract remains the leading cause of visual impairment. The etiology of cataract is multi-factorial and oxidative damage is one of the causes. **Aim:** To estimate oxidative stress by markers like enzymatic antioxidant Superoxide Dismutase (SOD) and lipid peroxidation product Malondialdehyde (MDA) in age related cataract (ARC) subjects and compare them with healthy control subjects. **Materials and Methods:** This case control study was performed in 100 subjects divided into 2 groups - 50 subjects with age related cataract and 50 age and gender matched healthy controls. Oxidative stress was assessed by estimation of erythrocytic SOD and serum MDA. **Results:** Serum MDA levels were significantly high and erythrocytic SOD levels were significantly low in age related cataract than controls ($P < 0.05$). There was no significant difference in these parameters in subjects with different subtypes of age related cataract. **Conclusion:** Oxidative stress with increased oxidants and decreased antioxidants defense mechanism participate in the etiopathogenesis of age related cataract. So, decrease in oxidative stress may be beneficial in the delaying the progression of age related cataract.

INTRODUCTION: According to the Global Data on Visual Impairments 2010 by World Health Organization, 80% of visual impairment including blindness is avoidable, out of which 33% is due to cataract¹. It has been reported by Andhra Pradesh Eye Disease Study (APEDS) that about 44% of the total blindness in India is due to cataract². Cataract is defined as any opacity of crystalline lens or its capsule, developmental or otherwise with impairment of vision.

Senile or age-related cataracts contribute to more than 80% of all cataracts. Incidence of age-related cataract increases linearly with increase in the longevity. In India, about 40% people over 50 years suffer from cataract³. There is no proven primary prevention or medical treatment for cataract and is conventionally treated with surgery.

In spite of the progress made in surgical techniques for cataract removal all over the world during last ten years; still cataract remains the leading cause of visual impairment. The etiology of cataract is multi-factorial like increased reactive oxygen species (ROS) production in the intraocular region due to oxidative stress, a low antioxidant defense capacity, high lipid peroxidation⁴, increased permeability of the lens membrane, excessive tissue sorbitol concentration, abnormal glycosy-

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lation of lens proteins, modification, aggregation and accumulation of proteins *etc.*⁵

The common cause of cataract is the normal aging process. The development of age related cataract is a slow and complex process; the exact mechanism has not been clearly defined⁶. There is increasing biochemical evidence that oxidative stress has been concerned with development of age related cataract⁷.

Oxidative stress in two different ways can lead to cataractogenesis: directly by reactive oxygen species (ROS) and indirectly by reactive products of lipid peroxidation (LPO)⁸. LPO products and ROS can diffuse across membranes, so can modify proteins localized far away from the initial site of formation. Reactive Oxygen Species (ROS) include mainly superoxide ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$), hydroperoxyl (HO_2), peroxy (ROO^{\cdot}), alkoxy (RO) as free radicals and hydrogen peroxide (H_2O_2) as non radicals. As ROS have low activation energy, they are able to react with biomolecules of all cellular structures and can cause chemical and physiological modifications⁹. ROS have physiological functions at low levels but toxic to the cells at high levels. They are easily generated in the course of normal metabolic activities and may also be produced by external agents such as electromagnetic and particulate radiation, air pollutants, tobacco use or through metabolism of drugs⁹. They can be produced and act inside the cell or they can be generated within the cell and released to extracellular space.

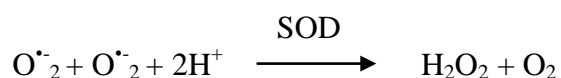
During normal physiological conditions there is a balance between oxidants and antioxidants. However, if this balanced is disturbed with either increased amounts of oxidants or decreased antioxidant activity, a condition known as oxidative stress arises. The body's defense mechanisms play an important role in the form of antioxidants that help to modulate the physiological effects of ROS and to minimize the damage which are caused by oxidative stress. The antioxidant defense systems include antioxidative enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase and antioxidant compounds (Vitamins A, C, E and reduced glutathione)¹⁰. Normally these antioxidant defense mechanisms protect the lens from oxidative stress and maintain lens transparency¹¹. When exogenous or endogenous factors increases

oxidative stress, there is diminution of these ROS scavenger enzymes and increased production of ROS within the lens epithelial cells, which are highly reactive and can damage macromolecules such as lipids, proteins and nucleic acids, causing cell death and cataract¹².

ROS can rapidly react with polyunsaturated fatty acids and start chain reaction leading to lipid peroxidation (LPO). LPO results in the structural alteration of the membrane with the release of the cell and the organelle contents and the loss of the essential fatty acids, with formation of cytosolic aldehyde and peroxide products. Malondialdehyde (MDA) is major end product of LPO¹³. Prolonged accumulation of ROS and lipid peroxidation products also damage the lens crystallines which aggregate and precipitates, forming lens opacities¹⁴. The LPO may be linked to the premature development of senile cataract¹⁵.

ROS are highly reactive compounds with a half-life of only seconds. Therefore, *in-vivo* determination of ROS is generally not feasible. Several biomarkers of oxidative stress have been used to assess it *in-vivo* and *in-vitro* such as MDA as biomarker of lipid peroxidation having longer half life¹⁶.

Superoxide dismutase (SOD) is one important antioxidant enzyme present in nearly all cells exposed to oxygen, with the main function to catalyze the dismutation of two superoxide molecules into oxygen and hydrogen peroxide¹⁷.



SOD detoxifies superoxide to less toxic H_2O_2 , which is then completely detoxified by other antioxidant enzymes like glutathione peroxidase and catalase. This protects the cell membrane from the damage which is caused by ROS. But the decreased SOD levels may lead to increased lipid peroxidation, resulting in cellular rigidity and deformability¹³.

The recent studies on cataract formation focus on the primary role of the systematic oxidative stress which is generated outside the lens. Studies on the antioxidant status of the lens and the blood in cataract patients have been reported in western

countries. However, very few studies have been conducted in India. The present study tried to find out the possible role of oxidative stress by estimation of the erythrocytic levels of the antioxidant enzyme SOD and serum levels of lipid peroxidation product, Malondialdehyde (MDA) in age related cataract with different types, mainly cortical, nuclear, posterior subcapsular and mixed cataract and compared these parameters with age matched control group in rural population.

MATERIAL AND METHODS:

Study Design: This was a cross sectional study, conducted on subjects attending ophthalmology OPD, Krishna Hospital, Karad, Maharashtra from January 2016 to December 2017.

Ethics: Ethical clearance was taken from Institutional Ethics Committee. IEC (Institutional Ethics Committee) approval number is ECR/307/Inst/MH/2013. Informed written consent was taken from every subject after explaining about the study.

Methodology: Based on the previous study done by Kaur J *et al.*,¹⁸ sample size was calculated as follows: to obtain mean difference in serum MDA level of 3.01 nmol/ml (2.42 ± 0.46 nmol/ml vs. 5.43 ± 1.69 nmol/ml) among controls and cataract patients with permissible error 10%, confidence interval 95%, power 80% it come around minimum 10 in each group by using formula:

$$n = (SD1^2 + SD2^2) (Z_{1-\alpha/2} + Z_{1-\beta})^2 / d^2$$

Open Epi, version 3, open source calculator was used.

Hence, in each group 50 subjects were included and the study consisted of two groups with total 100 subjects between age group 45-60 years. First group comprised of 50 cataract patients and second group comprised of 50 control subjects.

Inclusion and Exclusion Criteria:

Inclusion Criteria: Patients diagnosed with cataract, with age 45-60 years.

Age and sex matched subjects were included as controls.

Exclusion Criteria:

- Patients with congenital/complicated/traumatic/secondary cataract.

- Patients operated for cataract (pseudophakia, surgical aphakia).
- Pre-existing ocular diseases (glaucoma, uveitis, corneal opacity).
- Patients with systemic illness like hypertension, diabetes mellitus, liver, cardiac or renal diseases, pregnancy and history of any other substance abuse (alcohol, drugs).

Systematic sampling method was used for selection of subjects. According to inclusion & exclusion criteria and two groups, subjects were selected from the patients coming to ophthalmology OPD. Fifty age and sex matched subjects were included as controls. According to the group, every third patient was selected in the study after informed consent till completion of required sample size.

Written consent was taken after proper explanation of need of study from all the participants and a proforma was used to collect the base line data like demographic data, past history and medical history. A detailed medical and ocular history was taken and proper systemic and ocular examination was done. Complete ophthalmic examination, including best corrected visual acuity, tonometry, grading of lens opacities using slit lamp and the Lens Opacities Classification System III (LOCS III) and fundus examination after dilatation of pupil was done. A grading of \geq N II/C I/P I or a combination was considered to indicate significant cataract.

Pure nuclear (N), cortical(C), and posterior subcapsular (PSC) cataract subgroups had isolated cataract without the presence of other types. Mixed cataract included a combination of nuclear, cortical or PSC cataract. The opacity grade of the worse eye was considered for analysis.

Biochemical Investigation: After overnight fasting, 3 ml of venous blood sample was collected in plain bulb and 2ml of venous blood sample was collected in EDTA bulb with aseptic precautions from all the subjects. Blood was processed in Biochemistry laboratory of KIMSU, Karad. Serum was separated by centrifugation in plain bulb. Serum Malondialdehyde [as Lipid Peroxidation product (LPO)] estimated by Kei Satoh method¹⁹. Erythrocytic Superoxide Dismutase estimated by I.N.T. [2 (-4-iodophenyl)- 3- (-4nitrophenol)- 5-

phenyltetrazolium chloride] method by using RANSOD kit supplied by RANDOX Laboratory USA²⁰.

Statistical Analysis: Chi-square test, unpaired t test and ANOVA were used to find the significance of study parameters between different groups. The data analyzed using IBM SPSS Statistics, version 20. P value <0.05 was considered as statistically significant.

RESULTS:

TABLE 1: AGE, GENDER AND GROUP WISE DISTRIBUTION OF PARTICIPANTS

Group	No. of participants	Gender		Age in years Mean ± SD
		Male	Female	
Cataract	50	22	28	48.62 ± 3.54
Control	50	19	31	49.90 ± 3.35
Total	100	41	59	

Gender wise there was no significant difference in groups (Chi-square test = 0.372, P = 0.542). This shows that groups were sex matched. Age wise there was no significant difference in groups (Chi-square test = 1.85, P = 0.067). This shows that groups were age matched **Table 1**.

TABLE 2: COMPARISON OF SOD & MDA BETWEEN THE STUDY GROUPS

Study groups	E.SOD (U/ml of whole blood) Mean ± SD	S. MDA (nmol/ml) Mean ± SD
Cataract	326.72±32.71	2.31±0.34
Control	356.14±28.67	1.65±0.57
t value	4.78	6.99
P Value	<0.001	<0.001

On comparison of the mean values of erythrocytic SOD and serum MDA between the groups using unpaired t test, the values were found statistically significant (P<0.001). Serum MDA levels were significantly high and erythrocytic SOD levels were significantly low in cataract subjects than controls (P<0.001) **Table 2**.

TABLE 3: CORRELATION OF SOD WITH MDA IN GROUPS

	Cataract		Controls	
	®	P value	®	P value
SOD with MDA	-0.491	<0.001	-0.071	0.625

Correlation analysis showed that there was a significant negative relationship between SOD and MDA (® = -0.491, P<0.001) in cataract group and

no significant correlation was found between SOD and MDA in control (® = -0.071, P=0.625) **Table 3**.

TABLE 4: COMPARISON OF SOD AND MDA IN SUBTYPES OF CATARACT

Type of cataract	No. of cases	Biochemical parameters	
		E.SOD (U/ml of whole Blood) Mean ± SD	Sr. MDA (nmole/ml) Mean ± SD
PSC	17	326.35 ± 23.53	2.27 ± 0.22
Cortical	5	324.80 ± 39.98	2.35 ± 0.59
Nuclear	6	307.33 ± 11.67	2.55 ± 0.11
Mixed	22	332.72 ± 39.90	2.26 ± 0.37
Anova	F	0.953	1.221
	P value	0.423	0.313

On comparison of the mean values of erythrocytic SOD and serum MDA in subtypes of cataract using ANOVA test, the values were found statistically non significant (P = 0.423 for SOD and P = 0.313 for MDA) **Table 4**.

DISCUSSION: According to World Health Organization's (WHO) report, cataract is an ever - increasing problem and approximately 51% of the world blindness may be due to cataract, representing about 20 million people (2010)²¹. According to morphological classification age related cataract can be subdivided into three types: nuclear, cortical and posterior subcapsular cataract. The pathophysiology behind the age related cataracts is complex and it has yet to be fully understood. It is believed that oxidation is a very early or initial event in the overall process in the sequence of events which lead to cataracts²².

Present study showed that serum MDA levels were significantly high and erythrocytic SOD levels were significantly low in cataract subjects than controls. Our findings in serum SOD follow similar trend as observed by Chang D *et al.*,²³ who found that there was a significant decrease in the activity of SOD in serum of cataract patients, compared with normal controls. Similar results showed by Kaur J *et al.*,¹⁸ and Pradhan AK *et al.*²⁴ While other studies showed conflicting results with increased blood levels of the antioxidant enzymes in cataract patients when compared with controls^{25, 26}.

Decrease in the SOD levels may be either due to use of more amount of SOD to convert ROS like O₂ into H₂O₂ or due to inhibition of SOD activity

by increased H_2O_2 ²⁷. Decrease in SOD levels indicates that protective systems are not keeping pace with the insult due to oxidative stress causing damage to lens proteins.

Our findings in serum MDA follow similar trend as observed by Donma *et al.*,¹³ Atti SH *et al.*,²⁸ Kaur J *et al.*,¹⁸ and Chang D *et al.*²³ LPO is initiated by free radical attack on membrane lipids, generating large amounts of reactive products, which have been strongly implicated in the mechanisms of cataractogenesis²⁹.

The ocular lens is continuously exposed to light and is at a high risk of photooxidative damage which can result in cataract. Photo oxidative stress has important consequences in the lens because the lens never sheds its cells and there is no turnover of lens proteins throughout the life²⁸. Photo oxidative stress results essentially from light absorption by the constituents of the lens like structural proteins, enzymes, DNA and membranes. With aging components involved in protecting the lens from stress appear to decrease in activity²⁸. Also the ROS generated in photooxidation damages not only the normal crystalline lens proteins but also antioxidant defense enzymes¹⁸. Oxidation of membrane lipid causes polymerization and crosslinks between lens proteins and membrane. Damaged proteins accumulate and eventually opacification of lens occurs²⁸.

So, increase in the serum MDA levels indicates increase in oxidative stress that may be responsible for formation and progression of cataract. With increase use of self illuminated objects like mobile, tab, computers *etc.* photo oxidative damage to the lens may occur earlier causing early onset of cataract.

In the present study, a significant negative relationship was found between SOD and MDA in cataract group and no significant relation between was found between SOD and MDA in control group. This shows that oxidative damage plays an important role in the development and progression of ARC.

In the present study, no significant difference was found in the levels of erythrocytic SOD and serum MDA among different morphological subtypes of ARC. Similarly, Chang D *et al.*,²³ and Pradhan AK

et al.,²⁴ found no significant difference in subtype cataracts. While the POLA study showed a significant association of increased levels of the erythrocyte SOD with an increased risk of nuclear cataracts³⁰.

Our results confirmed that oxidative stress is present in all three types of cataract. There may be other factors which may be responsible for the different morphological subtypes in ARC. This suggests that oxidative stress might be responsible for initiation and progression of the cataract along with other factors.

Limitations: Our study was a cross-sectional approach so we cannot comment about longitudinal relationship.

Data on time of exposure to sunlight, exposure to indoor smoke and other confounders were not controlled. This could have influenced the result in type of cataract and biochemical parameters.

Further research is recommended to include other confounding factors.

CONCLUSION: The present study showed decrease in the levels of SOD and an increase in the levels of MDA in ARC patients compared to controls. This suggests increased oxidative stress with weakening of the body's antioxidant enzymes defense mechanism and increase in lipid peroxidation, which play important role in the etiopathogenesis of age related cataract. It was also observed that oxidative stress is increased similarly in all subtypes of age related cataract. So, decrease in oxidative stress may be beneficial in the delaying the progression of age related cataract.

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