STUDY OF OXIDATIVE STRESS & ROLE OF ANTIOXIDANTS IN SENILE CATARACT

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ABSTRACT: OBJECTIVE: Senile cataract is by far commonest cause of visual impairment and blindness globally and also in developing countries like India. It has been hypothesized that oxidative damage may be involved in the pathogenesis of cataract. The aim of our present study is to evaluate the role of oxidative stress in cataract.

METHODS: We tried to assess the role of oxidative stress in patients by estimating the levels of lipid peroxidation assessing plasma Malondialdehyde(MDA) levels and antioxidant status by reduced glutathione(GSH), Serum Glutathione-S-Transferase(GST) and Vitamin-C in blood. For this, we have taken 80 cases of senile cataract patients compared with 100 age matched controls. **RESULTS:** A significant increase in the levels of Serum MDA, whereas significant decrease in the levels of reduced glutathione, Serum GST and Vit-C were observed. **CONCLUSION:** This suggests that oxidative stress and reduced anti oxidant defense mechanism play an important role in the pathogenesis of Senile cataract which needs further studies.

KEYWORDS: Senile cataract, oxidative stress, antioxidants.

INTRODUCTION: Anatomically, an opacity in the lens or its capsule, whether developmental or acquired is called a cataract. Clinically, it is opacification of the lens which interferes with vision and which obstructs the normal red glow on direct or indirect ophthalmoscope. Cataract can be classified etiologically into congenital and acquired types whereas Senile or age related cataract is the most common type. According to WHO, there are about 42-45 million blind suffering from cataract and about 15 million of these are in developing countries like India and China. Over 50% of all cases of blindness can be attributed to cataract and more than 20 million people worldwide are affected(1,2).

Oxidative stress is implicated in the pathogenesis of a variety of human diseases(3). Oxidative damage occurs to biomolecules like lipids, proteins, carbohydrates and nucleic acids and other extracellular components like collagen and hyaluronic acid which are very deleterious(4) resulting in lipid peroxidation, mutagenesis and carcinogenesis. Free radicals 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 smoke or through the metabolism of drugs(doxorubicin)(5).They can be produced and act inside the cell or they can be generated within the and released to extracellular space. However, the body's defense mechanisms play an important role in the form of antioxidants that help to minimize the damages which are caused by oxidative stress. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their actions. Oxidative stress occurs when there is an imbalance between reactive oxygen species(ROS) and antioxidants reaction capacity which stimulate the development of a disease such as cataract. Oxidative damage can result in a number of molecular changes that contribute to the development of glaucoma, cataract, and other eye diseases(6). If the free radical theory of aging is applied to the eye, an altered oxidant/antioxidant balance should be evident for age related ocular diseases, such as age-related macular degeneration, cataract, and glaucoma.(7).

The present study was planned to evaluate the possible role of oxidative stress and antioxidant status in the pathobiology of senile cataract patients.

MATERIALS AND METHODS: The present study was conducted in the Departments of Ophthalmology and Biochemistry , S. V. Medical college, Tirupati, AP. 80 diagnosed senile cataract cases in the age group of 45-70 years were chosen for study. Out of 80, 60 were senile cortical cataract cases(30 cases of immature & 30 cases of mature) &20 patients are of senile nuclear(10 cases of immature nuclear&10 of mature nuclear) cases. 80% of the cases both female and male included population who are mainly manual labourers working under the sun.100 age matched controls having visual activity of 6\6 without any lens opacities in either eyes are taken . The controls included mainly population with life style where there is less exposure to sunlight. We have excluded the patients having diabetes mellitus, hypertension and patients having any other systemic illness like thyroid disorders. Informed consent was obtained from all individuals included in the study.

10ml of fasting blood samples were collected by venipuncture and for the separation of sera, 5ml of blood was centrifuged at 3000rpm for 5min and the remaining 5ml of blood was taken into a plain vial containing EDTA and was centrifuged at 3000rpm for 10min for the separation of plasma. Aqueous humor is also collected from each cataract case preoperatively with BCG syringe on same day of blood sample collection. The oxidation is mainly brought about by free radicals .Direct estimation of blood oxidant levels is difficult because of the very short half life of free radicals. So, the plasma MDA levels were estimated by using thiobarbituric acid reacting substances(TBARS) by the method of Yagi(8) and Sinnhuber et al(9).Reduced glutathione was determined by the method of Beutler et al(10). Serum GST is measured by following the increase in absorbance at 340nm using 1,chloro2,4 dinitrobenzene as substrate(11).Plasma ascorbic acid level is estimated by using 2,4 dinitrophenylhydrazine & reading the absorbance at 520nm(12). The findings were expressed as mean $\pm$ standard deviation & evaluated for statistical significance.

RESULTS: In this study significant increase in the levels of serum MDA is observed in senile cataract when compared with controls.. There is significant decrease in levels of GSH and serum GST levels in senile cataract and also in its cortical and nuclear types when compared with controls. There is significant decrease in plasma Vit-C levels in

senile cataract and also in its cortical and nuclear types when compared with control groups.

DISCUSSION: The current interest is in oxidative stress, nutrient & enzymatic antioxidant status in pathophysiology of senile cataract which is responsible for the largest number of blindness cases in the country. Oxidative stress has been implicated in cataractogenesis (13). In our study, a statistically significant relationship was found between the presence of cataract and plasma MDA levels. The plasma MDA level, a byproduct of lipid peroxidation, is a reliable and commonly used biomarker of overall lipid peroxidation. ROS causes damage to biopolymers including nucleic acids, proteins, PUFA & carbohydrates which are the basic mechanisms underlying diseases including cataract. The solid mass of the lens is 98% protein. Because these proteins undergo minimal turnover as the lens ages, they are subjected to chronic stresses of exposure to light and oxygen. So, these proteins are extensively damaged in aged lenses. Lens opacities develop as the damaged proteins aggregate and precipitate(14). Lipid damage to the fiber cell membrane is also associated with lens opacities(15). Smoking and ultraviolet light, which appear to induce oxidative stress(16), are also associated with elevated cataract risk(17). Photo oxidative stress has important consequences in the lens because the lens never sheds its cells & there is no turnover of lens proteins throughout the life. The constituents of the young lens differ chemically from the older lens. These differences between young and old lenses are a result of three major processes. First, there are post translational changes in protein in the inner region of the lens where protein synthesis is insignificant and particular protein macromolecules have been present for many years. There is posttranslational modification of proteins like racemization, glycation (18), COOH terminal degradation, deamidation and non-covalent aggregation. Second, the crystallins the major structural proteins are produced by many genes and any gene is not necessarily active all throughout life. Finally, with aging certain key metabolically active components involved in protecting the lens from stress appear to decrease in activity. In the normal, young human lens, there is no oxidation of the cytosolic protein and no oxidation in the membrane fraction. All thiols are buried in the interior of the macromolecular structure. In normal lenses, some membrane protein oxidation is apparent by the age of 60-65, but there is still no oxidation of cytosolic protein. At this stage, only about 50% of the protein thiols remain buried. In cataract, the picture is dramatically different. All thiols are exposed, and massive oxidation of thiol to both protein and mixed disulfides (probably with GSH) as well as cysteic acid is observed. . Oxidation of membrane lipids precedes high molecular weight protein aggregation. This often exposes buried functional groups & lead to conformational change. Oxidation of membrane lipids also causes polymerization & cross-links between lens proteins & membranes. If these damaged proteins accumulate, eventual opacification occurs. Cataracts are thought to result from photo oxidation of lens proteins that results in protein damage, accumulation, aggregation and precipitation in the lens(19).

Photo oxidative stress results essentially from light absorption by the constituents of the lens like structural proteins, enzymes, DNA & membranes. Direct protection is offered mainly by dietary antioxidants like Ascorbic acid, Tocopherols & carotenoids and antioxidant enzymes. So, the rise in plasma MDA levels indicate that oxidative stress may be responsible in the pathogenesis of cataract.

We also observed a significant decrease in the levels of reduced glutathione in the cases as compared to the controls. In young lenses, damaged proteins are usually

maintained at harmless amounts by defense systems. Primary defenses that directly protect the lens against the initial oxidative insult include small molecule antioxidants (eg, vitamins C and E and carotenoids) and antioxidant enzyme systems (eg, superoxide dismutase, catalase, and the glutathione redox cycle). The lens also has secondary defense systems, which include proteolytic enzymes that selectively identify and remove damaged or obsolete proteins. Accumulation of photo oxidized (and/or otherwise modified) proteins in older lenses indicates that protective systems are not keeping pace with the insults that damage lens proteins. This occurs in part because similar to bulk proteins, enzymes that compose some of the protective systems are damaged by photooxidation. The intracellular depletion of reduced glutathione can be either due to the formation of a direct complex with an electrophilic agent or due to the inhibition of synthesis or due to the subjection of the cell to oxidative stress(20). When a cell is subjected to oxidative stress, there is increased utilization of glutathione, thus leading to its depletion. Many enzymes are GSH dependent and their activity may be regulated by the thiol disulphide exchange. They are thus dependent on the GSH status. Another possible explanation for reduced GSH levels may be due to defective intracellular synthesis. The liver is the major site for GSH synthesis. The precursors necessary for this synthesis are L-glutamate, L-cysteine and L-glycine. Although both glutamate and glycine are important, it seems that the major determinant of the rate of GSH synthesis is the availability of the amino acid cysteine.(21). Cysteine results from the metabolism of homocysteine and any interruption in the homocysteine-cysteine pathway would result not only in the accumulation of homocysteine but also in less available quantities from the second amino acid which may affect GSH synthesis. So, reduced glutathione levels here indicate there is depletion which is because of increased defense mechanisms.

We also observed a significant decrease in the levels of Serum GST in the cases as compared to the controls. Glutathione -S- transferase (GST) is reduced which are dimeric, mainly cytosolic enzymes that have extensive ligand binding properties in addition to their catalytic role in detoxification(22,23). This reduction is due to the reduced levels of GSH.

There is also a decrease in the levels of non-enzymatic anti-oxidants such as Vit-C, which states that there is an increased defense mechanism against oxidative damage in cataract. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to an increased turnover for preventing oxidative damage in these patients, thus suggesting an increased defense against oxidative damage. Our results support the researchers who reported decreases in the antioxidant level and increases in lipid peroxidation level(24,25). Several other researchers showed over expression of antioxidants to be associated with cataract.(26,27).

In conclusion, the present study revealed an increase in the levels of MDA and decrease in the levels of reduced glutathione, serum GST and Vit-C in cases compared to controls suggesting the role of oxidative stress as a pathogenic mechanism in the development of cataract and further extensive studies are required in future to establish oxidative stress as a biomarker in the development of cataract.

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Table 1

Comparison of levels of MDA, enzymatic and non-enzymatic antioxidants in cases and controls

Parameters	Senile cortical cataract(n=60)	Senile nuclear cataract(n=20)	Controls(n=100)	P value
MDA (nmol /ml)	8.99±0.13	7.93±0.13	2.18±0.12	<0.001(highly significant)
GSH (mg/g Hb)	5.87±0.11	6.43±0.97	14.66±0.13	<0.001 (highly significant)
Serum GST (IU/L)	39.01±6.92	40.05±5.67	72.73±9.33	<0.001 (highly significant)
Vitamin-C (mg/dl)	2.14±0.19	2.32±0.84	7.58±0.16	<0.05 (significant)