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ROLE OF BIOCHEMICAL MARKERS FOR EVALUATION OF OXIDATIVE STRESS IN SENILE CATARACT

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ABSTRACT

BACKGROUND: Cataract formation is mostly considered to be a multi-factorial disease and oxidative stress might be one of the leading cause. Tissue oxidative stress may be due to increased oxidant production and/or a decreased antioxidant capacity in cells characterized by the release of free radicals, resulting in cellular degeneration. The imbalance between the rate of free radical production and the antioxidant defense causes cellular damage resulting in lipid peroxidation. Oxidative stress is involved in many ocular diseases. The effect of Reactive oxygen species as measured by lipid peroxidation product, Malondialdehyde and the efficiency of antioxidant capacity represented by Superoxide dismutase and Zinc and their role in pathogenesis of cataract. MATERIALS AND METHODS: A total number of 100 newly diagnosed cataract cases and 60 Age matched controls were included in the study. Biochemical markers like Serum malondialdehyde, Superoxide dismutase and serum zinc were estimated in the bloodsamples of all cases and controls. RESULT: Increased levels of Malondialdehyde and decreased levels of Superoxide dismutase and Zinc support that oxidative stress may be implicated in the development of senile cataract. SUMMARY AND CONCLUSIONS: In the present study Serum malondialdehyde is the best marker to identify oxidative stress followed by Superoxide dismutase. The diagnostic accuracy is represented by area under curve, which is highest for Malondialdehyde followed by Superoxide dismutase and Zinc.

KEYWORDS

Malondialdehyde, Oxidative stress, Reactive oxygen species, Superoxide dismutase.

INTRODUCTION

Cataract is one of the major cause of impaired vision and blindness worldwide and is defined as opacity of the crystalline lens [1]. According to the WHO report there are 40 million blind people in the world, of which 17 million are blind due to cataract and approximately 13 million live in developing countries. In India alone 4 million people per year become blind because of cataract[2]. The pathogenesis of cataract in old age has been found to be influenced by many factors including oxidative stress. A major threat to lens transparency lies in the accumulation of Oxidative products in the crystalline lens and other molecules over an individuals life time. It has been suggested that oxidative stress also may mediate damage from other types of insults, such as from ultraviolet light. Since peroxides are generated in fiber and anterior epithelial cells, the average concentration of H_2O_2 can be much higher in patients with cataract.[3]

Malondialdehyde (MDA) is one of the toxic product of lipid peroxidation and plays an important role in the evaluation of oxidative stress[4]. It is one of the most frequently used indicator of lipid peroxidation. Superoxide dismutase (SOD) exists as a family of

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metalloproteins and is widely distributed in mammalian tissues. Extracellular SOD which is a Cu-Zn-SOD that is immunologically distinct from the classical SOD, which is present in cytoplasm.[5] Zinc is an important trace element and is essential for more than 200 metalloenzymes, including the antioxidant enzyme superoxide dismutase [6]. The present study was aimed at determining the concentration of lipid peroxidation product in the serum such as MDA and antioxidant enzyme activity by estimating SOD and their role in the pathogenesis of cataract. Serum zinc was also estimated to evaluate its antioxidant role in the cataract.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, Osmania Medical College and Department of Ophthalmology, Sarojinidevi eye Hospital, Hyderabad, A.P.

Cases and Controls: The patients who were approaching the Department of Opthalmology, inpatient block were screened to be included in the study. A total number of 100 newly diagnosed cataract cases (50 female and 50 male) and 60 Age matched controls were selected (30 male and 30 female).

Inclusion Criteria; Cases and Controls are more than 50 years age with cataract.

Exclusion Criteria; History of Diabetes Mellitus, Hypertension, Liver or Kidney disorders, Refractive errors, any other systemic illness and use of any antioxidant or vitamin supplementations**.** The study was conducted with the approval of the institutional ethical committee. Informed consent was obtained from all 160 subjects after explaining the nature of the study. Participants were in the supine position for 5 to 10 minutes before venipuncture, and 3ml venous whole blood collected in 5 mg% EDTA bottle for MDA estimation, 3 ml venous blood in plain bottle and allowed to clot to separate serum for SOD and Zinc estimation. The following methodology was applied to the samples to obtain the required Biomarker levels

1) Estimation of serum malondialdehyde by Thiobarbituric acid reactive substance assay (TBARS) [7,8]

2) Superoxide dismutase by Sodium carbonate method [9]

3) Estimation of serum zinc by nitropaps method.[10]

Statistics: The data has been statistically analysed by using mean, standard deviation. Independent sample't' test and 'p' value were used to assess the significance of difference of means between the case and control groups. Pearson correlation was used to assess the correlation between different parameters in the groups analyzed by using SPPS – 14 program and Pearson – Correlation 2 tailed tests.

RESULTS

The parameters were analyzed in 100 cataract patients. The results were compared with 60 age and sex matched healthy controls.

Table 1. Representing Mean ± SD of MDA, SOD and Zinc in male, female and both male and female among controls and cases.

Parameter	Sex	Controls Mean ± SD	Cases Mean ± SD
MDA	F	334.46 ± 43.88	471.41±76.06
	M	322.84 ± 26.61	471.23±92.18
	M & F	328.75 ± 36.59	471.32±84.08
SOD	F	82.74 ± 13.81	58.73±8.50
	M	87.73 ± 12.11	61.87±8.18
	M & F	85.24 ± 13.12	60.30±8.45
Zinc	F	81.87 ± 14.08	68.60±20.13
	M	85.90 ± 15.21	63.04±17.19
	M & F	83.88 ± 14.674	65.82±18.83

The difference between means of SOD, MDA and Zinc among males and females is statistically not significant in controls and cases. P values for MDA, SOD and Zinc were 0.226, 0.142 and 0.226 for controls and 0.014, 0.905 and 0.954 for cases respectively. The mean for MDA in cases is high compared to controls and this difference is statically significant.(p <0.0001). Whereas the mean for SOD and Zinc in Cases is low compared to Controls and statistically significant in both parameters (p < 0.0001).

Table 2.Representing correlation between parameters in controls

PARAMETER	MDA	SOD	ZINC
MDA	1.0000	r= - 0.6587 P=0.0001	r= - 0.2789 p=0.0310
SOD	r= - 0.6587 P= 0.0001	1.0000	r= 0.1072 p=0.4189
ZINC	r= - 0.2789 P=0.0310	r= 0.1072 p=0.4189	1.0000

There is a negative correlation between SOD & MDA and it is significant where as no correlation observed between Zinc and other parameters like SOD and MDA.

Table 3: Representing correlation between parameters in cases

PARAMETER	MDA	SOD	ZINC
MDA	1.0000	r= - 0.5835 P=0.0001	r=0.05129 P=0.6123
SOD	r= - 0.5835 P=0.0001	1.0000	r= - 0.1270 p = 0.2079
ZINC	r= 0.05129 P=0.6123	r= - 0.1270 p= 0.2079	1.0000

There is a negative correlation between SOD & MDA and it is significant where as no correlation observed between Zinc and other parameters like SOD and MDA.

Table 4: Showing Best Cut-Off Value, Sensitivity and Specificity.

Parameter	Best Cut-Off Value	Sensitivity	Specificity
MDA	377.3 n mol / L	95%	90%
SOD	81.40 I.U / g protein	100%	73%
Zinc	55 micro g/dl	31%	97%

In order to assess the maximum sensitivity and specificity of various variables in indentifying abnormality, the best cut off values were calculated by using ROC curves. MDA is having more specificity and sensitivity where as Zinc is more specific but not sensitive and SOD is more sensitive but not specific.

Table 5: Showing Area under Curve and 95% Confidence limits.

Parameter	AUC	95% Confidence limits	
		Lower Bound	Upper Bound
MDA	0.9711	0.9490	0.9932
SOD	0.9278	0.8742	0.9815
Zinc	0.7785	0.7075	0.8495

The most sensitive markers were serum MDA and SOD when compare with Zinc.

DISCUSSION

Human senile cataract is a multifactorial disease but Oxidative stress is considered to be the most important risk factor. As a result of oxidative stress, a series of highly reactive intermediates are produced like superoxide radical, hydrogen peroxide, hydroxyl radicals and they can react with proteins, nucleic acids, lipids and carbohydrates of cell leading to peroxidative damage to biological membranes resulting in cataract. Exposure to light may lead to formation of reactive oxygen species by photosensitizing mechanisms. It has been reported that a significant proportion of lenses and aqueous humor taken from cataract patients have elevated H_20_2 levels.[11,12] Lipid peroxides formed are unstable compounds, they tend to degrade rapidly to a variety of sub products. MDA is one of the known secondary product of lipid peroxidation and it can be used as a marker of cell membrane injury. In the present study, there was no significant difference of MDA levels between males and females in both cataract cases and controls. The levels of MDA were significantly increased in cataract cases compared to controls. Several studies carried out determination of MDA in plasma and aqueous humor collected from patients with senile cataract and non cataract patients and they observed that MDA was elevated. The observations of present study are in agreement with previous studies.[13, 14, 15, 16]. As age advances, ROS cause increased peroxidative damage to unsaturated fattyacids present in phospholipids and glycolipids of lenticular membranes. Human lens lipids were also shown to have absorption maxima at 293 nm indicating their susceptibility to oxidative degradation. Lipid oxidation increased linearly and uniformly throughout the human lens with age. The relative and absolute amount of sphingolipids including dihydrosphingomyelin and sphingomyelin increased with age, whereas glycerolipids including phosphatidylcholine and two phosphatidylethanolamine-related phospholipids, decreased[17]. As a result composition, structure, lipid hydrocarbon chain order or stiffness increases. Increased membrane stiffness may increase light-scattering, reduce calcium pump activity, alter protein–lipid interaction and disruption of the homeostasis of the cell. These changes were substantially greater than the changes in lipid levels reported in any organ in association with any disease. So as age advances there is increased peroxidative damage to lenticular membranes by oxygen free radicals generated in eye fluids and tissues. Thus the continuing oxidative damage to lens results in elevated levels of MDA in cataract patients. To protect from Reactive Oxygen species the lens like other tissues, contains a series of defense mechanisms. Crystalline and other proteins in lens fibers do not turnover and must serve the lens for lifetime of a person. Thus, the lens is more dependent than most tissues on protection from oxidative damage. The antioxidant mechanisms in lens includes Superoxide dismutase.[18] In the present study there was no significant difference in SOD levels among males and females within the groups. Levels of SOD were significantly lower in cataract cases when compared to controls. This finding correlates with the previous studies. There may be two reasons for lowering of SOD. 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 H_2O_2 also causes inhibition of SOD activity. Second, with aging certain key metabolically active components involved in protecting the lens from stress like SOD, glutathione peroxidise, glutathione reductase, catalase will decrease in activity. In the present study, there was a negative correlation between MDA and SOD in cases. Similar results were observed in previous studies.[13,15,16] .The significantly decreased levels of SOD in cataract patients compared to controls detected in above studies and in present study would enhance photooxidation and induce lipid peroxidation as indicated by significant increased levels of MDA. In the present study there is no significant difference in Zinc levels among males and females within the groups. Zinc levels were significantly lower in cataract cases than controls. This finding correlates with the previous studies[16]. Superoxide dismutase requires zinc for its activity and zinc has antioxidant role per se and its deficiency may increase susceptibility to oxidative damage. Thus the decreased levels of Zinc will contribute to formation of cataract. Thus increased levels of MDA and decreased levels of SOD and Zinc support the hypothesis that oxidative stress may be implicated in the development of senile cataract. Best cut off values for different parameters as calculated from ROC curves are used to assess their discriminatory capacity. In the present study serum MDA is the best marker to identify oxidative stress followed by SOD. Though Zinc shows highest specificity it lacks sensitivity in identifying abnormality. The diagnostic accuracy is represented by area under curve (AUC) which is highest for MDA followed by SOD and Zinc. Thus MDA, SOD and Zinc can be used as markers of oxidative stress leading to senile cataract.

SUMMARY AND CONCLUSIONS

As age increases, cataract becomes a common disorder. Oxidant and antioxidant imbalance plays a key role in aging which was proved by many studies. It is strongly implicated that oxidative mechanisms play a major role in etiology and pathogenesis of cataract as concluded by many previous studies. Oxidative stress has been shown to cause cataract in in-vitro models. To evaluate the role oxidative stress in pathogenesis of cataract, estimation of MDA the secondary product of lipid peroxidation of lenticular membranes was carried in the study. MDA levels were increased in cases compared to controls, indicating oxidative sress. This oxidative stress damages lenticular membranes resuling in senile cataract. To evaluate the antioxidant status in senile cataract patients, SOD an enzyme involved in antioxidant defense mechanisms is estimated in the study. SOD levels were decreased in senile cataract patients compared to controls.The decreased levels of SOD is due to increased utilisation as a result of oxidative stress and decreased activity of enzyme in the advanced age. Zinc is considered to have an antioxidant role as it is part of antioxidant enzyme SOD and it also forms a part of metallothionein complexes. A decrease in the serum zinc levels were observed in cataract patients when compared to the controls. The decreased levels of Zinc contribute to senile cataract formation.

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