Original Article

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Lipid Peroxidation and the Total Antioxidant Status in the Pathogenesis of Age Related and Diabetic Cataracts: A Study on the Lens and Blood

Biochemistry Section

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ABSTRACT

Background: Cataract is one of the major causes of a visual impairment, which eventually leads to blindness. An oxidative damage to the lens proteins is a major factor which leads to cataract formation. Therefore, we intended to study the relationship between the biochemical markers of oxidative stress and various forms of cataracts.

Methods: We examined the lenses and the sera of 120 subjects who were aged 50 to 80 years, who were distributed in two groups, viz. the study group (90 patients) and the control group (30 subjects). The oxidative stress was assessed by estimating the lipid peroxidation product in the form of thiobarbituric acid reactive substances (TBARS), the antioxidant status by measuring the levels of vitamin E and the total antioxidant capacity (TAC). The study group patients were further divided into those with nuclear cataracts (30 patients), cortical cataracts (30 patients), and diabetic cataracts (30 patients).

Results: In this study, it was found that the levels of TBARS in the study group were significantly high ($p<0.001$), whereas the TAC ($p<0.001$) and the vitamin E ($p<0.001$) levels were significantly low, both in the lenses and the blood of the study group as compared to those of the control group.

Conclusion: Thus, the present study suggests that an imbalance between the oxygen free radicals and the antioxidants may lead to lipid peroxidation in the lens. Also, the elevated levels of glucose in the diabetic cataracts lead to the auto-oxidation of glucose and a non-enzymatic glycation of the lens protein. Thereby, the high molecular weight proteins aggregate in the cataract.

Key Words: Cataract, Lens, Lipid peroxidation, Vitamin E, Protein glycation, Total antioxidant status

INTRODUCTION

Any alteration in the optical homogeneity of the lens or a decrease in its transparency is known as a cataract. Cataract is one of the major causes of a visual impairment, which eventually leads to blindness [1]. The impact of lipid peroxidation on the development of maturity onset cataracts and the relationship of lipid peroxidation with diabetes have recently been noted. Lipid peroxidation, an event which is caused by an imbalance between the free radical production and the antioxidant defenses, may play a role in the cataractogenesis [2].

Given the extent of the disability which is caused by cataracts, it is important that some measures must be taken to slow down the development of the cataracts, as we cannot prevent them from occurring. A delay in the cataract formation for 10 years, will reduce the prevalence of the cataracts by 50%. Such a delay will enhance the quality of the life for much of the older population and it can reduce the economic burden which is caused by the visual disability and surgery [3].

In the lens, both the reactive oxygen species (ROS) and the glycation of proteins may initiate toxic biochemical reactions, leading to extensive damage. The antioxidants such as a-tocopherol and ascorbic acid, and the antioxidant enzymes which include super-oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are responsible for neutralizing the deleterious effects of the ROS [3,4].

So, the present study was undertaken to evaluate the contributions of the free radicals and the antioxidants to the pathophysiology of cataracts.

MATERIALS AND METHODS

This study was conducted in Sholapur (Maharashtra, India) on the lenses and the sera of 120 individuals who were aged 50 to 80 years, who were distributed in two groups, viz. the study group and the control subjects. The study group included 90 cataract patients. The study subjects were further divided into those with nuclear cataracts, cortical cataracts and diabetic cataracts, with 30 patients in each group. The control group comprised of 30 persons with a visual activity of 6/6 or better in both the eyes and to whom antioxidant medicines were not given. They were all healthy individuals without any systemic diseases and without any habits like smoking, alcoholism, etc. The patients with a history of ocular surgeries, trauma, infection and inflammation of the eyes were also excluded from the study. Prior to the start of the study, local institutional ethical clearances were obtained from the above mentioned institutes. Written informed consents were obtained from all the subjects at the times of their recruitment into the study.

Collection of the Lenses and Preparation of the Homogenates

The cataractous lenses were removed surgically by the PECCE + IOL (Planned extra capsular cataract extraction+ intra-ocular lens)

technique. The lenses were obtained and they were packed in ice-cold saline and immediately taken to the laboratory. Each lens was weighed and its wet weight (mg) was recorded. The lenses were homogenized with a Teflon rod and diluted to 1:10 (w/v) by adding 50mM potassium phosphate buffer (2mM EDTA), PH 7.00. The lens homogenates which were obtained were centrifuged at 10,000 rpm for 15 min at 40ºC. The supernatants which were obtained following the centrifugation were processed for the estimation of the biochemical parameters [5].

Blood Collection

Five ml blood samples were collected by venipuncture from the patients who were undergoing surgeries for cataracts on the same day. 3ml of this blood was collected in a plain bulb and the separated serum was used for the estimation of TBARS and vitamin E. The remaining 2ml of blood was collected in a heparinized bulb for the assessment of TAC.

TBARS Determination

The TBARS levels, as an index of lipid peroxidation, were determined by the thiobarbituric acid (TBA) reaction according to the method of Yagi [6]. The principle of this method depends on the measurement of the pink colour which is produced by the interaction of TBA with MDA. The sample (for the blank, H_2O is used), SDS, acetic acid, thiobarbituric acid, and H_2O were added to the test tubes, respectively. Then, they were incubated at 951ºC for 30 min in a water bath. After this incubation, the butanol-piridine (15 : 1) solution was added to the tubes. Then, the tubes were centrifugated at 4000 r.p.m. for 10 min. The butanol top layer was measured against the blank spectrophotometry at the 532nm wavelength. 1,1,3,3 tetraetoxypropane was used as the primary standard.

Vitamin E Determination

Vitamin E was estimated by the colourimetric method of Baker and Frank [7]. This method is based on the reduction of the ferric ions to ferrous ions, which form a red coloured complex with a-a' dipyridyl, that is read at 520nm.

Measurement of the Total Antioxidant Capacity

The TAC was measured by the Ferric Reducing Ability of Plasma (FRAP) Assay [8]. It was based on the antioxidant power to reduce ferric ions to ferrous ions at a low pH, that formed a coloured ferrous tripyridyltriazine complex and the absorbance of this complex was measured at 593nm.

STATISTICAL ANALYSIS

All the results were expressed in mean ± SD. One way Analysis of Variance (ANOVA) was used to test the significance of the differences and the Student's "t" test was used to test the significance of the differences between the two groups. A p-value of < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The present study demonstrated an enhanced lipid peroxidation both in the sera and the lenses of the cataract patients [Table/Fig-1 and 2]. A statistically significant increase in the level of lipid peroxide ($p< 0.001$) was observed, both in the sera and the lenses of the study group subjects as compared to those of the control subjects. The levels of TBARS were 2-3 folds higher in the lenses with the cataracts as compared to those in the normal lenses, the highest being in the nuclear cataracts, followed by those in the diabetic cataracts. The plasma vitamin E levels were significantly decreased in the nuclear cataracts as compared to those in the controls ($p<0.001$). But no significant decreases in the plasma vitamin E levels were observed in the cortical and the diabetic cataracts as compared to those in the controls. Further, the levels of the lens vitamin E were significantly decreased in the nuclear, cortical and the diabetic cataracts ($p<0.001$) as compared to those in the control lenses, the highest levels being observed in the diabetic cataract lenses. Whereas, the TAC was significantly decreased in all the cataractous patients as compared to that in the controls. The levels of both the plasma and the lens total antioxidants were significantly decreased in the cortical, nuclear and the diabetic cataracts ($p<0.001$) as compared to those in the controls.

The possible role of lipid peroxidation in causing the oxidative modification which occurs in the human lens plasma membrane

Parameters \ Groups	Controls (n=30) Mean ± SD	Cataract Patients		
		Nuclear (n=30) Mean ± SD	Cortical (n=30) Mean ± SD	Diabetic (n=30) Mean ± SD
TBARS (nmol/ml)	2.92 ± 0.50	5.14 ± 1.0*	3.95 ± 0.71*	4.34 ± 0.56*
TAC (nmol/L)	1.43 ± 0.1	1.2 ± 0.09*	1.28 ± 0.16*	1.27 ± 0.09*
Vitamin E (mg/dl)	1.26 ± 1.08	0.97 ± 0.16**	1.09 ± 0.25NS	0.82 ± 0.10#

[Table/Fig-1]: Levels of TBARS and antioxidants in **Blood** of control subjects and Patients with different cataracts.
n =No. of Subjects; # p=0.02; * p<0.001
NS Non significance; ** p<0.05

Parameters \ Groups	Controls (n=30) Mean ± SD	Cataract Patients		
		Nuclear (n=30) Mean ± SD	Cortical (n=30) Mean ± SD	Diabetic (n=30) Mean ± SD
TBARS (nmol/gm.lens)	1.08±0.13	2.6±0.39*	2.14±0.51*	2.74±0.66*
TAC (nmol/gm.lens)	0.86±0.19	0.62±0.16*	0.78±0.43*	0.52±0.21*
Vitamin E (mg/d gm.lens)	0.86±0.19	0.68±0.23*	0.81±0.24 NS	0.65±0.25*

[Table/Fig-2]: Levels of TBARS and antioxidants in **Lens** of control subjects and Patients with different cataracts.
n =No. of Subjects; NS Non significance
* p<0.001

during aging and senile cataract development, is currently a subject of intense interest [9].

Earlier studies have reported increased levels of the lipid peroxidation product, MDA, in the cataract subjects as compared to those in the controls, which was in accordance with our findings [5,10-13]. The high levels of TBARS in the cataractous lenses may, in turn, be the result of the lipid peroxidation of the lens cell membrane, which is produced locally or they may be the consequence of the migration of the products from the retina [14-16]. A peroxidative damage to the plasma membranes of the crystalline lens fibres may lead to disturbances in their permeability for ions, loss of the thiol groups of the membrane bound crystallines and also large protein aggregates with low solubilities in the lens [9].

The oxidative damage, as measured by TBARS, may be increased, because of ageing and exposure to hyperglycaemia, thus causing a nonenzymatic glycation of the proteins. The possible sources of oxidative stress in diabetes include the shifts in the redox balance, which result from altered carbohydrate and lipid metabolism, an increased generation of ROS, and decreased levels of the antioxidant defenses. The glycation product can be oxidized by the ROS, to give advanced glycation end-products (AGEs). This AGE formation can cause tissue damage. Both the glycated and the AGE-modified proteins can lead to oxidative stress [17,18]. Another mechanism which was proposed by Katta AV et al., hypothesized that a non-enzymatic glycosylation might enhance the susceptibility of the lens to sulphydryl oxidation and high molecular weight insoluble protein aggregation. These insoluble proteins are bound to the membranes and their oxidations have been shown to begin at the membranes. MDA, which is known to play a role in the lens opacification, can form cross-links between the membrane lipids and the proteins. The oxidative damage to the protein is one of the modifications that leads to severe failures in the biological functions and death [19, 20].

In diabetes, there are at least six different pathways through which the higher concentrations of glucose can lead to the accumulation of ROS within the lens. These ROS may induce lipid peroxidation in this tissue [21]. Since cataracts may be related to a tissue destruction process, it is reasonable to expect a raised lipid peroxide concentration in the serum and the lenses of the cataractous patients [9].

The levels of the lens vitamin E were significantly low in all the cataract groups as compared to those in the control lenses. This might be due to the fact that the vitamin E and vitamin C antioxidant vitamins act synergistically with the antioxidant enzyme system, i.e. high levels of one enzyme in the presence of low levels of another or high levels of one of the antioxidant vitamins in the presence of low enzyme levels may provide adequate protection [22]. Also Vitamin E, particularly α-tocopherol, functions in vivo as a chain breaking antioxidant and it is also a potent peroxyl radical scavenger. So, maybe, during the process of the free radical scavenge, vitamin E is used up and the level of vitamin E decreases in the lens [23]. But Pradhan AK et al., [18] and Garg et al., [10] did not find a much significant correlation between the serum or plasma levels of vitamin E and the age related cataracts.

In the present study, the TAC level was assessed by the FRAP assay, based on the antioxidant powers of the low molecular weight antioxidants such as vitamins (A, E andC) and trace elements (Zinc, Selenium and Copper). The Status of the Total Antioxidants in the present study was significantly decreased in all cataractous patients as compared to that in the controls. Our findings strongly support those of Paul K et al., [21] and Issa N [22]. A decrease in the TAC is a condition when the production of the ROS increases. This TAC might have participated in the defense against the injurious effects of different ROS which were released due to the increased lipid peroxidation, protein glycation and the aggregation of the high molecular weight proteins. Thus, the low levels of the extra-cellular and the membrane antioxidants make the lens tissue susceptible to different types of oxidations and their byproducts.

CONCLUSION

Cataract is an age related disorder and considering the extent of the disability which is caused by cataracts, it is important that some measures must be taken to slow down the development of cataracts, as we cannot prevent them from occurring. The oxidative stress is increased, both in the age related and the diabetic cataract patients. The results of our study showed a higher production of lipid peroxide and a decreased total antioxidant status in age related as well as diabetic cataract patients. But the diabetic patients were subjected to more oxidative stress at a much earlier age as compared to the senile cataract patients. Hence, the supplementation of an adequate dose of antioxidants to these cataract patients at a much earlier age may be beneficial in delaying the cataract complications.

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