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Oxidative Stress Products in Diabetic and Non-Diabetic Cataract Lens – A Pilot Study

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Abstract

Purpose: The study aims to evaluate the alterations in the levels of oxidative products and enzymatic antioxidants in the lenses of diabetic and non-diabetic cataract patients. **Methods:** The levels of malondialdehye (MDA), conjugated diene (CD), advanced oxidation protein products (AOPP) and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) were measured in the lenses of 30 diabetic and 30 non diabetic patients who underwent cataract surgery. **Results:** Our results show significantly higher MDA, CD and AOPP levels as well as decreased SOD and GPx activity in diabetic cataract when compared to nondiabetic cataract lenses. **Conclusion:** The study indicates an increased lipid peroxidation with correspondingly decreased antioxidant defense in diabetic cataract which suggests a vital role for oxidative stress in the pathogenesis of diabetic cataract.

Keywords
Cataract, malondialdehyde, conjugated diene, advance oxidation protein products, diabetes.

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INTRODUCTION

Loss of transparency of the lens, defined as the cataract is one of the important causes of unavoidable blindness in the world. Surgical extraction of the cataractous lens remains the only treatment despite some post-surgical complications. About 10.8 million were blind and 35.5 million were visually impaired due to cataract in 1990 to 2010.[1] Ocular complications are common in diabetes and cataract becomes the major cause of blindness in these patients.[2]

Lipid peroxidation due to reactive oxygen species (ROS) is one of the likely mechanisms during cataractogenesis that causes dysfunction of cell permeability and therefore reduced defense of the lens. Malondialdehyde (MDA), one of the important

by-products of lipid peroxidation is used as a common biomarker to study the extent of lipid peroxidation [3, 4] and conjugated diene (CD) serves as an initial marker for lipid peroxides.[4] Oxidative stress can also cause numerous protein modifications that result in the formation of high molecular insoluble aggregates that cause lens opacity.[5] Advanced oxidation protein product (AOPP) is identified as a new marker for protein oxidation. It has been reported to be elevated in patients with renal insufficiency, diabetes mellitus and diabetic retinopathy. [6, 7, 8] Oxidative stress has been shown to be linked with diabetes and its complications.[9, 10] ROS trigger cataract, [11] and is found to be one of the degenerative indications of diabetes.[12] Imbalances between free radical production and antioxidant levels generate oxidative stress damaging the cell proteins, nucleic acids and lipids.[12]

The lens has its own defense support against lipid peroxidation with low molecular mass compounds like glutathione peroxidase (GPx) and superoxide dismutase (SOD). These molecules reduce radical propagation by acting against peroxyl radicals and mediate reactions which then interrupt lipid peroxidation in an autocatalytic chain reaction. GPx activity decreases with the progress of cataract which causes the increased accumulation of lipid hydroperoxides in the lens with cataract. Increased activity of GPx and SOD may resist oxidative damage and therefore help to prevent the initiation of the cataract in the lens.[13]

In developing countries like India, there is an early occurrence of cataract and three times more prevalent rates. [14] Changes in the environmental and nutritional patterns have a role in oxidative stress and in cataract formation. The present study evaluated the enzymatic antioxidants and oxidative stress products in diabetic and non-diabetic cataract patients in South India. To the best of our knowledge, this is the first study to compare the CD and AOPP levels in the lenses of cataract and diabetic cataract patients.

METHODS

This comparative case-controlled study consists of 30 non diabetic cataract (control group) and 30 diabetic cataract patients (study group) who underwent cataract surgery. The study was approved by the institutional ethics committee (Ref No: IEC-NI/15/OCT49/61) and informed consent was obtained from all the participants prior to surgery. Patients who were diagnosed with cataracts, with and without diabetes were included in the study. Patients with a history of smoking or with any kind of ocular diseases except for cataract or under antioxidant therapy were excluded. Cataract was diagnosed by slit-lamp examination, visual acuity, direct and indirect ophthalmoscopy. Type I diabetes mellitus was confirmed by blood glucose and HbA1c levels.

Sample collection and processing

The lenses of both nondiabetic and diabetic cataract patients were collected after small incision cataract surgery or extra capsular cataract extraction and immediately stored at -80^{0}C. The color, wet weight and thickness of lenses were noted and the tissue was homogenized in 10mM sodium phosphate buffer (pH 7.2), 1mM Ethylenediaminetetraacetic acid (EDTA) and 0.2mM butylated hydroxytoluene (BHT) in 0.15% ethanol using a Teflon rod under cooling conditions and centrifuged for 10000 rpm for 15 minutes(mins) at 4^0 C. The resulting supernatant was immediately assayed for analysis.

Measurement of Malondialdehyde

Levels of MDA were estimated by the method proposed by Zamora, et al., [15] briefly 10mM sodium phosphate buffer (pH 7.2), 1mM EDTA and of 0.2mm BHT in 0.15% ethanol was added to 30µl of the homogenate. The reaction was started by adding TBA reagent (20% TCA (Trichloroacetic acid) - 0.5% TBA (Thiobarbituric acid) - 0.33N HCl), and the mixture was vortexed, boiled at (15mins, 100^{0}C) and immediately cooled. N-butanol was added with vigorous shaking and the flocculent residue was removed by centrifugation at 6000 rpm for 10 minutes. The optical density of the sample was read at 535nm. The values are expressed as µmol//g lens tissue.

Measurement of baseline conjugated diene

Baseline conjugated diene was measured by the method proposed by Chajes et al. [16] 100µl of homogenate was mixed with 100µl of double distilled water, followed by the addition of 3ml chloroform: methanol in 2:1 ratio (v/v) and further vortexed and centrifuged for 10 mins at 3500rpm (4^{0}C). The alcohol layer was removed and evaporated under nitrogen gas. The remaining residue was redissolved in 2ml cyclohexane and absorbance was measured at 234nm. The results were represented as relative units of optical density (OD_{234})

Measurement of Advanced oxidative protein products

AOPP was quantified by the method proposed by Witko-Sarsat et al.[17] Tissue homogenate was diluted in PBS in 1:5 ratios and placed directly in 96 microtitre plate. chloramine T solution (0 to 100 µmol/L) with 1.16M of potassium iodide were used as standard. Acetic acid was added to all wells

of standards and samples. The values are expressed as µmol/L of chloramine-T equivalents

Activity of Superoxide dismutase

SOD activity was assayed by the method described by Kakkar et al., [18] with slight modifications. The assay mixture contained 50µl of homogenate, 300µl sodium pyrophosphate buffer, 186µM phenazine methosulphate, 300µM nitroblue tetrazolium, 780µM NADH in a total volume of 525µl. The reaction was started by the addition of NADH followed by incubation on (30°C, 90 sec). Then glacial acetic acid was added and the mixture was vortexed with 2ml of n-butanol and allowed to stand for 10 mins at room temperature. The butanol layer was removed after centrifugation at 3500 rpm for 10mins and the absorbance was measured at 560 nm. One unit of enzyme activity is defined as the enzyme concentration required for inhibiting the chromogen production/absorbance by 50% in one minute under the assay condition and expressed as specific activity of SOD units/mg tissue

Activity of Glutathione peroxidase

The activity of GPx was estimated by the method described by Rotruck et al., [19] using H_2O_2 as substrate. The reaction components include 10mM sodium azide, 4mM reduced glutathione, 0.01M sodium phosphate buffer pH (7.0), 50µl of homogenate (enzyme source), 25mM H_2O_2 and 60µl of double distilled water. The assay was started by addition of 10µl of H_2O_2, incubated for 5mins at 37^{0}C and the reaction was stopped by the addition of 10% TCA. The reaction mixture was further centrifuged at 10,000 rpm for 15mins. To the supernatant, 0.3M disodium hydrogen phosphate buffer and Ellman's reagent were added and immediately the absorbance was noted at 0, 60,120,180 seconds at 412nm. The activity is expressed as GSH oxidized/min/mg tissue.

RESULTS

The present study consists of 30 nondiabetic cataract and 30 diabetic cataract patients with a mean age of 63 ± 9 and 60 ± 8 and the average weight of the lens were 102mg and 99.3mg respectively (Table 1).

The levels of enzymatic antioxidants and MDA are given in Table 2. The mean SOD activity in diabetic cataract lenses was found to be significantly decreased compared to controls (P =0.02). The GPx activity in diabetic cataract patients was slightly decreased compared to the nondiabetic group. Similarly the marker for lipid peroxidation MDA and CD were significantly increased in diabetic lenses (P< 0.0001 and P= 0.03 respectively). AOPP levels were also significantly increased in diabetic cataract compared to non-diabetic lenses (P=0.01) (Table 2).

Table: 1. Comparison of age, sex, lens wet weight of diabetic and non-diabetic cataract groups.

	Non-Diabetic cataract	Diabetic cataract
Sex (F/M)	13/17	15/15
Age in yrs (Mean ± SD)	63± 9	60± 8
Average Lens wet weight (mg)	102	99.3

***values are expressed as Mean ± SE**

Table: 2. Activity of SOD, GPx and levels of MDA, CD, AOPP in Nondiabetic and Diabetic cataract patients

Parameters	Non diabetic cataractous lenses (N= 30)	Diabetic cataractous lenses (N= 30)
SOD[a] (units/mg/tissue)	4.61 ± 0.54	2.92 † ± 0.44
GPx[b] (GSH oxidized/min/mg tissue)	19.82 ± 0.92	18.21 ± 0.64
MDA[c] (µmol//g tissue)	57.39 ± 2.79	85.61‡ ± 5.39
CD[d] (OD_{234})	0.40 ± 0.25	0.56 # ± 0.28
AOPP[e] (µmol/ml)	0.17 ± 0.02	0.18 $ ± 0.02

[a]Superoxide dismutase (SOD), [b]Glutathione peroxidase (GPx), [c] Malondialdehyde (MDA), [d]Conjugated diene (CD), [e]Advanced oxidation protein products (AOPP)

† P = 0.02, ‡ P< 0.0001, # P value equals 0.0374, $ P value equals 0.0122 values are expressed as Mean ± SE in Non-diabetic and diabetic cataracts.

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Declaration of interest
The authors report no conflicts of interest.

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DISCUSSION
In hyperglycemic condition, there exist at least six different pathways that involve in the accumulation of reactive oxygen species within the lens [20] and uncontrolled glycemia leads to an increased production of oxidative products in plasma and tissues of diabetes patients.[21]Cataract remains as one of the secondary complications of diabetes, with increased disease duration increasing in the risk for cataractogenesis.[22, 23]It has been well documented that free radicals damage proteins, lipids, and DNA under oxidative stress. Normal lens is fully equipped with antioxidants to strive against oxidative stress. Enzymatic antioxidants namely superoxide dismutase and glutathione peroxidase combat the toxic effects of ROS. [24]
In this study, we analyzed the oxidative stress products of lipids and proteins and enzymatic antioxidant levels in non-diabetic and diabetic cataract lenses. Lipid peroxidation has been found to be increased in diabetes [25, 26] and MDA, breakdown product of polyunsaturated fatty acids under peroxidation condition, is found to be a reliable, sensitive and stable marker to evaluate the oxidative stress *in vivo*. Hashim Z et al., [26] demonstrated a higher MDA level in the plasma of diabetic cataract when compared to non-diabetic cataract patients and the same has been found in the lenses, which is concurrent with our study.[27, 28] Babizhayav [29] and Chang et al., [30] have reported increased accumulation of conjugated diene in the lenses and serum of cataract patients respectively. CD levels were high in the early developmental stage of cataract and this diene act as an initiating factor which propagates later and affects the lens. [4] A higher degree of glycation and glucose autooxidation process may potentially induce the free radical-mediated lipid peroxidation in diabetes mellitus. Markers of protein oxidation have been associated with cataractogenesis [30] and we found an increased AOPP in diabetic cataract patients which supports the idea that frequency of protein damage is high in cataract patients with diabetes when compared to those without diabetes. These oxidative products may promote the progression of microvascular damage in diabetes and also alter the blood-ocular barrier.
In aging, enzymes are exposed to frequent post-translational modifications and also a decreased protein turnover rate in differentiated fiber cells.[31]
We found a pronounced decrease in SOD activity in diabetic cataract when compared to non-diabetic cataract which supports the idea that deactivation of antioxidant enzymes could mediate the development of diabetic complications. Deactivation of antioxidant enzymes could increase H_2O_2 and O_2^- level in the lens which may cause oxidative protein modification.[32, 33, 34] GPx reduce the oxidative damage at a cellular level under physiological conditions [35, 36, 37] and has the ability to combat the toxic effects of hydroperoxides.[38] Gpx activity was low in diabetic patients when compared to the non-diabetic group, but significance was not noted. Decrease in SOD level increases the super oxide radicals, which further inactivate the enzymes, catalase and glutathione peroxidase.[39, 40] Increased lens lipid peroxide and decreased antioxidant enzyme activities were earlier reported in cataractous lenses.[24, 41]The lens dry matter is mostly of crystalline protein which changes very slowly during normal eye life. If exposed to free radicals, lens transparency is affected due to carbonylation and glycation of the lens protein. [42]
Most of the lipids are bound to proteins thereby limiting their movement and enhancing lens opacity. In human cataract, the composition of lens lipids changes markedly and this process is very fast in diabetic patients with high plasma glucose level.
Oxidative stress plays a dominant role in opacification of the lens by increasing the lipid peroxidation and decreasing the anti-oxidant levels and thereby acts as a key element in the development of cataract in diabetes. Antioxidants that target on the lipids could help in decreasing the cataract incidence in diabetes as well as in nondiabetic cataract patients. There is a strong relationship between cataract in diabetic patients with peroxidation of lipids and diabetic patients are prone to cataract formation. Through GPx and SOD, lens protects itself from oxidants like MDA, CD, AOPP. Since India has a huge number of diabetes patients, the research on this path can suggest that

lifestyle modifications can be useful to prevent or delay the occurrence of cataract in these patients.

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