

# Evaluation of antioxidants and argpyrimidine in normal and cataractous lenses in north Indian population

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## Abstract

• **AIM:** To assess the level of glutathione, thioltransferase, and argpyrimidine in nuclear and cortical cataractous lenses as well as in the clear lenses in the north Indian population.

• **METHODS:** Human cataractous lenses were collected from the patients who underwent extracapsular cataract extraction surgery; clear lenses were collected from the freshly donated eye bank eyes. Antioxidant molecules such as glutathione and thioltransferase enzyme activity were measured; simultaneously in these lenses a blue fluorophore argpyrimidine, an advanced glycation end (AGE) product level was assessed using high performance liquid chromatography (HPLC).

• **RESULTS:** The protein concentration was found to be present at higher levels in the control lenses compared to cataract lenses. A significant decrease in the glutathione level was observed in the nuclear cataractous lenses compared to cortical cataractous ( $P=0.004$ ) and clear lenses ( $P\leq 0.005$ ), but no significant change in the level of antioxidant enzyme thioltransferase was observed. Further, argpyrimidine a blue fluorophore (AGE) was found to be significantly higher in the nuclear cataract ( $P=0.013$ ) compared to cortical cataract lenses.

• **CONCLUSION:** Antioxidants such as glutathione significantly decrease in age-related nuclear and cortical cataract and an AGE, argpyrimidine are present at significantly higher levels in nuclear cataract.

• **KEYWORDS:** age-related cataract; antioxidants; argpyrimidine; glutathione; nuclear cataract; cortical cataract; thioltransferase

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## INTRODUCTION

Cataract refers to the opacification of the crystalline lens resulting from alterations in cellular architecture of lens specifically in lens proteins<sup>[1]</sup>. According to the World Health Organization report (2004), cataract is the leading cause of blindness around the world<sup>[2]</sup>. It is an irreversible age-related process for which modern medical science has no effective pharmacological management<sup>[3]</sup>. The exact mechanism of cataract formation is yet to be defined. Various risk factors are known to be associated with cataract; these include age, by far the biggest risk factor, the incidence of all type of cataracts increases exponentially after 50y, cigarette smoking, diabetes mellitus, sunlight exposure, drug usage, educational status, and errors in refraction<sup>[4]</sup>.

The lens proteins undergo non-enzymatic post translational modification and cross-linking, which finally lead to light scattering in the lens resulting in cataract formation<sup>[5]</sup>. Crystallins ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) are the major proteins of the lens. The physiological function of eye lens depends on a balanced redox state in the micro-environment for maintaining its transparency, as lens cells are constantly exposed to reactive oxygen species<sup>[6]</sup>. Mitochondria is primary site for the formation of reactive oxygen species (ROS) as 95% of  $O_2$  which is generated during the normal metabolism of cells from the electron transport chain (ETC) in inner mitochondrial membrane<sup>[7]</sup>. These ROS are generated endogenously or exogenously from the environment. Redox homeostasis in the lens is achieved by scavenger antioxidant molecules and repair systems. The endogenous high level of reduced glutathione (GSH) plays a vital role as the first line of defense against reactive oxygen species. GSH is not distributed uniformly within the lens. Its concentration is higher in the cortex than in the nucleus<sup>[8]</sup>. The GSH level in the lens decreases progressively with age and during cataract formation in humans<sup>[9]</sup>. The second line of defense for lens is its intrinsic repair enzymes. The two important enzyme-repair systems include GSH-dependent thioltransferase and NADPH-

dependent thioredoxin/thioredoxin reductase. Thioltransferase (E.C.1.8.4.1) acts as a repair enzyme to prevent lens protein aggregation by restoring thiolated proteins to their normal reduced state by cleaving protein-thiol mixed disulfides<sup>[8]</sup>.

Lens protein undergoes various physicochemical changes during aging and cataract formation<sup>[10]</sup>. Long lived proteins including  $\alpha$ -crystallins are the most susceptible to form the advanced glycation end products. The Maillard reaction is one mechanism implicated in such changes, where aldehydes and ketones react non-enzymatically with the amino group of proteins to form an irreversible advanced glycation end (AGE) product<sup>[11]</sup>. An important AGE is argpyrimidine which is a blue fluorophore derived from the reaction of methylglyoxal (MG) with guanidine group of arginyl residues on proteins. Padayatti *et al*<sup>[11]</sup> reported the presence of argpyrimidine in the water insoluble fractions of crystallins at higher level in case of human brunescant cataractous lenses as compared to normal young lenses and aged lenses.

Ethnic variation in cataractogenesis is a widely known fact<sup>[12]</sup>. In the present study, an attempt has been made to assess the activity of thioltransferase, GSH and argpyrimidine in the lenses obtained from the north Indian patients having nuclear or cortical cataract and in control lenses and to find out correlation between the level of argpyrimidine and the antioxidants.

## SUBJECTS AND METHODS

**Patient Samples** This study was approved by the Institutional Review Board and all ethical guidelines were followed. A written informed consent was taken in every case as per the institute's guidelines. The study adhered to the tenets of the Declaration of Helsinki. All patients underwent detailed ocular examinations including type of cataract and fundus evaluation. The samples for the present study consisted of cataractous lenses from patients aged >40y, with visually significant cataract who underwent extra capsular cataract extraction surgery either for predominant cortical cataract ( $n=30$ ) and nuclear cataract ( $n=30$ ) from Advanced Eye Center, Post Graduate Institute of Medical Education and Research, Chandigarh, India. Control clear lenses were collected from donors >40y of age ( $n=15$ ) from the Eye Bank. Patients with history of diabetes mellitus, any form of traumatic cataract and any history of using systemic or topical steroids were excluded from this study.

**Preparation of Crude Human Lens Homogenate** Each lens was homogenized in 2 mL of 0.1 mol/L potassium phosphate buffer (pH 7.4). An aliquot of the lens homogenate was separated and used for glutathione assay and the remaining homogenate was centrifuged (10 000 rpm, 30min, 4°C). The supernatant was designated as the water soluble fraction and the pellet was designated as the water insoluble fraction. The water soluble fraction was used for the measurement of

thioltransferase activity and the water insoluble fraction was used for the assessment of argpyrimidine level.

**Detection of the Level of Glutathione in Cataractous Lenses and Control Lenses** The level of GSH in each lens homogenate was determined by the method of Cui and Lou<sup>[13]</sup> (1993). In all the assays, blank and different dilutions of standard (reduced glutathione as standard, with concentrations 20-100 nmol) were run in parallel. The concentration of the GSH in the samples was estimated from the standard curve plotted with the known concentrations of reduced glutathione against their respective optical density (OD) at 412 nm. Results were expressed as mmol/L.

Briefly, 20  $\mu$ L of 10% trichloroacetic acid (TCA) was added to 200  $\mu$ L of lens homogenate, the mixture was centrifuged (10 000 rpm, 30min) and the supernatant was used for the detection of glutathione level. The assay was carried out in flat-bottomed micro-titre plates. The reaction mixture consisted of 20  $\mu$ L of lens homogenate, 176  $\mu$ L of 0.2 mol/L  $\text{Na}_2\text{HPO}_4$  containing 2 mmol/L  $\text{Na}_2\text{EDTA}$  and 4  $\mu$ L of 10 mmol/L 5, 5'-dithiobisnitro-benzoic acid (DTNB). The OD was measured at 412 nm after 5min.

**Assay for Activity of Thioltransferase in Cataractous Lenses and Control Lenses** Thioltransferase activity was measured by the method of Mieyal and Chock (2012)<sup>[14]</sup>. The reaction mixture consisted of 100  $\mu$ L 0.1mol/L potassium phosphate buffer (pH 7.4), 100  $\mu$ L 0.2 mmol/L NADPH, 50  $\mu$ L 0.5 mmol/L GSH, 1.1  $\mu$ L 0.4 units of GSSG reductase and an aliquot of lens sample in a total volume of 1 mL. The reaction was carried out at room temperature and initiated after 5min by the addition of 100  $\mu$ L 2 mmol/L hydroxyethyl disulfide (HEDS). The decrease in absorbance of NADPH at 340 nm was monitored at 1min interval for 5min, using spectrophotometer (Beckman Coulter Inc., USA). Specific activity (mU/mg of protein) of the enzyme was determined from nmols of NADPH (molar extinction coefficient of NADPH 6.22  $\text{L}\cdot\text{mmol}^{-1}\cdot\text{cm}^{-1}$  under standard assay conditions) oxidized/min/mg of protein.

**Protein Estimation** The protein content in the lens homogenate as well as the water soluble fraction of the lens homogenate was determined using a Bicinchoninic acid assay<sup>[15]</sup>.

**Synthesis of Argpyrimidine** Since argpyrimidine was not commercially available, it was synthesized in the laboratory by the method of Shipanova *et al*<sup>[16]</sup> using methyl glyoxal and N- $\alpha$ -tertiary butyloxycarbonyl (t-BOC)-arginine. Briefly, 0.2 mol/L each methyl glyoxal and N- $\alpha$ -t-BOC-arginine at a molar ratio of 1:1 was dissolved in 9.6 mL 0.02 mol/L sodium phosphate buffer (pH 7.4). The reaction mixture was adjusted to pH 7.4 with 1.7 mL 1 mol/L NaOH and incubated at 55°C for 4d under sterile conditions. This was then treated with 10 mL 1 mol/L HCl for 2h at room temperature to release the t- $\alpha$ -BOC group from arginine. The resulting liquid mixture was concentrated

## Antioxidants and argpyrimidine in cataract

under vacuum and suspended in 8 mL of 80% methanol in water. This mixture was chromatographed on preparative silica plates (Silica 60, 20×20 cm, 2.0 mm, Altech associates, Inc., Deerfield, IL, USA) using solvent system of butanol: acetic acid: pyridine (5:2:2.5:2.5, v/v/v/v). The fluorescent band detected at  $R_f$  values 0.56 under long wave length ultra violet light was marked, scraped off the plate and then suspended in 10 mL distilled water. The suspension was further stirred overnight and then centrifuged (6500 rpm, 30min). The resultant supernatant was dried under vacuum, dissolved in 6 mL of water and filtered through a 0.4  $\mu$ m filter (Millex-GV Millipore, Japan). This was fractionated by preparative high performance liquid chromatography (HPLC, PerkinElmer, USA) using a C-18 reversed-phase column (4.6×25 cm, 10  $\mu$ m, Rustek, USA). The column was equilibrated with mobile phase A (water +0.1% TFA) with a flow rate of 1 mL/min. A gradient of 50% acetonitrile in water with 0.1% TFA was used as the mobile phase B. The gradient programme was as follows: 0-5min 0 B, 5-10min 30% B, 10-15min 50% B, 15-19min 70% B, 19-30min 100% B, 30-40min 0 B. The fluorescent compound was detected by an on line fluorescence detector set at excitation wavelength 320 nm and emission wavelength 380 nm. The absorption maxima of this compound at 231 nm and 335 nm were detected by an on-line diode array detector. A major fluorescent peak at retention time 11.5min was detected.

**Detection of Argpyrimidine in the Lens Samples** The water insoluble fraction of each lens homogenate was treated with 3 mL of chloroform: methanol (2:1) mixture for 3h with stirring and centrifuged (6500 rpm, 30min). The pellet was treated with 5 mL diethyl ether for 10min and centrifuged (6500 rpm, 30min). The residue obtained was dried in a desiccator and kept at 4°C. The 10 mg of WI protein fraction of each lens homogenate was treated with 10  $\mu$ L pronase E (2% w/w) in 200  $\mu$ L 0.05 mol/L phosphate buffer (pH 7.4) containing 0.02% sodium azide and incubated at 37°C for 24h. Digestion was continued with further addition of 10  $\mu$ L pronase E (2% w/w) for 16h. The digest was treated with 20  $\mu$ L aminopeptidase M (0.5% w/w) and 10  $\mu$ L carboxypeptidase Y (0.5% w/w) for 16h and 8h respectively. The digest was centrifuged (6500 rpm, 30min) to remove floating debris.

The argpyrimidine content in the digest was estimated by using HPLC. Briefly, 100  $\mu$ L of the digest was injected into C-18 reversed-phase column (Rustek, USA). Elution of argpyrimidine was achieved under the same conditions as described before. Results were expressed as the fluorescent intensity of the argpyrimidine in test samples. Further, for quantitative estimation of the argpyrimidine concentration in the test samples, different dilutions of the standard argpyrimidine (1 mg/mL) were run in HPLC under the same

**Table 1 Age of cataract patients and controls**

Groups (n)	Mean age $\pm$ SD (a)
Control lenses X (15)	47.20 $\pm$ 8.21
Nuclear cataract lenses Y (30)	71.25 $\pm$ 11.78
Cortical cataract lenses Z (30)	62.33 $\pm$ 10.73

**Table 2 Mean total protein levels in lenses of cataract patients and control and comparison**

Groups (n)	Total protein (mg/mL)	Comparison	P
Control lenses X (15)	12.1 $\pm$ 5.0	X vs Y	0.003
Nuclear cataract Y (30)	6.8 $\pm$ 3.4	Y vs Z	0.020
Cortical cataract Z (30)	10.09 $\pm$ 5.75	Z vs X	0.600

conditions. The argpyrimidine level was expressed in view of fluorescent intensity.

**Statistical Analysis** The mean, median and interquartile range for glutathione, thioltransferase activity, and argpyrimidine levels were calculated. For comparison of the individual parameters in different groups Mann-Whitney *U* test (comparison of median) were used. For the comparison of the interrelationship of each parameter within each group Pearson correlation, Spearman's rank correlation and linear regression analysis were employed.

## RESULTS

We analyzed 30 lenses with nuclear cataract and 30 lenses with cortical cataract from patients who underwent cataract extraction for visually significant cataract. There were also control 15 lenses obtained from donors from eye bank.

The total number and mean age of patients and controls in the present study was described in the Table 1.

The protein concentration in the supernatant of each lens homogenate was measured and results were expressed as mg/mL as shown in the Table 2. The protein concentration was found significantly reduced in nuclear cataract lenses as compared to control ( $P=0.003$ ) as well as cortical cataract lenses ( $P=0.020$ ).

The glutathione level (mmol/L) was measured in the supernatant of each lens. A significant decrease in the glutathione level (median value) was observed in the nuclear cataract lenses as compared to control as well as cortical cataract lenses (Figure 1A).

Although an increase in the TTase activity levels was observed (median level) in cortical and nuclear cataract groups compared to controls lenses, this change was statistically not significant between any of the groups Figure 1B.

**Synthesis of Argpyrimidine** A major peak with retention time 11.5min was detected at the emission wavelength, 380 nm in an on line fluorescence detector set at the excitation wavelength, 320 nm. This peak also showed absorbance maxima at 231 nm and 335 nm as obtained by an on-line

**Table 3 Correlation data for GSH levels, thioltransferase activity with total protein concentration**

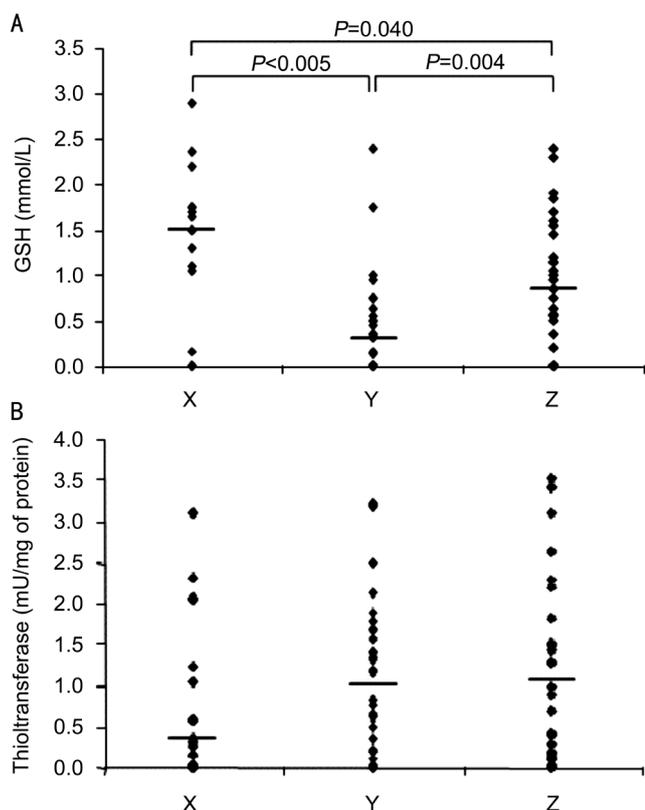
Group (n)	GSH vs total protein <i>r</i> ( <i>P</i> )	TTase vs total protein <i>r</i> ( <i>P</i> )
Control (15)	0.654 (0.011)	-0.276
Nuclear cataract (30)	0.225	0.370 (0.043)
Cortical cataract (30)	0.487	-0.244

TTase: thioltransferase.

**Table 4 Correlation data for GSH level, thioltransferase activity with argpyrimidine levels**

Group (n)	Argpyrimidine vs GSH <i>r</i> ( <i>P</i> )	Argpyrimidine vs TTase <i>r</i> ( <i>P</i> )	GSH vs TTase <i>r</i> ( <i>P</i> )
Control (15)	-0.011	0.230	-0.187
Nuclear cataract (30)	-0.166	-0.156	0.366 (0.043)
Cortical cataract (30)	0.215	0.489 (0.01)	0.186

TTase: thioltransferase.



**Figure 1 Glutathione level (A) and thioltransferase activity (B) in lens samples of patients and control samples** X consisted of clear control lenses ( $n=15$ ), Y and Z consisted of patients ( $n=30$ ) having predominantly nuclear cataract and cortical cataract patients.

diode array detector (data not shown). MALDI-TOF mass spectrometric analysis of this purified fraction revealed the presence of a molecule with  $m/z$  ratio of 256.95 which corresponded to the mass of argpyrimidine (data not shown). Further, using this argpyrimidine as a standard, the level of argpyrimidine in the lens samples of cataract patients and controls was detected using fluorescence intensity of the peak detected at the same retention time as that of standard argpyrimidine.

Enzyme digested control lens samples as well as the lens samples of patients having nuclear cataract and cortical cataract respectively for detection of the level of argpyrimidine in view

of the fluorescence intensity ( $\mu V/S$ ). A significant increase in the argpyrimidine level (median value) was noted in nuclear cataract as compared to cortical cataract lenses ( $P=0.013$ ).

**Correlation Study** A significant correlation was found between protein concentration and glutathione level in control lenses ( $P=0.011$ ). A significant correlation between protein concentration and thioltransferase activity was found in nuclear cataract lenses ( $P=0.043$ ) in Table 3. A significant correlation between glutathione level and thioltransferase activity was found in nuclear cataract lenses ( $P=0.043$ ). Further a significant correlation was found between thioltransferase activity and argpyrimidine level in cortical cataract lenses ( $P=0.01$ ). Further no significant correlation was found between the level of glutathione and argpyrimidine in any of the groups in Table 4.

#### DISCUSSION

Ageing has been established as a major risk factor for human cataract formation resulting in the increased oxidative stress and lower antioxidant capabilities in the lens<sup>[17]</sup>. Studies have shown that a substantial decrease in the rate of diffusion of small molecules between lens cortex and nucleus in the older lenses<sup>[18]</sup> with age this "diffusion barrier" was postulated to increasingly limit the transport of the intra-cellular antioxidants to the lens nucleus, which may result in the increased susceptibility of older lenses to the oxidative damage<sup>[19]</sup>. Cigarette smoking and smoky cooking fuel both implicated in the etiology of cataract, as cigarette smoke and firewood smoke condensate permeates the lens capsule and imparts opacification of lens in an light and dose dependent manner. Long term exposure to ultra-violet light (UV-B) is known to be associated with cortical cataract development<sup>[4]</sup>.

Human lens is differentiated into three different parts: nucleus, cortex and lens epithelium. The epithelial cells are present under the collagenous capsule surrounding the lens and are most metabolically active cells<sup>[20]</sup>. Some of these cells are dividing to form fibres cells where gene products of crystallins are formed. The outer layer of fibre cells consists of cortex and below the cortex the oldest nuclear cells are present<sup>[21]</sup>. Normal

human lens consists of protective agents and systems to combat oxidative stress. For decades chronic exposure to active forms of oxygen may lead to gradual erosion of antioxidants and their protective mechanisms of the lens. The major antioxidants of lens include GSH and ascorbic acid<sup>[22]</sup>. Any decrease in the antioxidant levels makes the lens prone to oxidative damage, which in turn results in accumulation of oxidized residues in the long lived lens proteins<sup>[23]</sup>. The present study also supports evidence that the enzyme responsible for protein thiol oxidation repair, including GSH, thioltransferase becomes less efficient during aging process. It is well known that GSH in the lens is vital for its maintenance in its transparency<sup>[24]</sup>. Studies have indicated an important hydroxyl radical-scavenging function for GSH in lens epithelial cells independent of the cells ability to detoxify H<sub>2</sub>O<sub>2</sub>. The synthesis and recycling of GSH falls with age, leading to a progressive loss of this molecule and a rise in its oxidized form (GSSG). This is partly due to a marked fall in glutathione reductase activity with age<sup>[25]</sup>. The relatively low ratio of GSH to protein-SH in the nucleus of the lens of adult individuals, combined with low activity of the GSH redox cycle makes the nucleus especially vulnerable to oxidative stress, as has been demonstrated in experimental animal models exposed to hyperbaric oxygen/UV-A as well as in the glutathione peroxidase knockout mouse<sup>[23]</sup>. Further loss of GSH occurs in cataractous lenses and over 50 percent of the methionine residues and nearly all of the cysteine residues in the proteins have been found to be oxidized<sup>[26]</sup>. Many reports suggests that mitochondrial dysfunction plays a vital role in the generation of reactive oxygens species (ROS) in mitochondria, which produces 95% of O<sub>2</sub> in the metabolism of normal cell *via* ETC in the inner mitochondrial membrane, which is susceptible to formation of free radicals, any damage to mitochondria of epithelial cells may result in ROS production which inturn may affect proteins and lipid plasma cell membrane of the underlying fibre cells in lens leading to cataract formation<sup>[7]</sup>. In the present study we observed that GSH was more depleted in nuclear cataractous compared to cortical cataractous and control lenses. We feel that these results were justified because since the occurrence of nuclear cataract was at higher age than cortical cataract. Since aging is a recognized cause of oxidative stress with evidence of GSH depletion, it might be possible that in nuclear cataractous lenses comparatively more GSH depletion occurs due to a synergistic effect of age and disease. It might be possible that GSH decrease in the diseased lenses is not solely due to cataract, but along with cataract other factors such as ageing, smoking, UV-light exposure, may act as contributory factors for the depletion of GSH and thioltransferase in the cataractous lenses as compared to control lenses.

The human lens has a peculiar anatomical structure *i.e.* presence of cellular part, acellular (fibrous) part and non-

nucleated portion. GSH depletion that results in oxidative stress, may not be associated with enhanced TTase induction resulting in increased TTase activity, which our observations suggest occur in lenses obtained from patients suffering from different types of cataract in relation to clear lenses.

The antioxidant GSH is depleted in the cataractous lenses with no statistically significant increase of the enzymatic counterpart (TTase) which is expected to generate serious oxidative stress resulting in the formation of advanced glycation end products (AGES). In relation to oxidative stress, the lens is shown to generate AGEs even in non-diabetic individuals<sup>[27]</sup>. Among the AGEs, argpyrimidine is of special concern since it has been detected from biological samples including cataractous lenses in a number of studies<sup>[28]</sup>. It was demonstrated that methyl glyoxal could modify arginine and form argpyrimidine in human lenses and this changes occurred at much higher rate in brunescens lens proteins than in either nuclear cataractous lenses or normal lenses. Further, the authors reported that lens crystallins showed argpyrimidine and covalently cross linked aggregates<sup>[10]</sup>.

In the present study we have observed that a statistically significant increase of argpyrimidine concentration was obtainable from the extracts of nuclear cataractous lenses, when compared to extracts of cortical cataractous lenses and clear lenses. This is obvious since we have also observed that GSH depletion was maximum in nuclear cataractous lenses and the patients who were considered in the nuclear cataract group were of more advanced age than the cortical cataract group. We believe that lack of enhancement of TTase activity might also contribute to increased production of argpyrimidine in the nuclear cataract group. Thus, it may be possible that more GSH depletion, increased age and lack of enhancement of TTase activity synergistically caused oxidative stress in the nuclear cataractous lenses resulting in more formation of argpyrimidine as compared to cortical cataractous lenses and clear lenses. It was observed that there is no significant difference in the weight of cataractous lenses and clear lenses (data not shown). Nuclear cataractous lenses, which contain significantly, lower concentration of protein as compared to cortical cataractous lenses and clear lenses. This is obvious because in nuclear cataractous lenses the oxidative stress was evidenced to be more due to GSH depletion, argpyrimidine formation and lack of enhancement of TTase activity. It is needless to mention that oxidative stress is universally accompanied by insults to biomolecules produced by ROS which may result in protein destruction.

It has been observed that in case of cortical cataract, a significant correlation exists between protein and GSH concentration of the lenses and which is not the fact in case of nuclear cataract. The increased argpyrimidine formation in the nuclear cataractous lenses indicates that there is more

methylglyoxal formation in this particular case. Methylglyoxal is known to form hemithioacetal adduct with cysteine<sup>[21]</sup>. It might be possible that due to this reason, GSH, which is a cysteine containing tri-peptide, has been comparatively depleted more than protein in nuclear cataractous lens, while a significant correlation was found between protein concentration and thioltransferase activity in the nuclear cataractous lens. This is because the nucleus of the lens is mostly acellular, this cannot induce TTase in response to oxidative stress. Therefore, oxidative stress in the nucleus of the lens is capable of protein destruction which in turn is expected to reduce TTase activity also. Furthermore, a significant correlation between GSH level and TTase activity may be possible in nuclear cataractous lens which is also a finding of the present study. It was also observed that GSH and argpyrimidine levels were not shown to be correlated significantly. This is expected, since methylglyoxal is known to form adducts with GSH and it also synthesizes argpyrimidine in GSH independent manner<sup>[29]</sup>. Further, we have also been observed that in case of cortical cataractous lenses, a significant correlation was found between thioltransferase activity and argpyrimidine level. This may be due to the fact that the cortex of the lens is a cellular structure, where oxidative stress can induce TTase formation as well as argpyrimidine generation.

In the present study, there was difference in the average age group in cortical and nuclear cataract groups compared to control group with no cataract as we could not obtain the clear lenses without cataract in the matching eyes of donors (eye bank) but these donors lenses were more than 40 years of age. Also in the present work evaluating the level of ascorbic acid might have given strong evidence for the antioxidant molecules role in the development of cataract, as we have not assess the vitamin C, which we feel were the limitations of the present study.

In the present study in view of decreased glutathione level and increased argpyrimidine formation in cataractous lenses indicate oxidative stress; to the best of our knowledge this is the first report from north Indian population regarding this, keeping in view the ethnic variations in cataractogenesis this study is of considerable current interest.

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