

# CHANGES IN GLUTATHIONE, GLUTATHIONE-LINKED ENZYMES AND HEXOSE MONOPHOSPHATE SHUNT ENZYMES IN SENILE CATARACT

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**Abstract :** Changes in glucose-6-phosphate dehydrogenase (G-6-PD), glutathione reductase (GSH-R), reduced glutathione (GSH), glutathione peroxidase (GSH-PO), transketolase (TK) and transaldolase (TA) were studied in lens and red blood cells (RBCs) to understand the possible biochemical mechanisms responsible for the development of senile cataract. The activity of G-6-PD was increased in lens, though not so in erythrocytes during cataractogenesis. A marked decrease was observed in GSH level and GSH-R activity in the lens and RBCs of the cataractous group. The activity of GSH-PO was remarkably high in lens but not in the erythrocytes during the maturity of cataract. The activity of TK decreased gradually in both the lens and erythrocytes. The activity of TA decreased in erythrocytes but increased in the lens with maturation of cataract.

**Key words :** senile cataract  
transaldolase

G-6-PD

glutathione  
transketolase

## INTRODUCTION

Cataract that develops in older people without a known cause is called senile cataract and is about 85% of all the known cataracts (1). The hexose monophosphate shunt (HMPS) is extremely active in lens and erythrocyte. The primary purpose of HMPS is to generate reducing power (NADPH+H<sup>+</sup>) which is utilised for various biosynthetic reactions. This reducing power is also used to maintain the antioxidant glutathione in the reduced state (2).

Depletion of reduced glutathione (GSH) and consequent formation of large protein aggregates with covalent disulphide crosslinks results in the protein insolubilization, which is the major reason for senile cataract. GSH-PO may play an important part in the lens metabolism by removing hydrogen peroxide through glutathione linked enzyme system (3).

Although oxidation is believed to be the initiation factor in senile cataract formation, the mechanism is still far from understood

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(3). An effective anticataract medication can be developed only by an expanded and intensified programme of cataract research directed towards understanding the physiology and chemistry of the normal and cataractous lens. The present investigation was designed to trace the HMPS pathway, glutathione and glutathione-linked enzyme systems in senile cataractous lens and erythrocytes with a view to explore the cause and effect relationship, if any.

## METHODS

Human blood and lens samples from normal and cataractous cases aged 40–85 years. Samples were obtained from the patients admitted for cataract extraction in the Ophthalmology Department, Medical College, Kottayam. Time interval between death and enucleation of normal eyes ranged from 1–6 h. Subjects had undergone clinical examination under dilated pupil with ophthalmoscope and slit lamp, and cataract had been graded as immature and mature (4).

- (a) Immature cataract: Visual acuity better than hand movement with counting fingers and presence of some optically clear space between the capsule and cataractous nucleus plus cortex.
- (b) Mature cataract: Visual acuity, hand movement and accurate projection of light. All layers of the lens fibre opaque without any change in the capsule.

NADP, NADPH, NADH, GSSG, glutathione reductase, t-butyl hydroperoxide,

fructose-6-phosphate, erythrose-4-phosphate, glycerol phosphate dehydrogenase, triose phosphate isomerase,  $\beta$ -mercaptoethanol, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and ribose-5-phosphate were purchased from Sigma Chemical Co. and all other reagents were of analytical grade and procured locally.

### Preparation of homogenate

The lenses were collected in bottles with ice cold saline. Weighed samples were homogenised with 10 parts of chilled buffer in a Potter-Elvehjem glass grinder. Enzyme assays were carried out in the supernatants obtained by centrifuging at 4°C at about 12000 × g for 10 min.

### Preparation of haemolysate

Venous blood specimens for the assays were anticoagulated with disodium EDTA, 1 ± 0.25 mg/ml of blood. The samples were centrifuged to remove the plasma and buffy coat. The remaining blood cells was washed with cold saline for thrice and resuspended in an equal volume of cold sodium chloride. To 0.2 ml of washed erythrocyte suspension in cold saline, 1.8 ml haemolysing solution was added and immersed in the freezing mixture until the contents were completely frozen and were repeatedly thawed. This was referred to as 1:20 haemolysate.

### Biochemical assays

Blood sugar estimation was done by the method of Cooper and Mc Daniel (5) to screen out diabetic cases. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)

activity of erythrocytes and lens were assayed by the method of Fairbanks and Klee (6). The increase in absorbance of NADPH<sup>+</sup> was measured at 340 nm in a spectrophotometer (Systonix 108). Glutathione reductase (EC 1.6.4.2) activity was measured using the method of Goldberg and Spooner (7), reduced glutathione by the method of Beutler et al (8) and glutathione peroxidase (EC 1.11.1.9) by the method of Beutler (9). Transaldolase activity (EC 2.2.1.2) was assayed by the method of Karl Brand (10). Macro method of Myron Brin (11) was followed to assay transketolase (EC 2.2.11). Protein and haemoglobin (Hb) were assayed by standard methods (12, 13). For statistical analysis, mean  $\pm$  standard deviation of measured values were evaluated by Student's t-test.

## RESULTS

Blood sugar level was normal showing that the cataractous cases were non-diabetic. G-6-PD activity of erythrocyte decreased significantly ( $P < 0.05$ ) in immature cataract (Table II) and the same was increased in lens significantly right from normal to mature cataract (Table I).

GSH-R activity in lens was significantly decreased ( $P < 0.01$ ) in immature and mature cataract in comparison to controls, the lowest being in mature cataract (Table I). The level of reduced glutathione in erythrocytes decreased significantly ( $P < 0.01$ ) in cataractous cases in comparison to controls (Table II). Significant decrease in GSH content in lens was observed in immature ( $P < 0.05$ ) and mature ( $P < 0.01$ ) cataract in comparison to controls. GSH

TABLE I: Levels of enzymatic activity of normal and cataractous lenses (Values are Mean  $\pm$  S.D. Numbers in parenthesis indicate the sample size).

Parameters	Control	Immature	Mature
GSH ( $\mu$ moles/g protein)	4.3 $\pm$ 0.10 (5)	2.5 $\pm$ 1.58 (7)	0.44 $\pm$ 0.24 (7)
GSH-R (units/g protein)	17.36 $\pm$ 1.59 (5)	16.3 $\pm$ 0.7 (9)	13.46 $\pm$ 0.55 (9)
GSH-PO (units/g protein)	0.07 $\pm$ 0.02 (4)	3.64 $\pm$ 0.62 (10)	4.92 $\pm$ 0.48 (10)
Glucose-6-phosphate dehydrogenase G-6-PD (units/g protein)	4.27 $\pm$ 0.61 (10)	10.5 $\pm$ 2.94 (15)	15.9 $\pm$ 8.72 (15)
Transaldolase TA (units/1 homogenate)	28.8 $\pm$ 9.60 (6)	53.7 $\pm$ 13.68 (10)	54.1 $\pm$ 13.05 (9)
Transketolase TK (units/1 homogenate)	30.02 $\pm$ 0.10 (5)	10.18 $\pm$ 2.81 (12)	10.19 $\pm$ 4.99 (11)

TABLE Ia: Levels of enzymatic activity of normal and cataractous lenses (Significant level).

Parameters	<i>P</i> value		
	C vs. IM	C vs. M	IM vs. M
GSH	0.05	0.01	0.01
GSH-R	0.01	0.01	0.01
GSH-PO	0.01	0.01	0.01
G-6-PD	0.01	0.01	0.01
TA	0.01	0.01	NS
TK	0.01	0.01	NS

C-Control, IM-Immature, M-Mature, NS-Not significant

content was significantly lower ( $P < 0.01$ ) in mature cataract vis-à-vis immature cataract (Table I). GSH content in erythrocytes decreased in immature cataract contrary to mature cataract where the same was increased (Table II).

There was no significant change in mean GSH-PO activity in erythrocytes between the cataractous groups and controls (Table II). GSH-PO activity of lens showed a significant ( $P<0.01$ ) and steep increase in all the cataractous groups. The increase in activity was highly significant ( $P<0.01$ ) in immature when compared to mature (Table Ia). Thus, there was a point to point relation between the maturity of cataract and the GSH-PO activity of lens.

TK activity in lens (Table I) and in RBCs (Table II) was significantly lower in immature and mature cataract ( $P<0.01$ ) in comparison to control group. A gradual decrease in TA activity in erythrocytes of the cataractous groups, which was significant, was observed in comparison to controls (Table II), though the degree

TABLE IIa: Enzymatic activity in erythrocytes of normal and cataractous subjects (Significant level).

Parameters	P value		
	C vs. IM	C vs. M	IM vs. M
GSH	0.05	0.01	NS
GSH-R	0.01	0.01	0.01
GSH-PO	NS	NS	NS
G-6-PD	0.05	NS	NS
TA	0.05	0.01	NS
TK	0.01	0.01	0.05

C-Control, IM-Immature, M-Mature, NS-Not significant

of significance varied considerably from immature ( $P<0.05$ ) to mature ( $P<0.01$ ) cataract (Table IIa). TA activity in cataractous immature and mature lenses increased considerably ( $P<0.01$ ) in comparison to controls (Table I).

## DISCUSSION

G-6-PD activity was observed to decrease in erythrocytes while it increased in lens in cataractous subjects as compared to controls. A negative correlation ( $P<0.05$ ) between erythrocyte and lens was observed in mature cataract (Tables I and II). The decrease in activity of erythrocyte G-6-PD found in the present investigation is supported by the observation of Westring and Pisciotta (14) and that of lens by Zinkham (15) and Bhat et al (16). The difference in the activity of G-6-PD in lens and erythrocyte is perhaps due to the difference in the frequency of degeneration and regeneration of cells. Thus, the ultimate effect of G-6-PD deficiency leads to lysis of membrane proteins in erythrocyte and liquefaction of lens fibres as put forward by Kinoshita (17).

TABLE II: Enzymatic activity in erythrocytes of normal and cataractous subjects (Values are Mean  $\pm$  S.D. Numbers in parenthesis indicate the sample size).

Parameters	Control	Immature	Mature
GSH ( $\mu$ moles/gHb)	9.39 $\pm$ 0.86 (7)	6.8 $\pm$ 1.04 (7)	7.15 $\pm$ 0.73 (10)
GSH-R (units/gHb)	13.7 $\pm$ 1.3 (9)	7.6 $\pm$ 0.8 (8)	6.9 $\pm$ 0.2 (9)
GSH-PO (units/gHb)	14.8 $\pm$ 3.80 (7)	13.2 $\pm$ 3.09 (10)	12.7 $\pm$ 5.11 (10)
Glucose-6-phosphate dehydrogenase G-6-PD (units/gHb)	18.18 $\pm$ 2.75 (10)	16.67 $\pm$ 2.59 (15)	16.81 $\pm$ 4.90 (18)
Transaldolase TA (units/1 haemolysate)	45.7 $\pm$ 10.93 (10)	41.1 $\pm$ 19.66 (10)	40.8 $\pm$ 11.53 (13)
Transketolase TK (units/1 haemolysate)	110.4 $\pm$ 28.67 (12)	47.7 $\pm$ 15.00 (9)	43.34 $\pm$ 21.75 (13)

NADPH generated by G-6-PD, is essential for the conversion of GSSG to GSH which controls the redox state and is needed for the maintenance of transparency of the lens, which is lacking in senile cataract.

Glutathione reductase activity in erythrocyte and lens decreased significantly ( $P < 0.01$ ) as cataract advances right from normal to mature which in turn is related to age. GSH-R protects the lens and erythrocyte constituents from oxidation and it maintains protein sulfhydryls and prevents membrane lysis (2, 18, 19). The decrease in GSH-R activity may be due to the various oxidative stresses (20) or due to the deficiency of flavin adenine dinucleotide (FAD), the coenzyme of GSH-R (21). Paniker, Srivastava and Beutler (22) observed a deficiency of GSH-R activity in erythrocyte. According to them, GSH-R deficiency may contribute to cataract formation due to the limiting role of this enzyme in the metabolism of lens.

In the present investigation, the concentration of GSH in blood as well as lens decreased in cataractous groups in comparison to controls (Tables I and II). Lens proteins contain reduced sulfhydryl groups (PSH). Oxidised disulphide groups (PSSP) may be reduced to PSH by GSH. Decrease in GSH limits its functional role to preserve the physicochemical equilibrium of lens proteins and red cell sulfhydryl groups (18). Thus, the decreased GSH or increased GSSG will result in the oxidation of PSH to PSSP resulting in protein

crosslinking and decreased solubility and transparency (23).

The high glutathione peroxidase activity in lens but not in erythrocyte indicate that the ocular tissue is highly affected by the harmful oxidant like  $H_2O_2$ . Increase in lipid peroxidation may be responsible for the increase in GSH-PO activity of the lens (24) as depicted in Table I. The increase in GSH-PO activity may inhibit lipid peroxidation and can protect lens from damage (25). But in the case of erythrocyte, low activity of GSH-PO leads to oxidation of Hb and finally causes lysis of erythrocytes. This observation is in agreement with the report of Meloni et al (19). The conversion of  $H_2O_2$  becomes difficult resulting in an increase in  $H_2O_2$  which in turn initiates the peroxidation of membrane lipids along with the oxidative damage to structural proteins. This may trigger the formation of cataract.

In the cataract(ous) group, the activity of TA in erythrocytes decreased while the same showed a significant increase in the lens as compared to the controls. This may be due to changes in membrane permeability or due to rupture or lysis of the lens membrane, thereby affecting leakage of various constituents finally leading to protein precipitation (26, 1). The activity of TK was found to be reduced in both erythrocytes and lens of cataract groups (Tables I and II). Disturbances in TK activity may lower the availability of pentoses required for nucleotide synthesis which in turn would affect protein synthesis leading to cataractogenesis.

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