FLUCTUATIONS OF $\gamma$-GLUTAMYL TRANSPEPTIDASE IN NORMAL AND CATARACTOUS HUMAN EYE

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Abstract: The most common form of cataract is the "Senile Cataract" occurring in the aged population, which has great socio-medical prevalence for many third world countries, where annually 2-3 million people become blind due to cataract. The cause and phenomenon of cataract development may be same but the changes related with $\gamma$-glutamyl transpeptidase (GTP) activity depends on age and types of cataract. Therefore, in present investigation the fluctuation of this enzyme was studied in the lens of patients suffering from senile cataract. The study shows an intensive decrease of GTP activity, which is 9.595 ± 0.094 n moles/min/mg (mean ± SD) in normal lenses as compared to 3.7 ± 0.216 n moles/min/mg (mean ± SD) in cataractous lens. Since GTP reacts very effectively with glutathione (GSH) amongst all the enzymes involved in the glutathione cycle it appears that GSH is entirely degraded within the lens. This study may help in finding degree, types and progress of cataract in patients.

Key words: Lens, $\gamma$-Glutamyl transpeptidase, Cataract

INTRODUCTION

Epidemiological literature indicates that the prevalence of cataract is related to geographical location, climate and sun hours [1,2], therefore a single primary cause of cataract is unlikely to exist. However, the most significant protective system in the lens involves reversible oxidation of glutathione (GSH). The lens contains high concentrations of GSH and its concentration in epithelium is 6 times higher than the whole lens concentration [3]. Normal lens maintains a steady state of concentration of GSH. However, this begins to drops in lenses undergoing cataract formation. This has been found to be true in almost all experimental cataracts and in human senile cataracts [4-6].

Although some oxidative changes are seen in the protein of the X-irradiated lens, it seems unlikely that these are the caused of the opacification. One of the functions of high level of reduced glutathione is probably to maintain protein sulphhydrals in the reduced form [7]. It appears that sulphhydr groups in both cytoplasmic and membrane bound proteins need to be in the reduced form for the proper functioning. Reduced glutathione probably acts in two ways in the maintenance of protein sulphhydr groups (i) by preventing their oxidation which has taken place in cataractous eye and (ii) being mobile molecules, it reacts with potential oxidants before they could interact with the lens proteins. GSH acts as a scavenger for any free radicals generated by ionizing radiation, UV and visible light, or univalent reduction of oxygen. In the process it would be oxidized to the disulphide. Any disulphide formed in the proteins can be reduced back to the sulphhydr by glutathione. This takes place through thiol exchange reactions with the intermediate formation of mixed disulphide of protein and glutathione [8].

Lens glutathione reductase is also capable of clearing mixed disulphide of glutathione and lens proteins. This provides the lens with a possible additional route for the regeneration of protein sulphhydrals. However, cataractous lenses contain substantial amount of mixed disulphide despite the presence of NADH or NADPH and active glutathione reductase. Therefore, it seems unlikely that glutathione reductase clears
the mixed disulphide under physiological conditions. All the constituents of the protecting glutathione system including glutathione redox cycle and its enzymes, glutathione reductase, glutathione-s-transferase, α-glutamyl transpeptidase, GSH, GSSG, NADPH, etc. have been reported in the lens [6]. Thus, GSH metabolism can be expected to be a significant factor in the defense of lens against cataractogenesis. Altered activity of the enzymes associated with the synthesis, catabolism and utilization of glutathione in the lens have been reported by Rathbun et al. [8] with the progression of cataract. Since α-glutamyl transpeptidase changes in lens along with age and types of cataract, its fluctuation, may help us to classify the stage of cataract development. It also helps in finding degree, types and progress of cataract in patients.

MATERIALS AND METHODS

Collection of material: The lenses of the patients who were undergoing cataract surgery at Nagari Eye Hospital, Ahmedabad, by medical Dr. involved in surgery, were collected. The cataract type in these patients was diagnosed with the help of slit-lamp biomicroscope. Several eyes with clear lens were obtained from C.H. Shamaria eye bank, Red Cross society, Ahmedabad, India.

Estimation of enzyme: The fresh lenses were washed, dried, weighed and homogenized in a known volume of buffer. The GTP activity was assayed by the method of Tate and Meister [9]. The assay mixture consisted of 0.2 ml 5 mM L- r-glutamyl-p-nitroanilidine, 0.2 ml 0.1 M glycylglycine and 0.3 ml 0.1 M tris-HCl buffer (pH 8.0). To this, 0.3 ml enzyme homogenate was added to initiate the reaction. Blank tubes were assayed without the enzyme homogenate and subtracted from the test readings to obtain correction for the spontaneous reaction. The release of p-nitroanilide was recorded at 410 nm at 37 °C. The activity was expressed as units/hr/g fresh lens, where a unit of GTP is equal to the µ moles of p-nitroaniline released per minute. Lenses were classified as per Chylack’s classification [10-12].

Statistical analysis: All results were expressed in mean ± SD. One way analysis of variance (ANOVA) was used to test the significance of difference and Bonferroni test to test the significance of difference between control and different cataract types. The p value less than 0.05 is considered as significant. The results are expressed graphically by considering values of control lens and AQH as control as 100%.

RESULTS

Fluctuation of GTP in normal eye lens: α-glutamyl transpeptidase activities in different age groups of normal human lenses are presented in table-1. It is evident from the study that the enzyme fluctuates in different age groups. As for example in last two age groups (71 to 80 and 81-90) the enzyme decreases significantly and it is minimum (7.231 n moles/min/mg) in the age group 81-90 years. Study also show that in sharp contrast to last two groups, in age groups 51-60 and 61-70, GTP increases significantly as compared to age group 41-50 years (Table 1).

Fluctuation of GTP in different types of cataracts: Table-2 shows GTP activity in different
table-1:

<table>
<thead>
<tr>
<th>Age in years Sample size</th>
<th>GTP activity (n moles/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41 -50 (5)</td>
<td>9.912 ± 0.11*</td>
</tr>
<tr>
<td>51 - 60 (5)</td>
<td>11.134 ± 0.09*</td>
</tr>
<tr>
<td>61 - 70 (4)</td>
<td>11.078 ± 0.14*</td>
</tr>
<tr>
<td>71 - 80 (5)</td>
<td>8.623 ± 0.18*</td>
</tr>
<tr>
<td>81 - 90 (4)</td>
<td>7.231 ± 0.09*</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD. p value * < 0.01

Table 2:

<table>
<thead>
<tr>
<th>Type of cataracts Sample size</th>
<th>GTP activity (n moles/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS 5</td>
<td>3.512 ± 0.219*</td>
</tr>
<tr>
<td>PSC 3</td>
<td>4.789 ± 0.318*</td>
</tr>
<tr>
<td>CS 3</td>
<td>4.998 ± 0.193*</td>
</tr>
<tr>
<td>NS, PSC 3</td>
<td>4.519 ± 0.177*</td>
</tr>
<tr>
<td>PSC, CS 4</td>
<td>3.875 ± 0.255*</td>
</tr>
<tr>
<td>NS, CS 4</td>
<td>4.200 ± 0.290*</td>
</tr>
<tr>
<td>NS, PSC, CS 5</td>
<td>3.111 ± 0.182*</td>
</tr>
<tr>
<td>CS, NS, PP 5</td>
<td>4.081 ± 0.131*</td>
</tr>
<tr>
<td>Mature 4</td>
<td>2.101 ± 0.171*</td>
</tr>
<tr>
<td>Brown 5</td>
<td>2.207 ± 0.180**</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD. p value * < 0.01, p-value ** < 0.05. Abb. used: NS = nuclear sclerosis, PSC = posterior sub capsular, CS= cortical spoke, PP = posterior polar.

Table 3:

<table>
<thead>
<tr>
<th>Normal or Cataractous lens</th>
<th>GTP activity (n moles/min/mg)</th>
<th>Percentage changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal 54 ± 11 Yrs. 9.595 ± 0.094 (n = 23)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Cataractous 58 ± 14 Yrs. 3.70 ± 0.216 (n = 41)</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD. p value < 0.01
types of cataracts. The enzyme level is significantly low in all types of cataracts studied as compared to the normal lenses of different age groups, as revealed in table 1. The maximum decrease is recorded in mature and brown cataracts (Table-2).

**Comparison of average GTP activity in normal and cataract lenses:** A comparison of GTP activity in control and cataract lenses is also made. In table-3 lens of normal individuals age 54 ± 11 Yrs is compared with cataracts patients of age 58 ± 14 Yrs. Study shows about 64 % reduction of enzyme in cataractous lens. The change in activity of this crucial enzyme is highly significant (Table-3).

**DISCUSSION**

The substrates for the GTP are glutathione, oxidized glutathione, s-substituted glutathione and other "glutamyl compounds [13,14]. Since GSH is entirely degraded within the lens [15], the GTP seems to play an important role in the lens. GTP reacts very effectively with GSH amongst all the enzymes involved in the GSH cycle. The activity of GTP under normal condition is very low compared to GR and GST. On account of that GSH level is maintained in lens [14].

There is no significant relationship between age and activity of GTP. The degradation of GSH by GTP is thought to be coupled with transport of amino acids across the membrane by the same enzyme. This mechanism is highly effective in the lens, since it has a rapid turnover of GSH and is able to transport amino acids in to the tissue [16]. Any change in such mechanism including low level of GSH, may affect negatively to GTP activity in the lens. The GTP activity level was found to remain steady in the initial stages of post-natal development and then there was a sudden surge in the activity in the adult lenses with increase in GSH [7].

The lowest GTP activity was noted in Mature and Brown cataractous lenses (33% compared to normal lenses) indicates strong oxidative damage. These could be due to the greater demand for GSH turnover including GSH transport and other processes related with this enzyme in the adult lenses. The rapid turnover of GSH would thus indicate rapid detoxification (oxidation).

Several epidemiological studies have claimed that antioxidants such as GSH and vitamin C have prevention role against the development of cataract [17-24]. Eventually the cumulative action of oxidative activities on GSH bringing about its oxidation could hamper the detoxifying mechanism causing reduction in the GSH levels. Besides, there is a fall in "glutamyl cysteine synthetase activity, hence the level of GSH decline rapidly in lenses with increase in age.

In an earlier study we have reported decreased GSH during ageing (slight) and cataractous (extensive) condition both in aqueous humour and lens [25]. The reduction in GSH level also depends on many other factors such as blood aqueous barrier, photo oxidation, ascorbic acid level in aqueous humour and lens, chemical oxidation, GSH-reductase and r-glutamyl cysteine synthetase level [26, 27]. Since GSH is an important substrate for GTP, its decrease would inhibit the feedback mechanism thereby lowering the activity. This indicates that GSH level is determinant factor for the activity of GTP. Further, the variations in the level of enzyme in different cataracts can also be taken as criteria of their stages of degeneration in different cataractous lenses.

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**REFERENCES**