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BIOCHEMISTRY OF AQH OF THE HUMAN EYE - A PHYSIOLOGICAL-AGING AND PATHOLOGICAL-CATARACTOUS STUDY

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ABSTRACT

In the aqueous humor of the normal and cataractous human eye respectively the concentration of ascorbic acid is 118.04 ± 3.26 µM / 100 ml. and 45.92 ± 1.24 µM / 100 ml; the concentration of GSH is 3.24 ± 0.87 µM / 100 ml and 1.65 ± 0.61 µM / 100 ml and the concentration of total proteins is 46.72 ± 4.76 mg/100 ml and 53.24 ± 5.08 mg /100 ml. Significant changes are found to occur between these three parameters under normal and cataractous condition. The concentration of these constituents varies in different types of cataract. This indicates that the changes in parameters are not entirely age dependent but are conditional in AQH of human eyes.

Key Words: Human AQH, Cataract, Normal, Ascorbic acid (AA) Glutathione (GSH), Total proteins.

INTRODUCTION

Cataract, the leading cause of blindness is not only an important public health problem but also a socio-economic one, especially in the tropical countries like India. Prevention or delay in the progression would present a major achievement for human welfare and is therefore one of the priorities of medical research in our country.Lens is avascular tissue with no innervations. Hence, the aqueous humor (AQH) serves as a nutrient medium for the lens and other ocular tissue. Alteration in the composition of AQH may have effect on the lens metabolism. Recent research has clearly shown that oxidative stress is a very important risk factor for cataract development (Truscott et al.,2005; Spector et al., 1995). The most significant antioxidant appers to be the GSH and Ascorbic acid (Reddy et al.,1984; Hum et al.,1987; Verma et al., 1988). It is reported that the uptake of plasma-derived glutathione (GSH) into the AQH is by simple diffusion and cellular uptake of GSH by the lens is carrier mediated. The protein concentration in AQH is much less as compared to that of blood. The protein spectrum of AQH shows that only low-molecular weight proteins are transported across the blood-aqueous barrier. The concentration of other nutrients also differs from that in plasma. The concentration of ascorbic acid (AA) is much higher in AQH compared to plasma. Alteration in the AQH may have effect on the lens metabolism which may lead to cataract (Spector et al., 1998). The concentration of antioxidant of the AQH decrease as the age increases (Taylor et al., 1995). AA reduces oxidative damage (Disiz et al., 1999; McCall et al., 1999). This is because of due to the active transport of AA from Plasma to AQH. It is difficult to obtain human cataractous lenses and AQH of the same cataractous eye, most of investigators used plasma or serum or erythrocyte to characterize prooxidant and antioxidant status in the patients with cataract (Casado et al.,2001; Prathan et al., 2004).Very less information on the age related variation of constituents of AQH in different types of cataracts is available in literature. Thus, the aim of the study on AQH was to obtain information about alteration in different biochemical parameters and its effect during aging and cataractogenesis. The biochemical parameters studied are GSH, AA and total proteins in AQH of normal and cataractous human eyes.

MATERIALS AND METHODS

The AQH samples were collected from the anterior chamber of eye, with the help of a micro-syringe The total quantity (0.3 – 0.4ml) obtained was put into a 2-ml tube and stored in liquid nitrogen until used. The samples were analyzed within six hours of collection. Ascorbic acid was estimated by spectrophotometric method (Pirie, 1965) that has been described in detail by Matsuda et al (1981), glutathione by Ellman (1959) procedure, as modified by Sedlak and Lindsay (Sedlak et al., 1968) and the total protein by a method of Lowry et al (Lowry et al., 1951).Estimation of ascorbic acid Ascorbic acid in the AQH was estimated by the method of Matsuda et al. (1981). One ml of standard 2, 6 – dichlorophenol indophenol was mixed with phosphate buffer (pH 6.6) and 2.8 ml distilled water. Extinction of the dye solution was measured at 600 nm. Known volume of aqueous humour was added to the dye mixture and further the absorbance was measured at 600 nm. The difference between the two readings was directly correlated with ascorbic acid standard curve.Estimation of GSH The fresh AQH samples were taken. All buffers and solutions were exposed to vigorous stream of nitrogen for 2-3 minutes prior to use. GSH was measured by mixing known volume of AQH with 0.2

ml distilled water and 50 µl 50% trichloro acetic acid (Merck, Darmstadt, Germany). The mixture was shaken intermittently for 15 minutes and centrifuged at 3000 g for 15 minutes at 4 °C. 0.2 ml of supernatant was mixed with 0.4 ml 0.4 M Tris buffer, pH 8.9 and 20 µl 0.01M DTNB. The tubes were shaken well and absorbance was read after 5 minutes at 412 nm.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 by considering values of normal AQH as control (100%).All the chemicals required and used for estimations are of analytical grade.

Types of cataracts studied and numbered are as follows:

1. Posterior sub-capsular (PSC)
2. Cortical spokes (CS)
3. Nuclear sclerosis (NS)
4. PSC, CS
5. PSC, NS
6. CS, Ns
7. PSC, CS, NS
8. Mature
9. Brown
10. CS, NS, Posterior polar

Cataracts are classified according to Chylack (1983 a, 1989 b, 1993 c) Eyes with clear lenses were used as normal samples.

RESULTS

As shown in Table 1, the level of AA decreased significantly ($p < 0.001$) in AQH of cataractous eye than in normal eye. In AQH of the normal eye, the value of AA is 118.04 ± 3.26 µM /100 ml, whereas in cataractous eye it is 45.92 ± 1.24 µM /100 ml. The amount of AA in AQH of the cataractous eyes shows a decrease by 61%. Such decrease in the level of ascorbic acid is earlier reported in the experimental cataract and human senile cataracts (Miratashi et al.,2001; Tessier et al.,1998). The table also indicates that the level of GSH is decresed significantly ($p < 0.001$) in AQH of cataractous eyes as compared to the normal eyes. In AQH of the normal eyes the value of GSH is 3.24 ± 0.87 µM /100 ml, whereas in cataractous eyes 1.65 ± 0.61 µM /100 ml. The amount of GSH in AQH of cataractous eyes is decreased by about 50%. As shown in the same table, the level of total proteins in AQH of the normal eyes is 46.72 ± 4.76 mg / 100 ml, whereas in cataractous eyes it is 53.25 ± 5.08 mg / 100 ml. Although the amount of total protein increases in cataract, there is no significant difference found have to occur when it is compared with normal.The age-related biochemical changes in AQH of normal eyes are shown in Table 2. The AA level decreases in AQH of normal eyes with age. However, a significant linear correlation does not exist. The table also shows that the correlation between the age and GSH level is again not linear and significant.The difference in the percentage values of different parameters in AQH of normal and cataractous eyes are as shown in table 3. The concentration of these three parameters in AQH of normal eyes is considered as 100 percentages and compared with cataractous AQH. Ascorbic acid significantly decreases in all types of cataract i.e. mean value of AA of all cataract are just 38.35 percentage as compared to normal (100 %). Similarly GSH significantly decreases in all types of cataract i.e. mean value of GSH of all cataract are just 51.47 percentage as compared to normal (100 %). But, level of protein is slightly and non-significantly increases just by 13.97 present as compared to normal.

Table 1 Biochemical changes in aqueous humor of normal and cataractous human eyes

AQH	Ascorbic acid (µM / 100 ml)	GSH (µM / 100 ml)	Total Protein (mg /100 ml)
Normal	118.04 ± 3.26 (n=40)	± 0.87* (n=43)	46.72 ± 4.76^{ns} (n=26)
Cataract	45.92± 1.24* (n=68)	1.65 ± 0.61* (n=65)	53.25 ± 5.08^{ns} (n=40)

* $p < 0.001$ All values expressed as mean ± SE

Table 2 Age-related biochemical changes in AQH of the normal eye

Age	Ascorbic acid (µM / 100 ml)	G S H (µM / 100 ml)	Protein (mg /100 ml)
31-40	131.6 ± 5.16 (8)	3.95 ± 0.11 (6)	42.06 ± 4.70 (4)
41-50	140.1 ± 4.31 (8)	2.55 ± 0.14 (8)	43.93 ± 4.24 (5)
51-60	114.3 ± 6.27 (8)	3.28 ± 0.10 (7)	44.25 ± 5.57 (6)
61-70	111.5 ± 5.03 (8)	3.40 ± 0.20 (7)	53.88 ± 4.47 (6)
71-80	95.5 ± 3.78 (8)	3.46 ± 0.05 (7)	49.50 ± 4.57 (5)
81-90	(NA)	3.16 ± 0.12 (8)	(NA)
Mean	**118.0* ± 3.26 (40)**	**3.24* ± 0.87 (43)**	**46.72 * ± 4.76 (26)**

* $p < 0.001$ All values expressed as mean ± SE

Table 3 Comparative percentages of AA, GSH and Total Proteins in AQH

Types of Cataract	Ascorbic acid	GSH	Protein
Normal	100.0 (40)	100.0 (40)	100.0 (26)
1	37.0 (8)	63.0 (6)	97.6 (5)
2	34.3 (6)	61.7 (6)	115.5 (5)
3	53.7 (7)	62.3 (7)	111.2 (5)
4	36.6 (9)	66.6 (6)	100.6 (5)
5	30.4 (6)	48.1 (6)	116.4 (5)
6	49.1 (7)	37.3 (7)	111.4 (5)
7	34.3 (7)	59.2 (7)	117.7 (5)
8	40.3 (7)	37.0 (8)	110.7 (5)
9	25.8 (5)	30.8 (6)	127.0 (5)
10	42.0 (6)	48.7 (6)	131.6 (4)
Mean of 1-10	**38.35**	**51.47**	**113.97**

DISCUSSION

The aging and cataractous lenses damage occurs due to oxygen and photo oxidation-derived free radical species which can be protected by antioxidant. The most significant of these appears to be the ascorbic acid and GSH (Augusteyn et al.,1981; Reddy et al.,1990). Early drop in the levels of AA in experimental cataract (Pandya A.V., 2014) is observed. AA inhibits lenses darkening in invitro in experimental uv-induced cataract (Zigman et al.,1973).Epidemiological studies proved that antioxidants such as AA have prevention role from chronic diseases such as cataract (Bates et al.,1996; Bleau et al., 1998; Kupfer et al.,1994; Carr et al., 1999; Diplock et al.,1998 ; Bunce et al., 1996; Malik et al., 1995; Ringvold et al.,1996) The amounts of GSH and AA are less in AQH of cataractous eye as compared to that of normal eye. This may be due to the defect in the transporting system, i.e. blood-aqueous barrier or may be due to an increased requirement (utilization) by the lens. Lack of supply of both, GSH and AA, may be one of the causes of cataract formation. Both of these are considered to be antioxidants preventing the oxidation of certain lens proteins during cataract formation. Hence, their requirement is likely to increase during cataractous condition. This can be one of the reasons for lower values of both GSH and AA in AQH of cataractous eyes.Table 3 indicates that the decrease in percentages of AA is more than GSH in all types of cataract except in CS+NS mixed type of cataract. This indicates that AA is utilized faster than GSH i.e. AA prevents the oxidation of GSH and gets oxidized itself. The percentage of AA and GSH in AQH is found affected even during the development to be to be similar in 8,9,10 numbered cataracts. It shows that both antioxidants are utilized to similar extent even though it could not prevent cataract development.The AQH contains fewer amounts of proteins. There is no significant difference in the level of proteins in AQH of normal and cataractous human eyes. This indicates the level of AQH protein is not significantly of cataract. Our results of proteins in AQH of normal eyes indicate slightly higher values than published by the following authors.

Heer (1957)	30.5 ± 4.1	(n=20)
Peretz & Tomasi (1961)	30 – 50 (n NA)	
Krause and Raunio (1970)	36	(n=4)

These values are tabulated from Documents Ophthalmologic. 53: 192-248 (1982). The age group that they have studied and the level of significance were not mentioned in it. Similarly, our results of ascorbic acid and GSH in AQH of normal eyes are also very close to the published values. AA and GSH seems to decrease with age but it is not significant and do not show linearity with increase in age. Increase in age does not seem to affect the level of proteins in AQH of either normal or cataractous eyes. But, it does have some effect on the levels of AA and GSH. The changes between 31 to 60 yrs. age and 61 to 80 yrs. age are totally different and indicative to the aging process i.e. low level of antioxidant and high level of extracellular protein in AQH of normal eyes. At the same time during cataractous condition the reduced level of ascorbic acid and GSH is very high and more significant. This indicates clear difference in normal and pathological biochemical changes in AQH. Since AQH is one of the body fluid like blood, CSF intra cellular, lymph etc. It's composition do not change with increase in age like other body fluid that itself indicates the changes are conditional specially for ascorbic acid and GSH and not age related. AQH formation and transport of nutrients across blood aqueous barrier is only affecting its composition. By any mean if these two parameters are maintained in AQH, certainly stops or slowdown the progress of cataractogenesis.

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