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A COMPARATIVE STUDY OF LENS PROTEIN GLYCATION IN VARIOUS FORMS OF CATARACT

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ABSTRACT

The aim of this study was to estimate and compare the levels of glycated protein in the lens extracted from patients with cataract of various etiologies. A total of 72 cataract lens were collected. The levels of total proteins, glucose and glycated protein in the lens were studied. Plasma protein and fasting glucose levels were also estimated. The amount of glycation of lens was significantly higher in case of hypermature senile cataract ($p < 0.01$) when compared with other types of cataracts. The levels of lens glucose between the various types of cataracts did not differ significantly. These results indicate that the level of lens glucose alone is not the only determining factor of lens protein glycation.

KEY WORDS

Protein glycation, Cataract and Diabetes

INTRODUCTION

The lens, which is behind the iris, refracts light entering the eye through the pupil, thus focusing it on the retina. The perfect physiochemical balance of the lens proteins gives it transparency. Any alteration in the optical homogeneity of the lens or decrease in its transparency is known as a cataract (1, 2). Senile cataract, a major cause of blindness worldwide, is an-age associated condition (3, 4). The term senile refers to the fact that no specific ophthalmic or metabolic diseases are known to precede or to be involved in this type of cataract (5). On the other hand, diabetes is also considered a significant risk factor accelerating cataract formation (6, 7).

Increasing experimental evidences suggest that glycation of lens proteins is involved in cataract formation (8-11). Glycation of lens proteins, whereby glucose or other reducing sugars react with the e-amino group of lysine residues or amino termini of proteins resulting in the formation of schiff base (SB). The SB undergoes an Amadori rearrangement via the Maillard reaction giving rise to a more stable ketoamine or Amadori product (early glycation products). At a later stage, the Amodori products undergo dehydration and rearrangement to form cross-links between adjacent proteins, resulting in protein aggregates or advanced glycation products (AGEs) (12,13).

Since the lens proteins are long-lived, they are highly susceptible to post-translational modification such as glycation. Glycation is believed to enhance protein unfolding, changing not only the physiochemical properties of lens proteins, but also their functions (14-16).

This study was undertaken to compare the extent of glycation in nuclear lens crystallins in diabetic and various stages of senile cataracts.

MATERIALS AND METHODS

Nuclear portions of lens were obtained from senile and diabetic individuals undergoing routine extra capsular lens extraction at the Ophthalmology department in JIPMER, Pondicherry, India. A total of 72 lenses were obtained. Of these patients, 5 were known to be diabetic. The remaining 67 had no history of diabetes and had normal serum glucose values (range 65-110 mg/dl). This senile group was categorized according to clinical guidelines as Matured senile cataract group [(MSC), 18-patients)], Immature senile cataract group [(ISC), 42-patients], and Hypermature senile cataract group [(HMSC), 7-patients]. The age range of all 72 cases was between 40-75 years.

The nuclei from diabetic and senile cataract cases were collected separately and after recording their weight and color, they were each homogenized in a 20 mM sodium phosphate buffer, pH 7.2 as described previously (17).

Lens total protein levels were measured by the method of Lowry (18) and protein nonenzymatic glycosylation by phenol sulfuric acid method (19).

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Lens glucose and serum glucose was estimated by glucose oxidation method (Auto analyzer Ciba Corning). Total serum protein was analyzed by Biuret's method (Auto analyzer Ciba Corning).

Statistical analysis

The data were presented as mean ± SD. Student's t-test for unpaired data was used to determine differences between diabetic and senile cataractous lenses. The limit of statistical significance was set at $p < 0.05$.

RESULTS

The levels of glycated protein in case of senile cataract were found to be significantly higher than in diabetic cataract ($p < 0.05$), even though the serum glucose was found to be higher in diabetic lens. When the levels of glycated protein was compared between the different groups in senile lens, the levels were found to be higher in hypermature group ($p < 0.05$), even though the lens glucose was significantly higher in immature and mature cataract than hypermature cataract. This observation indicates that hyperglycemia is not the only factor in determining the level of protein glycation in lens tissues.

DISCUSSION

A possible role of glycation of lens proteins has been implicated in the increase in coloration of lens from the pale yellow of the senile cataract patients to dark brown of diabetic cataract patients. This change in color may reflect a higher level of fluorescence of proteins caused by glycation in senile and diabetic lens. Glycation of long-lived proteins such as collagen and lens proteins in diabetes and ageing has been recognized as a major post-translational modification process, leading to the cross-linking, aggregation and insolubilization of such proteins (20-23).

Table 1: Biochemical characteristics of the study group

	Diabetic Senile Cataract	Immature Senile Cataract	Mature Senile Cataract	Hypermature Senile Cataract
Age (years)	56.8 ± 7.9	63.0 ± 8.7	57.7 ± 9.3	61.9 ± 5.8
Lens Glucose (in mg/100mg)	32.8 ± 2.8	38.6 ± 2.1	33.0 ± 3.4	27.7 ± 9.0*
Lens Protein (mg/100mg)	4.2 ± 1.5	5.0 ± 4.4	7.1 ± 1.2	5.4 ± 1.6
Serum Glucose (in mg/dl)	100.2 ± 2.0	87.9 ± 3.3*✦★	83.3 ± 2.4*✦★	90.3 ± 5.6*✦
Lens Glycation (in mg /100 mg protein)	3.8 ± 1.3	3.38 ± 0.7*✦★	4.3 ± 1.0*✦★	10.8 ± 8.6*✦

* $p < 0.05$ between Diabetic senile group and other groups.
✦ $p < 0.05$ between Hypermature senile cataract group with Immature and Mature senile groups
★ $p < 0.05$ between mature and immature senile cataract groups.

In this study, the phenol sulfuric acid method was used to measure nonenzymatic glycosylation in lens homogenates. High levels of nonenzymatic glycated proteins in the nucleus of diabetic lens, and to a greater extent in the nucleus of the senile lenses suggest that the factor involved in glycation of protein is more pronounced in aged lens than in diabetic lens. This is in contradictory to the observation of Duhaiman *et al* (17). A higher number of cases in the diabetic group could have helped to confirm this observation. This observation also indicates that hyperglycemia is not the only factor involved in the processes of glycation. Among the senile patients, HSC lens had significantly higher levels of glycated protein when compared with the other two groups. The levels of glycated protein in MSC were higher than in ISC. These observations suggest that glycation of lens proteins are age and disease-related in senile cataract.

In cataract pathogenesis, lens proteins are modified by glycosylation, ageing, heat, pH changes, radiation, free radicals and several chemical substances (1, 2). Modified proteins lose their biological activity; no longer perform their metabolic role and cause inflammation, necrosis or autoimmunity (24).

In summary, our observations suggest that glycation may accelerate the development of senile cataract, and to lesser extent in diabetic cataract patients. Further studies are warranted to confirm these observations.

REFERENCES

1. Cotlier, E. (1987) In Adler's Physiology of the Eye. (Moses RA, Hart WM, eds) pp. 268-90. St. Louis Washington, Toronto: The C.V. Mosby Company.
2. Davson, H. (1990) Davson's Physiology of the Eye pp. 139-92. London: McMillan Press.
3. Hoenders, J.H. and Bloemendal, H. (1981) In Molecular and cellular biology of the eye lens (Bloemendal H, ed.) pp.279-326. New York: John Wiley.
4. Leske, M.C. and Sperduto, R.D. (1983) The epidemiology of senile cataracts: a review. Am. J. Epidemiol. 118, 152-65.
5. Boutros, G., Koch, H.R., Jausen, R., Jacob, T.J. and Duncan, G. (1984) Effects of 8-methyoxypsorales on rat lens cations membrane potential and protein levels. Eye Eye Res. 38, 509-13.
6. Harding, J.J. and Van Heyningen, R. (1987) Epidemiology and risk factors for cataract. Eye 1, 537-41.
7. Harding, J.J. and Van Heyningen, R. (1988) Drugs, including alcohol that act as risk factors for cataract, and possible protection against cataract by aspirin-like analgesics and cyclopentathiazide. Br. J. Ophthalmol. 72, 809-14.
8. Beswick, H.T. and Harding, J.J. (1987) Conformational changes induced in lens alpha- and gamma-crystallins by modification with glucose 6-phosphate, implication for cataract. Biochem. J. 246, 761-69.
9. Lewis, B.S. and Harding, J.J. (1990) The effects of aminoguanidine on the glycation (non-enzymatic glycosylation) of lens protein. Exp. Eye Res. 50, 463-67.
10. Cherian, M. and Abraham, E.C. (1993) Glycation of human lens crystallins effect of age and aspirin treatment. Ophthalmic Res. 25, 349-54.
11. Cherian, M. and Abraham, E.C. (1993) *In vitro* glycation and acetylation (by aspirin) of rat crystallins. Life Sci. 52,1699-1707.
12. Bunn, H.F. and Higgins, P.J. (1981) Reaction of monosaccharides with proteins: possible evolutionary, significance. Science 231, 222-24.
13. Baynes, J.W., Watkins, N.G., Fisher, C.I., Hull, C.J., Patrick, J.S., Ahmed, M.U., Dunn, J.A. and Thorpe, S.R. (1989) The Amadori product on protein: structure and reactions. Prog. Clin. Biol. Res. 304, 43-67.
14. Kennedy, L. and Baynes, J.W. (1984) Non-enzymatic glycosylation and chronic complications of diabetes: an overview. Diabetologia 26, 93-98.
15. Kyons, T.J., Silver, G., Dunn, J.A., Dyer, D.E. and Bynes, J.W. (1991) Role of glycation in modification of lens crystallins in diabetic and non-diabetic senile cataracts. Diabetes 40, 1010-15.
16. Watala, C., Gwozdzinski, K. and Malek, M. (1992) Direct evidence for the alteration in protein structure and conformation upon in vitro non-enzymatic glycosylation. Int. J. Biochem. 24, 1295-302.
17. Duhaiman, A.S. (1995) Glycation of human lens proteins from diabetic and (non diabetic) senile cataract patients. Glycoconjugate J. 12, 618-24.
18. Lowry, C.H., Rosebrough, N.J., Farr, L. and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. J. Biol. Chem. 193, 265-75.
19. Nayak, S.S. and Pattabiraman, T.N. (1981) A new colorimetric method for the estimation of glycosylated hemoglobin. Clin. Chem. Acta 109, 264-274.
20. Monnier, V.M. and Cerami, A. (1981) Non-enzymatic browning *in vivo*: possible process for aging of long-lived proteins. Science 211, 491-93.
21. Stevens, V.J., Rouzer, C.A., Monnier, V.M. and Cerami, A. (1978) Diabetic cataract formation: potential role of crystallins glycosylation of lens. Proc. Natl. Acad. Sci. USA 75, 2918-22.
22. Dunn, J.A., Patrick, J.S., Thrope, S.R. and Baynes, J.W. (1989) Oxidation of glycated proteins: age-dependent accumulation of N epislon- (carboxymethyl) lysine in lens proteins. Biochemistry 28, 9464-68.
23. Abraham, E.C., Swamy, M.S. and Perry, R.E. (1989) Nonenzymatic glycosylation (glycation) of lens crystallins in diabetes and aging. Prog. Clin. Biol. Res. 304, 123-39.
24. Swamy, M.S. and Abraham, E.L. (1987) Lens protein composition, glycation and high molecular weight aggregation in aging rats. Invest. Ophthalmol. Vis. Sci. 28, 1693-701.