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Conformational Changes in Lens Protein: A Responsible Factor for Cataract Formation in Diabetic Patients

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Abstract
Background & objectives: *Cataract is considered a major cause of visual impairment in diabetic patient's. The present study addresses the progression of lens opacification by conformational changes and glycation of lens protein in diabetic individuals.*
Methods: *A sample size of 30 nuclear portion of the lens was divided equally between study group (diabetic individuals) and control group (non-diabetics). Serum glucose and lens glucose is estimated by GOD/POD method, total lens protein by Modified biuret; end point method, serum sodium and potassium by Automated Ilytanalyzer and total protein fractionation by electrophoresis.*
Results: *There was statistically significant decrease in value of lens glucose and lens total protein ($p< 0.01$), while slight elevation in the percentage of protein fractionation in diabetics as compared to non diabetics. Serum sodium level was also found to be statistically significantly elevated ($p<0.01$) in diabetics as compared to non diabetics, while no significant changes were found in serum potassium level.*
Interpretation and Conclusion: *This prospective cross- sectional study shows that cataracts are formed due to oxidative damage of lens, conversion of soluble low molecular weight protein to insoluble high molecular weight protein or due to glycation.*
Keywords: *Cataract, diabetes, glycation, lens protein.*

Introduction

Cataract, which can be defined as any opacity of the crystalline lens, results when the refractive index of the lens varies significantly over distances approximating the wavelength of the transmitted light. [1] This variation in the refractive index can result from changes in lens cell structure, changes in lens protein constituents, or both. [2] Cataract in diabetic patients is a major cause of blindness in developed and developing countries.

The pathogenesis of diabetic cataract development is still not fully understood. Recent basic research studies have emphasized the role of the polyol pathway in the initiation of the disease process. [3] The enzyme aldose reductase (AR) catalyzes the

reduction of glucose to sorbitol through the polyol pathway, a process linked to the development of diabetic cataract. Extensive research has focused on the central role of the AR pathway as the initiating factor in diabetic cataract formation. It has been shown that the intracellular accumulation of sorbitol leads to osmotic changes resulting in hydropic lens fibers that degenerate and form sugar cataracts. [4, 5] In the lens, sorbitol is produced faster than it is converted to fructose by the enzyme sorbitol dehydrogenase. The polyol pathway has been described as the primary mediator of diabetes-induced oxidative stress in the lens. [6] Osmotic stress caused by the accumulation of sorbitol induces stress in the endoplasmic reticulum (ER), the principal site of protein synthesis, ultimately leading to the generation of free radicals. These free radicals accelerate and aggravate cataract development. [7] Furthermore, increased glucose level in the aqueous humor may induce glycation of lens protein in which glucose or reducing sugar reacts with amino group of lysine resulting in Schiff base formation. This sorbitol undergoes amadori rearrangement via Millard reaction to form Amadori product. Later, this product undergoes dehydration and rearrangement to form cross-links of protein resulting in formation of Advanced glycation end product (AGEs). [8]

It has been shown that not only hyperglycemia but lens protein changes are responsible for development of diabetic cataract .The perfect physiochemical balance of lens protein gives it transparency.[9, 10]This balance is altered by conversion of soluble low molecular weight cytoplasmic lens protein to insoluble high molecular weight aggregates with concomitant decrease in total protein. The resulting protein change cause abrupt fluctuations in refractive index of lens scatter light rays and reduce transparency. [11]

It is hypothesized that lens electrolyte imbalance also place an important role in cataract formation. Normally lens has high content of potassium and low content of sodium. [12] These two cations are in balance with each other. Alteration in balance of these cations results in cataract formation. [13]

Materials & Methods

The material was obtained from Department of Ophthalmology. Institutional ethical clearance was also taken. Nuclear portion of lens were obtained from diabetic and non-diabetic individuals undergoing routine extracapsular lens extraction. A total of 30 lenses were obtained. Among these 15 were non diabetics and remaining 15 had no history of diabetes and had normal serum glucose level. The nuclei of lens were collected in normal saline and stored at 0-2 degree centigrade. After recording of color and weight, the lens nucleus were homogenised in normal saline by homogenizer machine. Then the samples were incubated and filtrates were obtained.

Under all aseptic measures 2 ml of venous blood samples were obtained from the study group. The samples were incubated for 20-25 minutes to separate out serum. Then these serum samples were stored at 4 degree centigrade to conduct the biochemical tests.

Serum glucose and lens glucose was measured by GOD / POD method. Glucose oxidase oxidizes blood glucose to give gluconic acid and H_2O_2.The H_2O_2 thus produced reacts with enzyme peroxidase present in the system to liberate 0_2. The liberated 0_2 reacts with chromogen system to produce quinonimine, the pink colored complex. Intensity of color is directly proportional to the amount of glucose in sample is measured colorimetrically at 505nm.

Total protein lens was measured by modified biuret, end point method. The peptide bonds of proteins react with cupric ions in alkaline solution to form a colored chelate; the absorbance is measured at 578nm.

Serum Na & K was measured by Automated Ilytanalyzer method. The Ilyte measures sodium and potassium in biological fluids, using ion selective electrode technology. Protein fractionation of cataract lens was measured by electrophoresis method.

Statistical analysis was performed by independent sample t-test using SPSS 21.00 version.

Results

In the present study, the value of lens glucose & lens total glucose were found to get decreased in comparison to non diabetic patient. Serum glucose was increased (160.00±20.11**) in diabetics than in non diabetics. Slight elevation in the percentage of α1, α2, β1, β2 &γ were found in diabetics in comparison to non diabetic patients. This difference in the levels may be due to the different mechanism of cataract formation between the two groups. (Table 1)

From table 2 Serum sodium level was found to be elevated (154.55±3.62*) or significant in diabetics in comparison to non diabetic individuals while no significant changes were found in serum potassium level.

Table 1. Showing the concentration of lens glucose, lens total protein and fractionation of proteins in healthy control and diabetic retinopathy patients.

S. No.	Parameters	Healthy Control	Diabetics Retinopathy Patients	P value
1	Lens glucose (mg/dl)	22.93±1.20*	11.55±1.62	.015
2	Lens total protein (mg/dl)	1.66±0.25**	1.00±0.07	.000
3	α_1 (%)	1.01±0.45	3.14±0.18*	.016
4	α_2 (%)	1.25±0.44	1.67±0.89*	.018
5	β_1 (%)	1.34±0.79	1.77±1.08*	.004
6	β_2 (%)	1.66±0.74	3.49±0.48*	.007
7	Γ (%)	4.32±0.65	4.99±1.32*	.006

Values Expressed as Mean ±SD
**Significant
***Highly Significant
NS Non Significant.

Table 2. Showing the concentration of serum biochemical indices in healthy control and diabetics retinopathy patients.

S. No.	Parameters	Healthy Control	Diabetics Retinopathy Patients	P value
1	Serum Glucose (mg/dl)	82.50±8.42	160.00±20.11**	.000
2	Serum Sodium (m mol/L)	145.30±2.44	154.55±3.62*	.010
3	Serum Potassium (m mol/L)	3.96±0.27	4.10±0.27^{NS}	.888

Values Expressed as Mean ±SD
**Significant
***Highly Significant NS Non Significant

Discussion

In the study, serum glucose was analyzed in diabetic and non diabetic patients having cataract. It was found that in diabetic cataract patients, the serum glucose level was increased .Under ordinary dietary conditions; glucose is the only sugar present in the free state in blood plasma. In diabetes, it is found that blood glucose concentration increases due to deficiency or diminished effectiveness of insulin and also due to hyperactivity of the thyroids, pituitary and adrenal glands, emotional stress.

The value of lens glucose and lens total protein was found to get decreased in diabetics in comparison to non diabetics. Wada et al and Broekhuyse (1968) [14] reported that the concentration of lens glucose decreased with the development of cataract in diabetic patients. It is

so because under physiological condition, the lens crystallins produces covalently cross linked crystalline which leads to cataract formation.

Since Heyningen's report (1959) [15], polyols of ocular lenses have been evaluated by many scientists, they found that sorbitol, glucose and fructose accumulated in cataractous lenses of diabetic animals as well as in human diabetics. The accumulation of polyols in a lens presumably raises osmotic pressure, increased water volume causes lens opacification.

In diabetic patients, glycation of ε amino groups of lysine in the crystalline lens protein offers a new mechanism for formation of diabetic cataracts. Glucose or glucose-6-phosphate reacts with protein via disulphide bond formation which resulted in an increased absorbance of light and an increased formation of high molecular weight aggregates. Conformational change was conformed either by increased susceptibility of proteins to tryptic digestion or due to unfolding of Hb to form Heinz bodies.

Serum Sodium value in cataract patients was found to be elevated. Multiple studies have done to clarify the relationship between human biochemical elements and cataract formation. One of the proposed risk factors for cataract formation is serum sodium level; normally lens has high content of potassium and low content of sodium. Lens K+ level is125 mmol/kg of lens water and lens Na+ is 14-26 mmol/kg of lens water. These two cations are in balance with each other, which is mainly due to Na+-K+ ATPase pump and lens permeability. Alteration in either of these ions leads to cation imbalance in lens which results in cataract formation. In this study no significant changes were found in serum potassium level in study and control group.

Conclusion

From this study we can conclude that conformational changes in lens along with elevated serum glucose and sodium level contributes to cataract formation in diabetic individuals. Strict glycemic control with protein supplementation may prove a boon to prevent the progression of diabetic cataract.

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