Antioxidant and calcium levels in mature and immature diabetic cataract lens

Deepa K 1, *, Manjunatha Goud B.K 2, Suma M.N. 1, Devaki R.N. 1, Nandini M. 3 and Sudhir 4

1 Department of Biochemistry, JSS Medical College, JSS University, Mysore, India. 2 Department of Biochemistry, Ras Al Khaimah Medical and Health Sciences University, UAE. 3 Department of Biochemistry, Kasturba Medical College, Mangalore, India. 4 Department of Community Medicine, JSS Medical College, JSS University, Mysore, India.

INTRODUCTION

Worldwide more than 285 million people are affected by diabetes mellitus. This number is expected to increase to 439 million by 2030 according to the International Diabetes Federation.

Diabetes mellitus is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation (Aragno et al., 1999). Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between ROS production and cellular defense mechanisms (Bonnefont et al., 2000).

Diabetic cataract is more prevalent in diabetic adults compared to non diabetics. It depends on duration of diabetes and glycemic status. It progresses rapidly if the blood sugar levels are not maintained within normal range. The mechanism behind the diabetic cataract is mainly due to hyperglycemia induced oxidative stress. Excessive levels of glucose reaching the mitochondria leads to an overdrive of electron transport chain, resulting in over production of superoxide anions, normally scavenged by mitochondrial superoxide dismutase. When latter fails oxidative stress develops and it is proposed that this mechanism is responsible for activation of all major pathways responsible for diabetic complications of which includes, activation of sorbitol pathway, glycation and protein kinase activation (Brownlee, 2000).

Hyperglycemia can also stimulate oxidative stress by the auto oxidation of glucose in the presence
of transition metals as well as generation of ROS during the process of glycation. Oxidative stress also affects the expression of antioxidant enzymes by its action on signal transduction. Hyperglycemia can also cause inactivation of the existing enzymes by glycation. Thus oxidative stress may develop by insufficient antioxidant activity even if ROS production is within physiological range (King et al., 1996).

Calcium is an important extracellular cation in the body, total calcium in the body is about 1-1.5kg. 99% is in bone and 1% in extracellular fluid. It is present both in extracellular and intracellular compartments. The cell membrane is generally impermeable to calcium ions. Normally the calcium levels are maintained at low concentration inside the cell. This is maintained by Na-K+ exchanger; Na-Ca+ exchanger and calcium ATPase pump (Galvan, 1988). Optimum intracellular calcium is necessary to maintain lens transparency. Calcium is maintained in micromoles range in the cytoplasm by regulatory systems. In normal lens, the ionized calcium is less than 1% of the total concentration and below 0.1% of the level in surrounding aqueous humor (Tomlinson et al., 1991).

Metal ions are known to play an essential role in living systems, both in growth and in metabolism. Impaired metabolism of trace elements is observed in diabetic patients. Alteration in the capacity to maintain normal calcium homeostasis have been suggested to underlie the reduced cellular functions characteristic of aging process and predisposes the senescent organism to diverse pathologies viz Cancer, Heart disease, Neurodegenerative disease including Cataract (Thomas et al., 2000; Ernesto et al., 1987) Altered calcium homeostasis due to oxidative stress leads to increased intracellular calcium levels and in turn leads to cataract formation (Wride, 1996; Nakamura et al., 2000).

The aim of the study was to evaluate the total antioxidant activity and calcium levels in diabetic patients lens depending on type of cataract and to correlate the levels of calcium with the antioxidant activity in progression of cataract formation.

MATERIALS AND METHODS

The study included fifty diabetic subjects having cataract, aged between 45-70 years of either sex. Later they were grouped into group 1- immature diabetic cataract patients and group 2- mature diabetic cataract patients based on ophthalmoscopic examination by ophthalmologist. Patients with history of steroid intake, other ophthalmic disease, and systemic disorders were excluded from the study. The study was approved by the Institutional Time Bound Research Committee. A written informed consent was taken from the subjects. Postoperatively cataractous lens were collected in ice cold 0.9% Normal saline container and transferred in ice box to the place of analysis and analyzed on the same day immediately for the following parameters: For determining the antioxidant activity and calcium levels in lens, the lens was homogenized with 1.2ml ice cold 0.01M phosphate buffer pH 7.4. Mixture was centrifuged at 2500rpm for 10mins. 100µl supernatant was used for analysis. The total lens antioxidant activity was determined by Koracevic's method (Koracevic et al., 2001) the assay measures the capacity of antioxidant to inhibit the production of thiobarbituric acid reactive substances from sodium benzoate under the influence of the oxygen free radicals derived from Fenton reaction. The reaction was measured spectrophotometrically at 535nm. Calcium in the lens was determined by Cresolphthalein complexone method (Stern, 1957) using calcium kits from Aspen Lab Pvt Ltd. Cresolphthalein complexone a metal complex dye reacts with calcium ions in alkaline medium forming a purple color. Intensity of the color formed is directly proportional to the calcium concentration and was measured photometrically at 570nm. Since we analyzed in lens tissue we need to express the levels of total antioxidant activity and calcium in milligrams of proteins. So protein content was determined by the Lowry's method (Lowry et al., 1951) blue color obtained was measured spectrophotometrically using Bovine albumin as standard.

Statistical Analysis

The data was statistical analysis by epi-info software using Analysis of Variance [ANOVA]. Since two way ANOVA showed that the interaction between cataract and maturity is significant, the comparison between the type of cataract and maturity was done separately using independent ‘t’ test. The results were expressed as Mean ± Standard deviation.

The significance level at p<0.05 was considered statistically significant. To correlate the levels of calcium with the antioxidant activity, Pearson's correlation co-efficient was worked out.
RESULTS

The comparison of the antioxidant activity and calcium levels between mature and immature cataractous lens are presented in Table 1. A significant reduction in the Antioxidant activity in mature cataractous lens was observed with the mean value of 0.82 mg/mg of proteins against the value of 1.32 mg/mg of protein seen in immature lens. A significant increase in the lens calcium was observed in mature cataract. The mean value being 1.024 mg/mg of protein against 0.805 mg/mg of protein observed in immature cataract. A negative correlation was seen between lens antioxidant activity and lens calcium and r value was -0.966.

DISCUSSION

Dysfunctional neuro-endocrine–endocrine interactions contribute to the disturbances in trace element metabolism and cause severe complications in diabetes mellitus (Tallman et al., 1999). The present results showed that the levels of calcium were statistically increased in the mature cataractous lens compared with immature diabetic cataract patients (p<0.001), and the antioxidant activity is statistically decreased in mature diabetic cataractous lens (p<0.001).

Aksoy et al. (2001) have reported, a reduced total antioxidant status in aqueous humor in patients with diabetic cataract than those with senile cataract (Aksoy et al., 2001). A much reduced antioxidant activity in diabetic patients could be explained by hyperglycemia induced complication such as reduced gene expression of antioxidant enzymes and inactivity of existing enzymes by glycation (King et al., 1996). Thus resulting, overall reduction in antioxidant levels which fails to defend oxidative stress, there by accelerating the process of cataractogenesis. A number of studies have focused on the individual antioxidants. A decreased level of erythrocyte glutathione peroxidase, glutathione reductase has been reported in diabetic cataract. Likewise decrease levels of SOD, Catalase, GSH; Glutathione peroxidase has also been reported in lens (Nishikawa et al., 2000; Chandrasena et al., 2006). The role of calcium in lenticular metabolism has been studied by many workers in animal models and their study results are as follows:

P.G. Biju (Biju et al., 2007) and Lifei Wang (Wang et al., 2001) studies showed that the increased lenticular calcium is due to altered calcium ATPase Pump activity due to oxidative stress, which in turn leads to activation of calpain proteases which is responsible for proteolysis. In Fein et al. studies, increased lenticular calcium causes aggregation of high molecular weight proteins (Fein et al., 1979). In G-Duncan et al, KR Hightower et al, (Duncan et al., 1993; Hightower et al., 1980) studies the altered calcium homeostasis is due to altered calcium ATPase and sodium calcium exchange mechanism and thereby affecting calcium transport across lens. Investigations by Tang (Tang et al., 2003) have shown that the traces of calcium in lens are in some way related to the maintaince of normal permeability and regulation of dynamic equilibrium between ionic constituents of its lens and its surrounding fluid.

Thus there is a significant increase in calcium content with the onset of maturity is due significantly decreased antioxidant levels. Altered calcium homeostasis, due to oxidation of the regulatory proteins or by damage to the plasma membrane by lipid peroxidation, which thereby affects the membrane functions and structure, may be the cause for increase in calcium levels in these patients. In lens, calcium is essential for various lens fiber cell processes including its differentiation. An optimum concentration is essential for activation of Ca-ATPase pump. Altered calcium homeostasis due to oxidative

Table 1. Comparison of parameters between immature and mature cataract.

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<th>N</th>
<th>Immature</th>
<th>N</th>
<th>Mature</th>
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<tbody>
<tr>
<td>Dry weight of lens</td>
<td>24</td>
<td>56±1.79</td>
<td>26</td>
<td>70±2.57**</td>
</tr>
<tr>
<td>Total Proteins (mg/lens)</td>
<td>57±0.56</td>
<td>65±0.66*</td>
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<tr>
<td>Antioxidant Activity (mg/mg of protein)</td>
<td>1.320±0.208</td>
<td>0.820±0.085***</td>
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<tr>
<td>Calcium (mg/mg of protein)</td>
<td>24</td>
<td>0.805±0.416</td>
<td>26</td>
<td>1.024±0.056***</td>
</tr>
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***=p<0.001
stress leads to increased intracellular calcium levels and in turn leads to cataract formation by following mechanisms, increased intracellular calcium interacts with cytoplasmic proteins causing protein oxidation and aggregation of high molecular weight proteins. This leads to increase in soluble protein aggregates. Elevated calcium levels leads to widespread activation of proteases particularly calpain, leading to proteolysis of crystallin. Increased calcium reduces the calcium ATPase pump activity and sodium potassium ATPase activity thereby altering the membrane permeability of lens (Thomas et al., 2003).

The present data revealed that hyperglycemia produced marked oxidant impact as evident by the significant decrease in antioxidants status in lens. This definitely proves that oxidants play an important role as well in genesis of cataract. Hence, estimation of total antioxidant activity can serve as good predictive marker in assessing the disturbed redox status which is responsible for opacification of lens and also calcium plays an important role in causation of diabetic cataract.

REFERENCES


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