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Lipid peroxidation, copper and vitamin C in lens and serum of people with diabetes and senile cataracts: A comparative evaluation

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Abstract

Background: Oxidative stress is implicated in the pathogenesis of diabetic cataract. Copper, known to be a pro-oxidant and vitamin C, an antioxidant, and have far reaching implications in pathogenesis and therapeutic approach to cataract. This study is aimed at comparing the lipid peroxidation, copper and vitamin C levels in diabetic cataract and senile cataract patients.

Methods: In this hospital-based study, cataracts of grade I nuclear stenosis and above were included. Study subjects were forty eight patients with senile cataract and twenty six patients with diabetic cataract. In serum and lens homogenate, levels of malondialdehyde (MDA), the marker of lipid peroxidation, copper and vitamin C were assayed with standard spectrophotometric methods.

Results: Levels of copper, MDA were significantly higher, and vitamin C level was significantly lower in the lens of diabetic cataract patients when compared to senile cataract patients. The levels of MDA and copper in serum did not differ significantly between the two groups. Serum vitamin C was significantly lower in diabetic cataract group when compared to senile cataract patients.

Conclusions: We observed oxidative stress in the lens of diabetic cataract patients indicated by increased lipid peroxidation and copper, and decreased vitamin C. Future studies with larger sample size and involving antioxidant treatment of cataract are required.

Keywords: Diabetic cataract, senile cataract, copper, vitamin C, lipid peroxidation

1. Introduction

Global data has conclusively shown that cataract is a major cause for visual impairment and blindness, and that this preventable malady accounts for 48% of the cause of blindness all over the world more so in developing countries according to the WHO 2010 data (Pescosolido *et al*, 2016) [10]. This means an estimated 20 million are visually impaired and blind due to cataract. From mechanistic perspective, cataractogenesis is multifactorial and is an outcome of interplay between myriad intrinsic and extrinsic risk factors (Pollreisz and Schmidt-Erfurth, 2010) [11]. Excess exposure to UV Radiation, diabetes, cigarette smoking, alcohol consumption, increasing age, use of steroids and occupational exposure to heat and radiation are among the few significant factors which have been shown to increase the incidence of cataract in individuals (Pollreisz and Schmidt-Erfurth, 2010) [11].

Initiation and propagation of cataractogenesis is significantly high in people with type II diabetes mellitus and has been implicated as a major risk factor for the development of cataract (Pescosolido *et al*, 2016) [10]. Diabetic patients develop age-related lens changes that are indistinguishable from non-diabetic age-related cataracts, except that these lens changes occur at a younger age in patients with diabetes than in non-diabetic individuals (Pollreisz and Schmidt-Erfurth, 2010) [11]. Pathogenesis of diabetic cataract is proposed to be due to multiple mechanisms such as increased formation of free radicals and oxidative stress, aberrant glycations, formation of advanced glycation end products, and increased formation of sorbitol in lens (Pescosolido *et al*, 2016; Pollreisz and Schmidt-Erfurth, 2010) [10, 11].

Oxidative stress is implicated in the etiopathogenesis many diseases. It is due to excessive production of reactive oxygen species or depletion of antioxidants, or both (Pollreisz and Schmidt-Erfurth, 2010) [11]. Reactive oxygen species are known to cause oxidative damage to

our vital biomolecules DNA, proteins and lipids. Antioxidants protect the cells against detrimental effects of free radicals. Vitamin C is the major extracellular antioxidants. Copper and iron are considered prooxidants by virtue of their property to induce formation of free radicals (Halliwell and Gutteridge, 2006) [6]

Previous studies have reported reduced levels of antioxidants, and increased lipid peroxidation products in the plasma of diabetic patients with cataract when compared to non-diabetic cataract patients and normal healthy controls (Ugurlu *et al*, 2013; Donma *et al*, 2002; Saygili *et al*, 2010) [15, 5, 13]. There is paucity of studies which assayed oxidant-antioxidant status in lens, and correlated it with that of blood in diabetic cataract patients. Present study aims to assess lipid peroxidation, vitamin C and copper levels in lens and serum of patients with diabetic cataract in comparison to patients with senile cataract.

Materials and Methods

This study was carried out in Ophthalmology and Biochemistry from June 2014 to December 2015 in the departments of Father Muller Medical College Hospital. The study protocol was approved by Institutional Ethics Committee. The study population consisted of diabetic and senile cataract patients. Cataracts of Grade I Nuclear sclerosis and more were taken for the assay. Patients who were known diabetics for 2 years or more and who were on regular treatment in the form of oral medication and insulin were included in this study. The surgery performed in these patients was in the form of small incision cataract surgery with IOL implantation, a suture less variant of extra capsular cataract extraction. This was done in order to be able to collect the nucleus for biochemical assay.

Informed consent was obtained from all the study subjects. Subjects with history of cardiovascular diseases, renal dysfunction, liver diseases, thyroid disorders, hypertension, and any other systemic diseases were excluded from the study. Subjects taking any antioxidant supplements, chronic alcoholics and chronic smokers were excluded. Blood samples were drawn pre-operatively in plain vacutainers, sera separated and stored at -4°C . In cataract patients (Group I. and II.), lens was obtained during extracapsular cataract extraction followed by intraocular lens implantation. Lens was collected in cold 0.9% saline and stored. Lens was homogenized 1: 10 (w/v) in cold phosphate

buffer (0.2 M, pH 7.2). The homogenate was centrifuged in a cold centrifuge, and the supernatant was used for biochemical estimations.

Assays

In serum and lens homogenate, biochemical assays were performed. Copper was estimated by a spectrophotometric method based on reaction of copper with dithiocarbamate (Burtis); assay of vitamin C was based on oxidation of ascorbic acid by cupric ions and finally formation of dinitrophenylbishydrazone of dehydroascorbic acid which was photometrically quantitated (Burtis *et al* 2006) [2]. Malondialdehyde, the sensitive marker of lipid peroxidation, was estimated by the method of Ohkawa *et al*, which was based on formation of thiobarbituric acid reactive substances (TBARS) (Ohkawa *et al* 1979) [9].

Statistical Analysis

The values were expressed as mean with standard deviation. Significance of the difference of the values between the groups was evaluated by using Student's independent "t" test.

Results

Results of this study are presented in table 1. Cataract patients (diabetic and senile) shown to have higher levels of malondialdehyde and copper, lower level of vitamin C in serum when compared to known control values. The changes were more pronounced in diabetic cataract patients. Among the cataract patients, subjects with diabetes had significantly higher levels of copper and lower levels of Vit C in both serum and lens when compared to non diabetic senile cataract patients. Malonyldialdehyde (MDA) levels, the hallmark of oxidative stress was found to be significantly higher in the lens of diabetic cataract as compared to senile cataract, while the serum MDA was not significantly different between senile cataract and diabetic cataract groups. In the lens homogenates, levels of malondialdehyde and copper were significantly higher in diabetic cataract patients in comparison to senile cataract patients. Level of Vit C was significantly lower in diabetic cataract patients when compared to senile cataract patients.

Table 1: Levels of MDA, copper and vitamin C in serum and lens

	Senile cataract (n=48)	Diabetic (n=26)	P value
MDA, Lens (nmol/g tissue)	9.48 ± 1.15	20.83 ± 2.34	0.001
MDA, serum (nmol/dl)	232.08 ± 19.31	363.38 ± 26.38	0.095
Vitamin C, lens (mg/g tissue)	0.90 ± 0.15	0.51 ± 0.078	0.05
Vitamin C, serum (mg/dl)	0.65 ± 0.19	0.35 ± 0.09	0.018
Copper, lens (micrograms/g tissue)	1.14 ± 0.15	2.56 ± 0.42	0.001
Copper, serum (micrograms/dl)	131.63 ± 8.14	161.85 ± 9.91	0.225

Discussion

The present study revealed significant elevation in lipid peroxidation and copper along with a concomitant decrease in the levels of vitamin C in cataract patients. When compared to non-diabetic, senile cataract patients, people with diabetic cataract showed more pronounced elevation of lipid peroxidation and copper, and depletion of vitamin C. We observed higher levels of malondialdehyde, the marker of lipid peroxidation in the serum of cataract patients when

compared to senile cataract patients. The increase in MDA was more pronounced in diabetic cataract than non-diabetic senile cataract. In lens, diabetic cataract patients showed higher levels of MDA than non-diabetic senile cataract patients.

The process of cataractogenesis is complex and multifactorial, and oxidative stress is one of major mechanisms proposed to be responsible for development of cataract. Diabetes mellitus is the major cause of cataract.

Imbalance in oxidant-antioxidant status in favor of oxidants, is implicated in the pathogenesis of diabetic complications such as cataract (Ugurlu *et al* 2013; Zoric, 2003; Heydari *et al*, 2012; Cekic *et al*, 2010 ; Yildirim *et al*, 2009; Seygili *et al*, 2010; Donma *et al* 2002) [15, 19, 7, 3, 18, 13, 5]. Previous studies by have shown a significantly increased serum MDA and advanced oxidation products in diabetic senile cataract patients when compared to healthy controls (Yildirim *et al*, 2009) [18]. Reports also suggest that a cataract patients have high levels of plasma and lens MDA and that a correlation with opacity grades of lens existed (Tarwadi and Agte V, 2004) [14]. Studies also indicate that diabetics with cataract have increased levels of MDA and that a positive correlation with calcium and negative correlation with total antioxidant status exists (Deepa *et al*, 2011) [4]. However contradictory observations also does exist which indicate that the levels of MDA were unaltered, while an increase in the levels of conjugated dienes and xanthine oxidase was observed in cataract patients (Miric *et al*, 2013) [8].

Copper is considered a pro-oxidant by virtue of its involvement in generation of free radicals by Haber Weiss and Fenton reactions. However, copper-requiring enzyme superoxide dismutase is considered to be an antioxidant. The present study observed significant elevation in copper levels in serum and lens in cataract patients. Copper levels in lens were significantly higher in diabetic cataract in comparison to senile cataract. Aydin *et al*. reported significantly higher level of copper in serum, aqueous humor and lens in diabetic patients when compared to non diabetics (Aydin *et al*, 2005) [1], indicating association of copper with the development of lens opacification.

In this study we observed significantly lower vitamin C levels in serum in diabetic cataract patients when compared to senile cataract patients. In addition in lens, vitamin C levels were significantly lower in diabetic cataract than in senile cataract. A Study by Valero and co workers observed that high plasma level of vitamin was associated with reduced risk of cataract in a Mediterranean population characterized by high intake of vitamin C. Studies suggest protective role of vitamin C on the ageing lens (Valero *et al*, 2002) [16]. Level of vitamin C in serum was below normal in cataract patients, and the level was lowest in low-income patients (Tarwadi and Agte, 2004) [14]. An inverse relation of plasma vitamin C with cataract in older Indian population has also been reported (Ravindran *et al*, 2011) [12]. A meta-analysis of association of vitamin C and risk of cataract revealed inverse relation of higher vitamin C intake and higher serum vitamin C level (Wei *et al*, 2016) [17].

Conclusions

In this study we observed oxidative stress in the lens of diabetic cataract patients indicated by increased lipid peroxidation and copper, and decreased vitamin C. Future studies with larger sample size and involving antioxidant treatment of cataract are required.

Authors' Contribution

Conceived and designed the experiments: ARS, MSB; Performed the experiments: PD, NN, VS, MD; Analyzed the data: PD, MD, ARS, SS, MSB; Contributed reagents/materials/analysis tools: ARS, MSB; Wrote the paper: PD, MD, ARS

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