Journal of Diabetes and Its Complications 26 (2012) 275–279

Contents lists available at SciVerse ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Osmotic stress induced oxidative damage: Possible mechanism of cataract formation in diabetes☆

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ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form 15 March 2012
Accepted 8 April 2012
Available online 17 May 2012

Keywords:
Osmotic stress
Oxidative imbalance
Cataract
Diabetes
Polyol pathway

ABSTRACT

Chronic hyperglycemia causes increased level of reactive oxygen species which is thought to be involved in the pathogenesis of diabetes associated complications including cataract. In diabetic cataractous lens, over production of free radicals and decreased capacity of antioxidant defense system are the major contributors to oxidative damage by polyol pathway and advanced glycation end products. The current study focused on analysis of factors associated with osmotic imbalance and oxidative stress in aging and diabetic human cataractous lenses. We examined activities of polyol pathway enzymes, G6PD and glutathione system in lenses from subjects suffering from cataract due to aging and diabetes. We observed elevated activities of aldose reductase and sorbitol dehydrogenase while G6PD and glutathione system enzyme activities were found to be lower in cataractous subjects suffering from diabetes. The findings from the current study support the premise that osmotic imbalance, AGEs formation and oxidative stress contribute synergistically to the development of lens opacity in hyperglycemia.

1. Introduction

Cataract is the most common cause of visual impairment throughout the world. Almost 25 million people suffer from visual loss worldwide due to cataract formation (Apple, Ram, Foster, & Peng, 2000) including 0.57 million from Pakistan (Jadoon et al., 2007). Cataract is a multi-factorial optic disorder associated with various risk factors such as malnutrition, drugs, UV light exposure, aging and diabetes. So far, aging and diabetes remain the most debated and significant contributors (Vinson, 2006). Diabetic patients are prone to develop visual complications such as retinopathy, corneal epitheliopathy and cataract (Sjølie et al., 1997). Onset of cataract in subjects suffering from diabetes is reported to be 20 years earlier than non-diabetics (Vinson, 2006). Although removal of cataract is the most common and successful ophthalmic surgery, methods to prevent or delay cataract formation still remain a hot area of research. Apart from the possibility of developing post-operative complications, cataract surgery itself poses a major economic burden. In developing countries, cataract surgery either is unaffordable or is inaccessible emphasizing the importance of alternative methods.

Earlier studies have indicated that oxidative stress plays an important role in the pathogenesis of long-term diabetic complications (Chandrasena, Chackrewarthy, de Perera, & Silva, 2006; Ha & Lee, 2000). Chronic exposure to high glucose is expected to increase metabolic flux inducing mitochondrial superoxide production which damages electron transport chain resulting in accumulation of glycolytic intermediates (Nishikawa et al., 2000). Excessive production of reactive oxygen species (ROS) is likely to produce oxidative stress by increasing hexosamine and polyol pathway, formation of advanced glycation end products (AGEs) and glycation (Rolo & Palmeira, 2006). Substantial evidence indicates the involvement of polyol pathway in the pathophysiology of diabetic cataract. Polyol pathway enzymes, aldose reductase and sorbitol dehydrogenase catalyze the conversion of glucose into sorbitol followed by conversion to fructose (Chylack & Friend, 1990; Kinoshita, 1990). In normoglycemia, 5% of glucose enters into polyol pathway while in hyperglycemia, production of sorbitol increases up to 6 folds (Morrison, Clements, Travis, Oski, & Winegrad, 1970). Sorbitol accumulation leads to altered membrane permeability that in turn causes cell lesion (Oishi et al., 2002).

The level of oxidative stress is dependent on scavenging capability of antioxidants. Like other cells of the body, lens also has an antioxidant enzyme system for protection against damage caused by excessive ROS. The key enzymes involved in this process include superoxide dismutase, catalase (Hashim & Zarina, 2006; Ozmen et al., 2002), Paraoxonase (Hashim & Zarina, 2007) and glutathione system (Fecondo & Augusteyn, 1983; Ganea & Harding, 2006). In subjects suffering from senile and diabetic cataract, the activities of these

☆ Conflict of Interest: None.

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doi:10.1016/j.jdiacomp.2012.04.005

enzymes were shown to decline implicating role of oxidative imbalance in the loss of lens transparency. The extent of decrease was greater in subjects having diabetic cataract (Donma, Yorulmaz, Pekel, & Suyugül, 2002; Hashim, Ilyas, Saleem, Salim, & Zarina, 2009; Shin, Oh, & Park, 2006). In hyperglycemic subjects, the efficiency of these enzymes might be compromised due to decreased availability of glutathione and/or competition for NADPH by various substrates (Lee & Chung, 1999). Supply of reduced $NADP^+$ is ensured through hexose mono phosphate pathway (HMP). The activity of Glucose-6-phosphate dehydrogenase (G6PD), the key regulatory enzyme of HMP, is shown to decline during diabetes hence contributing to oxidative stress (Nishikawa et al., 2000). Another possible reason could be glycation of these enzymes which is known to cause structural changes resulting in reduced activity (Shin et al., 2006). The process of glycation is known to be accelerated in the presence of ROS (Loske et al., 2000), hence the term glycoxidation has been coined indicating the possible interplay of AGEs and ROS. We have earlier reported oxidative stress biomarkers (MDA, SOD, Catalase and Paraoxonase) and possible role of glycation in blood and lens samples from senile and diabetic subjects suffering from cataract (Hashim & Zarina, 2006; 2007; 2011; Hashim et al., 2009). The objective of the current study was to examine the activities of enzymes involved in keeping oxidative balance intact within the ocular lens with particular reference to polyol pathway and G6PD to delineate their role in osmotic stress and diabetes. To the best of our knowledge, this is the first report on G6PD activities in human senile and diabetic subjects suffering from cataract.

2. Material and Methods

2.1. Subjects and sample collection

Cataract lenses from non-diabetic ($n=50$) and diabetic ($n=50$) subjects were removed by extracapsular cataract extraction followed by intraocular lens implantation. Before surgery, all patients underwent complete eye examination in an ophthalmic clinic. All experiments were performed as per the guidelines and approval of the institutional ethics committee, and written Informed consent was obtained from all the participants.

Study protocol was divided into two groups, non-diabetic and diabetic cataract patients. [Non-diabetic subjects: 23 males and 27 females; age (mean ± SD) 59.98 ± 8.71 and 63.26 ± 9.15 years, respectively and diabetic subjects: 24 males and 26 females; age (mean ± SD) 61.61 ± 11.75 and 60.88 ± 10.99 years, respectively].

Cataract patients with diabetes consisted of individuals who were suffering from type 2 diabetes mellitus and were using oral hypoglycemic drugs for the past 5 years. Non-diabetic cataract patients were subjects who had normal blood glucose level with no history of diabetes. Smokers and patients who were suffering from any systemic disorder, hepatic disease, cardiovascular disease, renal dysfunction or anemia or with toxic or traumatic cataract were excluded from the study.

2.2. Activities of polyol pathway enzymes

2.2.1. Aldose reductase and sorbitol dehydrogenase

Activities of aldose reductase and sorbitol dehydrogenase were determined in non-diabetic and diabetic cataractous lenses according to the method of Kubo, Maekawa, Tanimoto, Fujisawa, and Akagi (2001). 10 mM DL-glyceraldehyde and 10 mM fructose were used as substrate for aldose reductase and sorbitol dehydrogenase respectively. Decrease in absorption of NAD(P)H was measured at 340 nm. Enzyme activity unit was defined as the amount of enzyme that oxidizes μmol of NAD(P)H per minute.

2.3. Activities of Glucose-6-Phosphate dehydrogenase and Glutathione system

2.3.1. Glucose-6-phosphate dehydrogenase

Activity of Glucose-6-phosphate dehydrogenase in cataract lenses from diabetic and non-diabetic individuals was measured by Randox kit method (cat no. PD 410). Change in absorption was measured at 340 nm.

2.3.2. Glutathione

Content of GSH was determined according to the method of Moron, Fierre, and Mannerwick (1979) in cataract lenses from non-diabetic and diabetic subjects with 5-5′ dithio-2-nitrobenzoic acid (DTNB). Each lens was homogenized in 5% Trichloroacetic acid followed by centrifugation at 3500 rpm for 10 min. The absorption was read at 410 nm.

2.3.3. Glutathione peroxidase

Enzymatic activity of Glutathione peroxidase was measured by Randox kit method (RANSEL cat no. RS 504) in diabetic and non-diabetic cataractous lenses. Decrease in absorption was measured for 5 min at 340 nm.

2.3.4. Glutathione reductase

Glutathione reductase was assayed in cataractous lenses as described earlier by Carlberg and Mannervik (1975). The enzyme utilizes nicotinamide adenine dinucleotide phosphate (NADPH) to convert oxidized glutathione to its reduced form. The change in absorbance was recorded at 340 nm for 2 min with 30 s interval. The unit of enzyme activity was expressed as nmol of NADPH oxidized/min/mg protein.

2.4. Statistical analysis

Data were analyzed using SPSS software (SPSS® for Windows® 10.0) and reported as means ± SD. Statistical significance of the difference between non-diabetic cataractous group and diabetic cataractous group was evaluated by two-tailed student's t test. $p<0.001$ was accepted as statistically significant.

3. Results

Figs. 1A and 1B represent the activities of polyol pathway enzymes. Data show that activities of aldose reductase (Fig. 1A) and sorbitol dehydrogenase (Fig. 1B) were significantly higher ($p<0.001$ and $p<0.01$ respectively) in diabetic cataractous lenses when compared with cataractous lenses from non-diabetic individuals. Increased activities reflect availability of excessive glucose as a result of hyperglycemia.

Fig. 2 shows the levels of reduced glutathione (GSH) measured in ocular lenses from diabetic and senile subjects suffering from cataract. As expected, reduced glutathione levels were found to be significantly ($p<0.001$) greater in senile subjects as compared with diabetics. The activities of enzymes of the glutathione system (Glutathione reductase and Glutathione peroxidase) along with G6PD are shown in Fig. 3. Data show significant decline in activities ($p<0.001$) of all three enzymes in diabetic cataractous lenses in comparison with non-diabetic cataractous lenses.

4. Discussion

Diabetes mellitus is the most common disorder affecting more than 285 million people throughout the world including 6.9 million from Pakistan. Diabetes is a major risk factor for producing lens opacity or cataract which is ultimately the leading cause of visual