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The Relation Between Advanced Glycation End Products and Cataractogensis in Diabetics

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Abstract: Background advanced glycation end products (AGEs) play a pivotal role in cataractogenesis. Production of AGEs takes place throughout the normal aging process but its accumulation is found to be accelerated in diabetes. Advanced glycation end products formation and cataract progression are extremely slow processes and are triggered by the presence of free radicals. Oxidative stress along with AGEs may integrate resulting in acceleration of cataract formation. Methods in the present study 20 patients with diabetic cataract, 20 patients with senile cataract, as well as 20 healthy "non- diabetic subjects" (Age and sex matched healthy controls) were selected from outpatient clinic in the Research Institute of Ophthalmology RIO. Malondialdehyde (MDA) (Oxidative marker), Total antioxidant capacity (TAC), reduced glutathione (GSH), Superoxide dismutase (SOD) Antioxidant markers and AGEs were estimated in all studied groups. Results there were significant decreases in TAC, GSH, SOD activities in both the senile and diabetic cataract groups compared to the control group. There was statistical significant increase in plasma MDA and AGEs levels in both the senile and diabetic cataract groups compared to the controls. Conclusion this study demonstrated increased accumulation of AGEs and increased lipid peroxidation products along with impaired antioxidant status in patients with both diabetic and senile cataract.

Key words: Advanced Glycation End Products (Ages) · Senile Cataract · Diabetic Cataract · Aging · Oxidative Stress · Antioxidants

INTRODUCTION

Cataract is a visual impairment resulting from opacification or optical dysfunction of the lens crystallin. It occurs in diabetic patients (osmotic cataract) as well as in non- diabetic patients (senile cataract) where age is the main predisposing factor [1].

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [2]. It is estimated that by the year 2030, Egypt will have at least 8.6 million adults with diabetes [3]. Long-term hyperglycemia is involved in the pathogenesis of diabetic microvascular and macro-vascular complications and cataractogenesis [4].

Glycation is a non-enzymatic process in which glucose covalently binds to a protein in a high glucose concentration [5]. Advanced glycation end products (AGEs) play a pivotal role in loss of lens transparency. AGEs production occurs throughout the normal aging process but its accumulation is found to be more rapid in diabetics [6]. Advanced glycation end products formation is accelerated by oxidative stress as reactive oxygen species enhance cross-linking between carbohydrate moiety and amino group of protein [7].

Lipid peroxidation of cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in microvascular complications of diabetes mellitus [8]. The toxic effects of the reactive oxygen species are neutralized in the lens by antioxidants

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such as ascorbic acid, vitamin E, glutathione peroxidase and reductase, superoxide dismutase and catalase [9]. Hashim *et al*. [10] reported an inverse relationship between levels of antioxidants and Glycation.

The aim of this study is to investigate the potential role of advanced glycation end products (AGEs) and oxidative damage in age-related (Senile) and diabetic cataract patients.

MATERIALS AND METHODS

The study was performed on forty patients of both sexes selected among those attending the outpatient clinics of The Research Institute of Ophthalmology (RIO). A group of twenty age and sex matched healthy subjects with no history of ophthalmologic or medical disease was served as a control group. Ethical Committee Approval of the Research Institute of Ophthalmology was obtained.

- All subjects have performed an ophthalmic examination, refraction, best corrected visual acuity, intraocular pressure, anterior segment slit lamp examination and dilated funduscopic examination. Fluorescein angiography was performed when clinically indicated. Medical examination and routine laboratory investigations, liver function, kidney function tests were performed
- Subjects were classified into the following groups:

Group I: Diabetic Cataract Group: Included 20 diabetic patients with cataract their mean age±S.E of 58.8 ±1.63, (7 males and 13 females).

Group Ii: Senile Cataract Group: Included 20 patients with senile cataract their mean age±S.E of 61.4±1.50, (12 of them males and 8 females).

Group III: Control Group: Included 20 healthy "non-diabetic subjects" (Age and sex matched healthy controls) with a mean age±S.E of 59.05 ±1.63, (11 of them males and 9 females).

Exclusion Criteria:

- Only those patients who did not have hepatic or renal diseases were selected. Any patient with serum creatinine >1.2 mg / dl or urinary albumin excretion > 150 mg / 24 hrs was not included in this study. Also any patient with local eye disease such as cataract, glaucoma or uveitis was excluded from the study.

Biochemical Parameters Assayed in this Study:

- The concentrations of blood hemoglobin (Hb) was assayed by the chemical method according to Betke and Savelsberg [11] & glycosylated hemoglobin (HbA1c) was performed by Ion Exchange Resin method using a commercial kit provided by NS Biotec, (Egypt).
- The determination of reduced glutathione (GSH) was determined by the chemical method according to Beutler *et al*. [12] & superoxide dismutase (SOD) was assayed by the colorimetric method using a kit provided by Biodiagnostic, (Egypt).
- Estimation of glucose was performed using a kit supplied by BioMe´rieux, CA 61-269; (France), malondialdehyde (MDA) was assayed by a colorimetric method using a kit supplied by Biodiagnostic, (Egypt), total antioxidant capacity (TAC) by colorimetric method using a kit provided by Biodiagnostic, (Egypt). The advanced glycation end products (AGEs) were determined by the enzyme linked immunosorbent assay (ELISA) procedure using a kit supplied by Cell Biolabs, Inc, CA 92126, San Diego, (USA).

Statistical Analysis: Statistical analysis was carried out using Microsoft excel (Version 10) and statistical package for social sciences (SPSS) software (Version 20). All values are expressed as mean±standard error (S.E) [13]. Continuous variables from more than two groups were compared with one-way analysis of variance (ANOVA) [14]. The P-values were considered statistically significant at: $P > 0.001$= highly Significant, $P > 0.05$= significant and $P < 0.05$= non significant (N.S).

RESULTS

- Basically, patients with diabetic cataract had significantly higher fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) compared to both the control and senile cataract groups (P-value <0.001). No significant difference was observed in fasting blood glucose (FBG) and glycosylated hemoglobin in the senile cataract group compared to control group (P-value >0.05) (Table 1).
- Both senile and diabetic cataract groups showed a highly statistical significant increase in plasma malondialdehyde (MDA) levels compared to controls (P-value <0.001). Moreover, there was statistically significant increase in plasma MDA level in diabetic cataract group compared to the senile cataract group (P-value <0.05) (Table 2).

Table l: Characteristic features of all the studied groups:

Group	Sex (M/F)	Age	(BMI) (kg/m^2)	Fasting blood glucose (mg/dL)	Blood hemoglobin (g/dL)	$HbA1_c$ (%)
Control (n=20)	11/9	59.051.63a	24.69±0.55a	93.05±2.97a	13.19±0.27a	4.95±0.32a
Senile cataract (n=20)	12/8	61.41.50a	24.60±0.53a	105.45±3.62a	12.6± 0.37a	4.86±0.42a
Diabetic cataract (n=20)	7/13	58.8 1.63a	26.07±0.36a	219.75±15.83b	13.24±0.43a	9.24±0.40b
F ratio		0.814	2.806	53.684	0.930	42.464
p value		NS	NS	**	NS	**
		0.448	0.069	0.000	0.400	0.000

Groups with different letters have a statistically significant difference. p*= significant at P-value <0.05, p**= highly significant at P-value <0.001 and NS= non-significant at P-value >0.05.

Table 2: Malondialdehyde level (nmol/mL), total antioxidant capacity (mM), Glutathione (mg/dl), superoxide dismutase and Advanced Glycation End products µg/ml in all the studied groups

Group	MDA (nmol/mL)	TAC (mM)	GSH (mg/dL)	SOD (U/g Hb)	AGEs (µg/mL)
Control (n=20)	1.53±0.09a	1.86±0.13a	82.20± 2.27a	1803.5±43.69a	7.07± 0.58a
Senile cataract (n=20)	2.13±0.10b	1.13±0.07b	57.70± 3.25b	1182.50±33.03 b	12.82± 0.54b
Diabetic cataract (n=20)	3.05±0.14 c	1.01±0.07 b	46.14±3.41 c	1148.00±38.20 b	14.94± 0.57 c
ratio	45.887	24.113	37.150	91.553	52.437
p value	** 0.00	** 0.000	** 0.000	** 0.000	** 0.000

Groups with different letters have a statistically significant difference. p*= significant at P-value <0.05, p**= highly significant at P-value <0.001 and NS= non-significant at P-value >0.05.

- Both senile and diabetic cataract patients were found to have a highly statistical significant decrease in plasma total antioxidant capacity (TAC), erythrocyte reduced glutathione (GSH) concentration and erythrocyte superoxide dismutase (SOD) activity compared to the controls (P-value <0.001). There were no significant decrease in plasma TAC level and SOD activities in the diabetic cataract group compared to senile cataract group (P-value >0.05). While, there was statistically significant decrease in erythrocyte GSH concentration in the diabetic cataract group compared to the senile cataract group (P-value <0.05) (Table 2).
- Both senile and diabetic cataract groups showed a highly statistical significant increase in plasma advanced glycation end products (AGEs) level compared to control group (P-value <0.001). Moreover, there was a significant increase in plasma AGEs in the diabetic cataract group compared to the senile cataract group (P-value <0.05) (Table 2).

DISCUSSION

Chronic elevation of blood glucose in diabetes plays a critical role in the development and progression of diabetic cataract. Prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism and long-term irreversible changes in stable macromolecules. The injurious effects of hyperglycemia are character-istically observed in non insulin dependent tissues for glucose entry into the cell (E.g. lens crystalline) and, hence, they are not capable of down-regulating glucose transport along with the increase of extracellular glucose concentrations [15].

The present study showed statistically significant increase in both fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) in diabetic cataract patients as compared to senile cataract patients and controls. These observations are similar to that reported by Khaw *et al*. [16], Gul *et al*. [17] and Miric *et al*. [18]. The development of cataracts in diabetics is due to chronic elevation of blood glucose and its conversion to sorbitol by the aldose reductase enzyme via the polyol pathway. Sorbitol does not easily cross cell membranes and it can accumulate in cells and cause damage by disturbing osmotic homeostasis [19].

Oxidative stress originates due to an imbalance between the generation of reactive oxygen species (ROS) and their scavengers (Antioxidant defense system). Reactive oxygen species also initiate lipid peroxidation of polyunsaturated fatty acids resulting in the production of reactive carbonyl compounds, malondialdehyde (MDA) is most abundant. Several studies reported higher MDA levels in plasma and cataractous lens samples from cataract patients [20, 21].

A highly significant increase in plasma MDA was detected in both senile and diabetic cataract patients compared to controls. Also significant increase was also found in plasma MDA in the diabetic cataract patients compared to the senile cataract. This result agreed with that of Hashim and Zarina [22] and Artunay *et al*. [23], which supported the hypothesis that both diabetic and

age-related cataract development were associated with increased oxidative stress markers and decreased antioxidant enzyme activities [21, 24]. Furthermore, Vaya and Aviram [25] and Deepa *et al*. [26] detected that the increased levels of lipid peroxidation product (MDA) in diabetics are due to increased production of reactive oxygen species (ROS) caused by the hyperglycemic status, hyperinsulinemia and hyperlipidemia. Hence, it can be concluded that cataract patients have higher levels of oxidative stress markers compared with controls [27].

There is a growing evidence to support a link between increased levels of ROS and disturbed activities of enzymatic and non-enzymatic antioxidants with increasing age and in diabetics [28]. Due to the importance of the imbalance in the oxidant to antioxidant ratio, the measure of total antioxidant capacity of serum has increasingly become used as an index of the antioxidant status [29].

In the current study, a highly statistically significant decrease was found in plasma total antioxidant capacity (TAC) in both senile and diabetic cataract patients compared to controls. This result agreed with that reported by Jacques *et al*. [30], Knekt [31], Nourmohammadi *et al*. [32] and Gul and Rahman [33] they detected that the total antioxidant status, which includes the chain breaking antioxidants such as ascorbate, urate and bilirubin and the membrane preventive antioxidants such as β-carotene and vitamin E, were lower in cataractous patients which indicates an increased risk for cataract formation. It was suggested that a decrease in the antioxidant capacity of the erythrocytes may increase the oxidative damage in tissues, including the oxidative modification of lens proteins observed in cataract [20, 34] the reduced antioxidant activity in diabetics could be explained by hyperglycemia induced complications such as reduced gene expression and inactivation of antioxidant enzymes by glycation [35]. Thus, this confirms the decrease in total antioxidant capacity (TAC) in human cataract patients. On the other hand, this study demonstrated no statistically significant decrease in plasma TAC in the diabetic cataract group compared to the senile cataract group. This result agreed with that reported by Saygili *et al.* [36]. While, Deepa *et al.* [26] found that the total antioxidant activity in serum as well as in the lens was reduced significantly in diabetic cataract compared to senile cataract.

The reducing compound, GSH, exists in an unusually high concentration in the lens where it functions as an essential antioxidant vital for the maintenance of the lens transparency [37]. It plays a major role in the regulation of the redox status of the cell and protects tissues from lipid peroxidation [38]. Reductions of GSH levels are responsible for the development of cataracts. GSH-dependent mechanisms function in protecting against this oxidative damage [39]. Also, the size of GSH pool is reported relatively low in the aged lenses or lenses under oxidative stress [37].

The present study illustrated a highly statistical significant decrease in erythrocyte GSH in both the senile and diabetic cataract groups compared to the control group. This was in agreement with Donma *et al*. [20] and George *et al*. [40].

Miranda *et al*. [41] reported that the lens contains a high concentration of reduced GSH, which maintains the thiol groups in the reduced form. These contribute to lens complete transparency. Glycation slowly inactivates GSH-related enzymes. In addition, GSH can be also glycated and, therefore, contributes to the opacification of the lens in these patients [41]. In the current study, there was also a significant decrease in erythrocyte GSH in the diabetic cataract group compared to the senile cataract group. This result agreed with Donma *et al*. [20]. Moreover, Kamei [42] and Babizhayev & Costa [43] detected a more pronounced decrease in both blood and lens GSH levels in diabetic cataract.

Superoxide dismutase (SOD) enzyme is part of the first line of defense against free radicals and thus it is expected that the activity of this enzyme may be affected by oxidative stress before the other antioxidant enzymes [44]. Many studies detected decreased SOD activities on serum of cataract patients [45, 46] and in cataractous lens tissue itself [22, 34]. Moreover, Maurya *et al*. [46] reported that the decrease in serum levels of this anti-oxidant enzyme lead to early cataract formation in diabetic patients.

In the current study, a highly statistically significant decrease was found in erythrocyte SOD in the senile and diabetic cataract groups compared to the controls. There was no significant variation between the activities observed in both the diabetic and the senile cataracts. This result agreed with that of Donma *et al*. [20]. Artunay *et al*. [23] reported that this reduction in the activity may be due to enhanced generation of superoxide radicals in vivo during aging and cataractogenesis. Reduced erythrocyte SOD activities reported in the diabetics suggest that superoxide scavenging activity is reduced and these results were caused by non-enzymatic glycation due to the negative association between HbA_{1c} levels and SOD activities in erythrocytes. The present study contradicted with that performed by

Nourmohammadi *et al*. [32] and Delcourt *et al*. [47] they reported increased blood levels of antioxidant enzymes to be associated with cataract. This is thought to be a compensatory defensive response to increased levels of oxidation within the body.

Normal aging is accompanied by a progressive increase of advanced glycation end products (AGEs) in lens crystalline [48]. Chronic hyperglycemia accelerates non-enzymatic glycation reactions between reducing sugars and free reactive amino groups of proteins (Maillard reaction) [49]. This reaction leading, first to the formation of the reversible Schiff bases, then to the Amadori product and finally, after slow and complex rearrangements, to irreversible AGEs [50], which are characterized by a brown color, fluorescence and cross-linking [49].

In this study, there was a highly significant increase in plasma AGEs in both senile and diabetic cataract compared to controls. Also, this study demonstrated a significant increase in plasma AGEs in the diabetic cataract group compared to the senile cataract group. These results were consistent with those of Gul *et al*. [17], Abiko *et al*. [51] and Zarina *et al*. [52] They postulated that the role of AGEs in diabetic and non-diabetic senile cataractogenesis were potentially important, because it induces both the structural and functional implications. In lens protein, non-enzymatic glycation of lens proteins by chronic hyperglycemia may cause conformational changes resulting in the formation of protein aggregates that precipitate in the lens leading towards lens opacity. Also, the action of highly reactive dicarbonyl compounds such as glyoxal and methylglyoxal is enhanced in diabetes and aging, leading to AGE cross-links on lens crystallins with resultant loss of chaperone activity, increased Dβ- crystallin content and dense aggregate formation [53]. Therefore, it can be speculated that glycation occurs during normal aging but to a greater degree in diabetes in which it plays a major role in cataractogenesis [54]. However, in contrast to our results [55] found that there was no relationship between serum AGEs level and diabetic complications.

CONCLUSION

this study suggests that AGEs formation and progression of cataract both are extremely slow processes and both are triggered in the presence of reactive oxygen species. Oxidative insult along with AGEs may integrate resulting in acceleration of cataractogenesis.

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