

Role of Advanced Glycation End Products (AGEs) and Obesity in Diabetic Cataract Rats

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Abstract: *Background:* obese subjects have a more elevated degree of oxidative stress than normal as increased body fat stimulates excessive reactive oxygen species (ROS) production. Also, obesity is associated with serious morbidities including a high incidence of type 2 diabetes and cataractogenesis. *Methods:* in the present study the body mass index (BMI) was evaluated. Fasting blood glucose (FBG), glycosylated hemoglobin (HbA_{1c}), Malondialdehyde (MDA), Antioxidant markers (Total antioxidant capacity (TAC), reduced glutathione (GSH), Superoxide dismutase (SOD) and Advanced glycation end products (AGEs) were assayed. Also, determination of total protein and electrophoretic analysis of lens proteins were estimated in 40 rats divided into four groups of 10 animals each: control (group I); diabetic (group II) injected with a dose of 40 mg/kg by streptozotocin; high fat diet (Group III) were access to high fat diet and (Group IV) were access to a high fat diet and injected with a dose of 40 mg/kg by streptozotocin. *Results:* there was a statistical significant increase in FBG, HbA_{1c}, MDA and AGEs levels in diabetic and HFD groups compared to control group. Meanwhile, there were statistical significant decrease in GSH and SOD activities in both diabetic and HFD groups compared to control group. On the other hand, there were statistical significant decrease in TAC level and total lens proteins in diabetic groups compared to control group. Sodium dodecyl sulfate (SDS) electrophoresis showed aggregation of lens proteins in the diabetic groups compared to HFD and control groups. *Conclusion:* this study clarifies increased accumulation of AGEs and increased lipid peroxidation products along with impaired antioxidant status in obesity and at accelerated rate in diabetics. Proper control of hyperglycemia, blocking of AGEs pathways by AGEs-inhibitors and low fat diet may be beneficial to delay diabetic cataractogenesis.

Key words: Obesity • Diabetic Cataract • Advanced Glycation Endproducts (Ages) • Oxidative Stress • Antioxidants

INTRODUCTION

Cataract is most simply defined as opacity of the crystalline lens [1]. One of the prominent characteristics of human and experimental cataract is a massive increase in water insoluble protein fractions which are made up of protein polymers. These polymers are characterized by brown coloration and fluorescence appearance in such lenses, which is important for cataractogenesis [2].

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [3]. The prevalence of diabetes mellitus is increasing rapidly in association with the increase in obesity. Worldwide, more than 285 million people are affected by diabetes mellitus. This number is expected to increase to 439 million by 2030 [4]. It is also estimated that by the year 2030, Egypt will have at least 8.6 million adults with diabetes [5].

Diabetic cataract, is considered as a complication of diabetes mellitus and the cause of visual impairment that can affect in individuals at younger ages [6]. Chronic hyperglycemia and the duration of diabetes are considered to be the major risk factors for these diabetic complications, Di Benedetto *et al.* [7]. Several factors, such as polyol pathway, advanced glycation end products (AGEs) and oxidative stress have been implicated in the development of diabetic cataract [8].

There is growing evidence that AGEs and RAGE (Receptor for AGEs) interaction stimulates oxidative stress and tissue damage in diabetes [9,10]. Oxidative stress is increased in diabetes mellitus owing to the increase in the production of oxygen free radicals and /or deficiency in antioxidant defense mechanisms [11].

Obesity is an energy-rich condition associated with overnutrition, which impairs systemic metabolic homeostasis and elicits stress [12]. Egypt is one of the countries in the world where the problem of obesity has been nearing an epidemic level. Nearly 70% of adult women and 48% of men in Egypt are overweight or obese [13]. Obesity is a major public health problem and its impact on ocular health is increasingly recognized. Association of obesity with cataract has been reported with varying degree of certainty. The inconsistency of results combined with the deficiency of robust data; suggest that further investigations are required to clarify this association [14].

The aim of this study is to determine the potential role of advanced glycation end products (AGEs), oxidative stresses and obesity in the development and progression of diabetic cataract.

MATERIALS AND METHODS

Forty white albinos Sprague Dawley rats of body weight (120 - 150 g) comprising both sexes and purchased from the animal house colony in the Research Institute of Ophthalmology.

Experimental animals in this study were divided into four groups (10 for each):

Group I: Animals fed on standard diet that served as a control.

Group II: Animals injected individually with a dose of 40 mg/kg by streptozotocin (STZ).

Group III: Animals were access to high fat diet (HFD).

Group IV: Animals were access to high fat diet and injected individually with a dose of 40 mg/kg by streptozotocin (STZ).

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Rats after overnight fasting were injected intraperitoneally with STZ (40 mg/kg body weight), dissolved in 0.05 M citrate buffer, pH 4.5, immediately before use) to induce type 2 diabetes [15].

Experimental Diet:

- A semisynthetic nutritionally adequate diet was prepared and fed to rats according to the designed protocol. The composition of diets was prepared according to Hong *et al.* [16].
- The experiment continues for 3 months, at the end of the experimental period, the body weight was recorded for each animal and the animals were kept fasting for 12 h and the blood samples were collected from the retro-orbital venous plexus on an anticoagulant agent.

Biochemical Analysis:

- Blood hemoglobin (Hb) was evaluated by the chemical method according to Betke & Savelsberg [17] & glycosylated hemoglobin (Hb_{1c}) was assayed by Ion Exchange Resin method using a kit provided by NS Biotec, (Egypt).
- The determination of reduced glutathione (GSH) was performed by the chemical method according to Beutler *et al.* [18] & superoxide dismutase (SOD) assay by colorimetric method using a kit provided by Biodiagnostic, (Egypt).
- The determination of glucose using a kit supplied by BioMe'rieux, CA 61-269; (France), malondialdehyde (MDA) assayed a colorimetric method using a kit supplied by Biodiagnostic, (Egypt), total antioxidant capacity (TAC) measured by colorimetric method using a kit provided by Biodiagnostic, (Egypt) & advanced glycation end products (AGEs) were done by Enzyme linked immunosorbent assay (ELISA) procedure using a kit supplied by Cell Biolabs, Inc, CA 92126, San Diego, (USA).
- After blood collection, the rats were scarified and the eye ball was removed. The lens was separated from the eye by making a cission in the cornea using a

sharp blade. The weight of the lenses was determined. The lens was homogenized in distilled water (84 mg lens tissue/ 1 ml distilled water). This was used for determination of total protein by the method of Lowery *et al.* [19] and electrophoretic analysis of proteins according to Laemmli [20].

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) [21]. Continuous variables from more than two groups were compared with one-way analysis of variance (ANOVA) and Post hoc-LSD [22]. 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

- Body weight decreased significantly in group II (Diabetic) compared to control (Group I) (*p*-value <0.001). While, there was a high significant increase in body weight in group III (HFD) compared to control (Group I) (*p*-value <0.001) and there was no significant change in body weight in group IV (HFD + diabetic) compared to control (Group I) (*p*-value >0.05) (Table 1).
- Both groups II and IV (Diabetic and HFD + diabetic) had significantly higher fasting blood glucose (FBG) and glycosylated hemoglobin (HbA_{1c}) compared to control (Group I) (*p*-values <0.001). On the other hand, there was a significant increase in fasting blood glucose (FBG) and glycosylated hemoglobin (HbA_{1c}) in group III (HFD) compared to control (group I) (*p*-value <0.05) (Table 1).
- Both groups II and IV (Diabetic and HFD + diabetic) showed a high statistical significant increase in plasma MDA level compared to control (Group I) (*p*-values <0.001). On the other hand, there was a

significant increase in plasma MDA level in group III (HFD) compared to control (Group I) (*p*-value <0.05) (Table 2).

- Both groups II and IV (Diabetic and HFD + diabetic) showed significant decrease in plasma total antioxidant capacity (TAC) level and a highly statistical significant decrease in erythrocyte reduced glutathione (GSH) concentration and erythrocyte superoxide dismutase (SOD) activity compared to control (Group I) (*p*-values <0.05 and *p*-values <0.001), respectively. On the other hand, there was no significant decrease in plasma total antioxidant capacity level and a significant decrease in erythrocyte reduced glutathione concentration and erythrocyte superoxide dismutase (SOD) activity in group III (HFD) compared to control (Group I) (*p*-value >0.05 and *p*-value <0.05), respectively (Table 2).
- Both groups II and IV (Diabetic and HFD + diabetic) had significantly higher plasma advanced glycation endproducts (AGEs) compared to control (Group I) (*p*-values <0.001). While, there was a significant increase in plasma AGEs level in group III (HFD) compared to control (Group I) (*p*-value <0.05) (Table 2).
- Both groups II and IV (Diabetic and HFD + diabetic) showed a high statistical significant decrease in total lens protein compared to control (Group I) (*p*-values <0.001). On the other hand, there was no significant decrease in total lens protein in group III (HFD) compared to control (Group I) (*p*-value >0.05) (Table 2).

Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis: SDS electrophoresis of soluble lens protein showed aggregated band in both groups II and IV (Diabetic and HFD + diabetic) at 35 KDa with clear disappearance of this band in HFD and control groups. This aggregation

Table 1: Body weight, fasting blood, glucose, Blood hemoglobin and HbA_{1c} in all studied groups:

Group	Body weight (g)	Fasting blood glucose (mg/dL)	Blood hemoglobin (g/dL)	HbA _{1c} (%)
Control (n=10)	229.9±3.24a	90.4±3.59a	10.52±0.18a	4.57±0.47a
Diabetic (n=10)	195.80±2.03b	186.20±3.79b	7.23±0.10b	10.46±0.55b
High fat diet (HFD) (n=10)	298.40±3.56c	105.00±3.00c	9.84±0.16c	7.88±0.51c
High fat diet (HFD) + Diabetic (n=10)	226.40±3.09a	201.90±3.13d	6.88±0.12b	12.02±1.06b
F ratio	203.364	275.251	158.096	22.164
<i>p</i> value	** 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.

Table 2: Plasma malondialdehyde level and TAC, GSH, SOD and AGEs levels in all the studied groups

Group (n=10)	MDA (nmol/mL)	TAC (mM)	GSH (mg/dL)	SOD (U/g Hb)	AGEs (µg/mL)	Total lens protein (mg/g wet. wet)
Control (n=10)	0.90±0.07a	1.80±0.19a	85.98±4.68a	1497.90±83.29a	0.73±0.07a	251.43±9.72 a
Diabetic (n=10)	2.14±0.10b	1.13±0.10bc	40.46±3.94b	687.60±69.74b	1.41±0.10bc	182.9±7.41b
High fat diet (HFD) (n=10)	1.32±0.08c	1.52±0.18ab	65.38±2.15c	1068.80±90.17c	1.18±0.09b	243.18±9.72a
High fat diet (HFD) +Diabetic (n=10)	2.41±0.07d	1.03±0.11c	35.84±4.03b	621.10±72.42b	1.48±0.11c	144.05±3.10d
F ratio	73.202	5.716	37.261	25.946	12.353	39.046
p value	** 0.000	* 0.003	** 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.

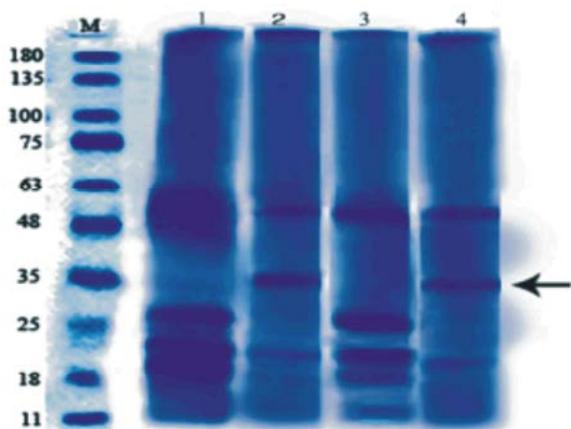


Fig. 1: Typical SDS-polyacrylamide gel electrophoretic pattern of lens proteins.

- M — protein marker (kDa),
- Lane 1— control,
- Lane 2 — diabetic group,
- Lane 3 — HFD group,
- Lane 4 — (HFD + diabetic) group.

and insolubilization of lens proteins in diabetic groups is an indicator of cataractogenesis (Fig. 1).

DISCUSSION

Obesity is considered to be a disorder of energy balance, occurring when energy expenditure is not in equilibrium with daily energy intake, so as to ensure body weight homeostasis [23]. Although the etiology of obesity is complex, dietary factors, particularly the consumption of a high fat diet (HFD), is considered the major risk factor for its development [24].

The present study revealed a highly significant reduction in body weight in group II (Diabetic) compared to control (Group I). This is in agreement with the study of Sajithlal *et al.* [25], Duzguner & Kaya [26] and Balakumar *et al.* [27]. Also, Eleazu *et al.* [28] reported that

in diabetes, the destruction of the pancreatic β -cells accompanied with insulin deficiency leading to increased synthesis of ketone bodies which are excreted in urine. The increased synthesis of ketone bodies coupled to lipolysis leads to a severe body weight loss.

The current study showed that body weight increases significantly in the HFD group compared with the control group. This is in accordance with the study of Lee *et al.* [29], Amin & Nagy [30] and Hussein [31]. It is usually assumed that high calorie and/or high fat diets can lead to obesity [32]. Many reports [33- 35] showed that high fat diet can lead to visceral obesity in rodent animal models.

Moreover, this study demonstrated no significant difference in body weight in group IV (HFD + diabetic) compared to control (Group I). This result agreed with that of Ugochukwu & Figgers [36]. While, Kim *et al.* [37] found out elevated body weights of the Zucker diabetic fatty (ZDF) rats to approximately 78% compared to controls. Also, Mega *et al.* [38] found that the obese diabetic ZDF rats exhibit an 8.7% reduction in their body weight compared with the lean control rats.

The cytotoxic action of STZ is associated with the generation of ROS causing oxidative damage that leads to β -cell destruction through the induction of apoptosis and suppression of insulin biosynthesis [39]. Although the β -cells cytotoxic activity of STZ is not fully understood, it is thought to be mediated by the inhibition of free radical scavenger-enzymes thereby enhancing the production of the superoxide radical which can damage pancreatic β -cells [40]. In addition, the intracellular metabolism of STZ produces nitric oxide that hastens DNA fragmentation, leading to severe necrosis of the β -cells, thereby the rate of insulin synthesis is diminished that ultimately resulting in hyperglycemia [41].

In the present study, a highly statistical significant increase in both fasting blood glucose (FBG) and glycosylated hemoglobin (HbA_{1c}) was observed in group II (Diabetic) compared to control (Group I). This result is in agreement with that of Sajithlal *et al.* [25], Duzguner &

Kaya [26], Balakumar *et al.* [27] and Civelek *et al.* [42]. These findings reflected the induction of diabetes mellitus in STZ administered rats.

Also, a significant increase in both fasting blood glucose (FBG) and glycosylated hemoglobin (HbA_{1c}) was detected in group III (HFD) compared to control (Group I). This result agreed with that of Amin & Nagy [30], Hussein [31] and Brockman *et al.* [43].

In the current study, there was a high statistical significant increase in both fasting blood glucose (FBG) and glycosylated hemoglobin (HbA_{1c}) in group IV (HFD + diabetic) compared to control (group I). This result agreed with that of Mega *et al.* [38], Kim *et al.* [37] and Mahmoud *et al.* [44] confirming the glycemic deregulation.

Diabetics usually exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes free radicals generation [45]. Oxygen free radicals could react with polyunsaturated fatty acids which lead to lipid peroxidation (LPO) [46, 47]. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors [48]. As a by-product of lipid peroxidation, MDA reflects the degree of peroxidation in the body [44].

The present study detected that plasma malondialdehyde (MDA) level shows a high significant increase in group II (diabetic) compared with their level in control (Group I). This is supported by Duzguner & Kaya [26], Balakumar *et al.* [27], Kakkar *et al.* [49] and Sailaja *et al.* [50] and all reported an increase in plasma MDA by hyperglycemia-induced glucose autoxidation and glycation of proteins.

The present study showed that there was a significant increase in plasma malondialdehyde (MDA) in group III (HFD) compared to control (Group I). This finding is supported by Belobrajdic *et al.* [51]. Several studies have also shown elevated lipid peroxidation products in obesity [52- 54]. Zhang *et al.* [55] suggested that high intake of dietary fat directly enhanced ROS overproduction which increased lipid peroxidation [55]. Also, in the present study a high significant increase in plasma malondialdehyde (MDA) was found in group IV (HFD + diabetic) compared to control (Group I). This result agreed with that reported by Mahmoud *et al.* [44] and Zhang *et al.* [56].

In this study, there was a significant decrease in plasma TAC in group II (Diabetic) compared to control (Group I). This result agreed with that reported by

Ndisang & Jadhav [57], Osman *et al.* [58] and Matsinkou *et al.* [59]. This severe depletion of TAC may be explained by the glycemic deregulation in diabetic rats.

Also, there was a significant decrease in plasma TAC in group IV (Diabetic and HFD + diabetic) compared to control (Group I). This is in agreement with that reported by Mahmoud *et al.* [44].

The notable decline in the key cellular non-enzymatic antioxidant defense system extensively provokes the susceptibility to oxidative stress [60].

The present study showed that there were a high significant decrease in both erythrocyte reduced glutathione (GSH) concentration and erythrocyte superoxide dismutase (SOD) activity in group II (Diabetic) compared to control (Group I). This was in agreement with that reported by Sajithlal *et al.* [25] and Duzguner & Kaya [26]. However, to the contrary of this study, Taheri *et al.* [61] reported that animal and human studies have shown contradictory results on the influence of diabetes on SOD activity. Both increase and decrease in SOD activity were reported in erythrocytes, whereas increased activity was seen in plasma and the retina and reduced activity in the pancreas. Rauscher *et al.* [62] found that SOD activity was increased in diabetic rats after 32 weeks of treatment. The variation in results from animal studies may be due to differences in selection of gender, duration of diabetes, tissues investigated and species of animals used [61].

Also, the significant decrease in both erythrocyte reduced glutathione concentration and erythrocyte superoxide dismutase (SOD) activity in group III (HFD) compared to control (group I). These results are in agreement with that of Elhadi *et al.* [63] who found that ROS production was increased in parallel with fat accumulation. Also, Grundy [64] found that the production of ROS increased selectively in adipose tissues of obese mice. The decline shown in plasma reduced/oxidized glutathione ratio and in the antioxidant enzymes may be a consequence of the ability of free fatty acids (FFAs) to increase ROS formation. Moreover, Toborek *et al.* [65] suggested that FFAs not only induce a state of oxidative stress, but also impair the endogenous antioxidant defenses by decreasing intracellular glutathione. Also, Brownlee [66] suggested that hyperglycemia in the HFD group activates different pathways leading to increased oxidative stress coupled to inhibition of the pentose phosphate pathway due to insulin deficiency resulted in decreased intracellular levels of NADPH, which is required for regeneration of GSH from its oxidized form GSSG. The net result was non-

enzymatic disruption of H₂O₂ and increased levels of cellular superoxides, hydroperoxides, hydroxyl radicals as well as other radicals [30].

The current study showed highly significant decrease in both erythrocyte reduced glutathione concentration and erythrocyte superoxide dismutase (SOD) activity in group IV (HFD + diabetic) compared to control (group I). This result is in agreement with the results reported by Mahmoud *et al.* [44], Zhang *et al.* [56] and Gokce & Haznedaroglu [67]. Clarify that the decrease in the activities of SOD and GSH in HFD/STZ-induced diabetic rats could be due to inactivation caused by STZ-generated ROS [44].

In the present study, there was a high significant increase in plasma AGEs in group II (Diabetic) compared to control (Group I). This result was in agreement with those obtained by Civelek *et al.* [42], Tanaka *et al.* [68] and Wan & Khalid [69]. One of the major consequences of hyperglycemia is the formation of AGEs [70]. Oxidative damage may be increased because of chronic hyperglycemia causing non-enzymatic glycation of proteins. Glycation products can be oxidized, e.g. by ROS, to give advanced glycation end products (AGEs) which cause oxidative tissue damage. Both glycated and AGE-modified proteins can lead to oxidative stress [71].

In the present study, a significant increase in plasma AGEs was observed in group III (HFD) compared to control (group I), this result agreed with that of Li *et al.* [72]. They provide evidence that high fat diet induced obesity is associated with enhanced serum and visceral AGE levels. This indicated that high fat diet induced tissue damage may be associated with high fat diet induced accumulation of visceral AGEs leading to apoptosis and oxidative damage [72].

Also, a highly statistical significant increase in plasma AGEs was detected in group IV (HFD+diabetic) compared to control (group I). This result agreed with that reported by Kim *et al.* [37], Kim *et al.* [73] and Matsui *et al.* [74].

Sulfhydryl oxidation is thought to be one of the main pathological events in cataractogenesis, through disulfide cross-linking and molecular aggregation leading to protein precipitation, insolubilization and lens opacification [75, 76]. Kyselova *et al.* [77] showed that diabetic cataract is characterized by marked decline in the soluble lens protein. Hyperglycemia-induced oxidative stress is considered as the most likely cause of changes in the sulfhydryl status of lens protein during diabetic cataractogenesis [78].

Moreover, non-enzymatic glycation of lens protein has been considered as one of the major factors responsible for diabetic cataract [79]. Glycation induces

protein conformational changes inducing protein aggregation and cross-linking leading to protein insolubilization [80, 81]. Hence, the degree of glycation in the soluble protein fraction determined the intensity of cataract as shown in the SDS electrophoresis (Fig. 1).

In conclusion, this study clearly demonstrated increased accumulation of AGEs and increased lipid peroxidation products along with impaired antioxidant status in obesity and at accelerated rate in diabetes. Proper control of hyperglycemia, blocking of AGEs pathways by AGEs-inhibitors and low fat diet may be beneficial to delay diabetic cataract development.

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