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Relation of Advanced Glycation End Products and Primary Open Angle Glaucoma Progression

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Abstract

The purpose of this paper is to investigate the relation between oxidative stress and advanced glycation end products in patients suffering from different stages primary open angle glaucoma (POAG) and complications of glaucoma progression. Forty five patients suffering from POAG classified into three stages; mild, moderate and advanced as well as fifteen healthy "non- diabetic subjects" (age and sex matched healthy controls) were selected from the outpatient clinic in the Research Institute of Ophthalmology (RIO) Giza Egypt.nitric oxide (NO), malondialdehyde (MDA), ascorbic acid (vitamin C), α-tocopherol (vitamin E), catalase activity (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and advanced glycation end products (AGEs) were estimated in all studied groups. A significant increase in MDA, NO and AGEs levels was detected in mild, moderate and advanced glaucoma compared to control, also significant decreases in vitamin C, vitamin E, GSH, and SOD activities were found in mild, moderate and advanced glaucoma compared to control. No significant change was found in catalase activity in all groups compared to control. Statistical significant positive correlations were found between intra ocular pressure (IOP) and disease severity. this study clearly demonstrated increased accumulation of AGEs, lipid peroxidation products along with impairment of the antioxidant status in patients with primary open angle glaucoma.

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We suggest that AGEs measurement could be used as a diagnostic marker in primary screening programs for diagnosis and prediction of the development and progression of glaucoma.

Key words: (Primary open angle glaucoma; advanced glycation end products; intraocular pressure; visual field; glaucoma progression; oxidative stress; antioxidants).

1. Introduction

Glaucoma is the second most common cause blindness after cataracts in the world. Primary open-angle glaucoma accounts for around 70% of the total glaucoma cases worldwide. Glaucoma is a term describing a group of ocular disorders with multi-factorial etiology united by a clinically characteristic intraocular pressure-associated optic neuropathy [1].

Actis and his colleagues in [2] confirm that intraocular pressure (IOP) remains the main risk factor for glaucoma progression; age and family history are great risk factors as underlined in the last decades; female sex can be an important risk factors.

If the condition is detected early enough, it is possible to arrest the development or slow the progression with medical and surgical means. Blindness occurs as a gradual loss of visual field resulting from the death of retinal ganglion cells. The underlying mechanism for open-angle glaucoma involves degeneration of the trabecular meshwork, usually by unknown causes, that lead to aqueous backup and chronically elevated eye pressure. With prolonged high pressure, the ganglion nerves in the retina atrophy [3].

An imbalance between pro-oxidants and antioxidants, in favor of the former, results in oxidative stress. This insult, in turn, may lead to damage of a variety of macromolecules, such as proteins, lipids, sugar residues, or DNA, and thereby leads, in extreme cases, to growth arrest, growth modulation, or cell death, such as death of retinal ganglion cells in glaucoma. Oxidative stress has been proposed to play a role in the pathogenesis of glaucomatous optic neuropathy [4].

Advanced glycation end products is receiving considerable attention as a possible modulator in visual disorders including glaucoma. The present study aims to investigate the association between AGEs in POAG based on the disease severity (mild, moderate and advanced) and its progression.

2. Subjects and Methods

The study was performed on forty five patients suffering from POAG of both sexes. The study was performed between 2015 and 2017; all cases were selected among those attending the outpatient clinics of Research Institute of Ophthalmology, Giza, Egypt. A group fifteen of age and sex matched healthy subjects with no history of ophthalmologic diseases or any other health complications, served as controls. Their mean age was 25.13±1.67 years, five males and ten females. Ethical Committee approval of the Research Institute of Ophthalmology, Giza, Egypt was obtained.

Patients in this study were classified into three main groups according to visual field; Group one: mild group: MD<(-)6.00 dB include fifteen patients with a mean age 55.73±3.15 years, five males and ten females. Group two: moderate group: MD > (-) 6.00 dB < (-)12 dB, include fifteen patients with a mean age 60.13 ±1.94, seven males and eight females. Group three: advanced group: MD > (-) 12.00 dB, include fifteen patients with a mean age 64.27±2.29, six males and nine females.

2.1. Ophthalmological examinations

Ophthalmic examination was done for patients and controls. Visual acuity, slit lamp biomicroscopy using 90 D lens, gonioscopy using Goldmann three mirror contact lens, intraocular pressure measurement using Goldmann applanation tonometer and visual field by the Humphrey automated visual field analyzer 24-2 program were performed. A detailed medical history was taken for each patient. Strict criteria were used to recruit patients for this study. Patients with acute and chronic infections, malignancy, liver diseases, acute and chronic nephritis and cirrhosis were excluded from the study.

2.2. Biochemical analyses

Blood samples were withdrawn from each subject. Whole blood, serum and plasma were prepared. Whole blood was used to determine reduced glutathione (GSH) according to the method described by Beutler and his colleagues in [5]. The activity of superoxide dismutase (SOD) was determined according to the method described by the authors in [6]. Plasma catalase activity was determined using the kit obtained from Bio-diagnostic, (Egypt) according to the method described by Aebi in [7].

Serum was used to determine malondialdehyde (MDA) according to the method described in [8]. Quantitative determination of nitrite oxide in serum was carried out by a spectrophotometric method according to Moshage and his colleagues in [9]. Colorimetric technique was performed for estimation of vitamin "C" using folin phenol reagent as described by the authors in [10]. An improved micro procedure for the determination of tocopherol in serum is reported in [11].

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).

2.3. Collection of aqueous humor samples

Collection of aqueous humor was performed during cataract or glaucoma surgery. Aqueous humor 0.1-0.2 ml was aspirated at the beginning of surgery through a paracentesis using a 27 gauge needle on a tuberculin micro syringe. Blood contamination or iris touch was avoided. All aqueous humor samples were immediately stored at -70˚C till assays.

2.4. Statistical Analysis

Data were presented as mean ± standard error (SE). All values were statistically analyzed using Microsoft excel

(Version 10) and statistical package for social sciences (SPSS) software (Version 10)**.** One-way analysis of variance (ANOVA; with post-hoc LSD analysis) was used to compare the groups on continuous variables. The degree of association between the variables was assessed using Pearson's correlation coefficient (r). For all statistical tests, P <0.05 was considered as the level of significance.

3. Results

Table 1 shows that the mean values±S.E. of age in the control group was 25.13±1.673 years, in the group of patients of mild 55.73±3.152, the moderate 60.13±1.944 and in the advanced group 64.26±2.285 years, indicating very high statistical significant increase in age in all groups compared to control group. The mean values±S.E. of intraocular pressure in control, mild, moderate and advanced groups were 14.40±1.046 mmHg, 31.53±3.258 mmHg, 37.20±2.736 mmHg and 40.53±3.141 mmHg respectively (figure 1).

Table 1: The mean ± S.E. and P values of sex (M/F), age (year), and IOP mmHg in the controls, mild, moderate and advanced glaucoma groups.

Parameters	**Sex**	**Age**	**IOP**
Groups	**(M/F)**	**(year)**	(mmHg)
Control	5/10	25.13 ±1.673b	**14.40 ±1.046b**
Mild	5/10	55.73 ± 3.152a	**31.53±3.258a**
Moderate	7/8	60.13± 1.944a	**37.20±2.736a**
Advanced	6/9	64.26 ± 2.285a	**40.53±3.141a**

Groups with different letters have a statistically significant difference.

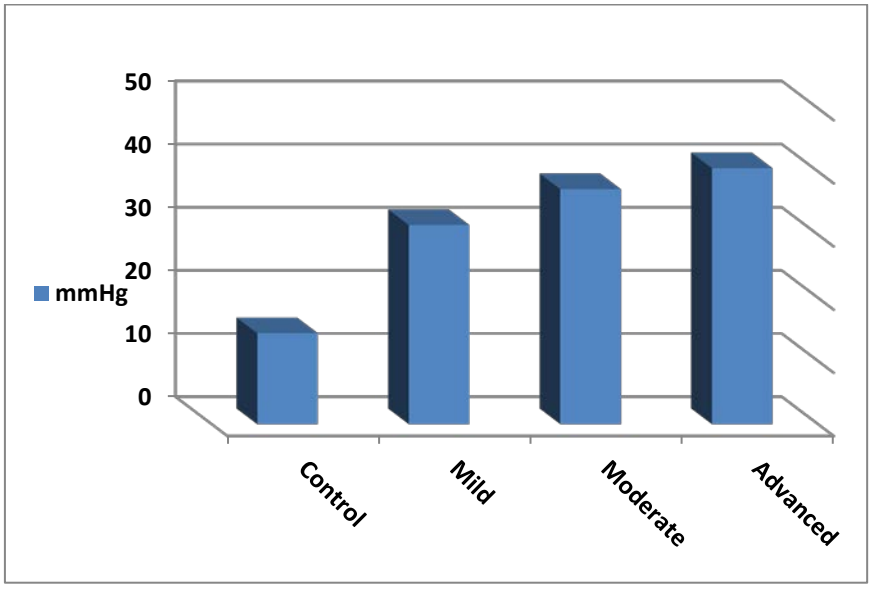

Figure1: The mean of IOP in different groups.

Table 2: Mean±S.E. and P values of MDA and NO in the control, mild, moderate and advanced glaucoma groups.

Groups	Parameters	MDA (µmol/L)	NO (µmol/L)
Control	mean ±S.E.	1.24± 0.086 b	26.27± 4.614b
Mild	mean ±S.E.	1.83 ± 0.148a	36.73 ± 4.709a,b
(n=15)	*%Change	47.6	39.8
Moderate	mean ±S.E.	1.92± 0.134a	38.53± 4.563a,b
(n=15)	*%Change	54.8	46.7
Advanced	mean ±S.E.	2.01 ± 0.097a	47.67± 5.907a
(n=15)	*%Change	62.1	81.5

Groups with different letters have a statistically significant difference.

% Change: Percentage change from normal control.

As shown in table 2, there was highly statistical significant increase in serum MDA level in the mild group (*p*-values<0.01). Also, there was very highly statistical significant increase in serum MDA level in the moderate and advanced groups compared to control group (p-values<0.001).

In addition to statistical significant increase in plasma NO level in the advanced group (*p*-values<0.05).

In table 3, there was high statistical significant decrease in serum vitamin C and E levels, GSH and SOD activities in all groups compared to control (p-values<0.001).

While, there was non-significant decreased in plasma CAT activity in all groups compared to the control group (p-values>0.05).

There was very high statistical significant increase in both blood and aqueous humor AGEs levels (table 4) in all groups compared to the control group (p-values<0.001).

Also there was statistical significant increase in blood and aqueous humor AGEs level in mild and moderate groups compared to advanced group (p-values<0.05).

Table 3: The mean ± S.E. and P values of vitamin C, vitamin E, CAT, GSH and SOD in the control, mild, moderate and advanced glaucoma groups.

Groups	Parameters	Vitamin C (mg/dl)	Vitamin E (mg/dl)	CAT (U/L)	GSH (mg dl)	SOD (U/ml)
Control (n=15)	mean± S.E.	0.62± 0.114a	1.09± 0.075a	486.92± 44.207a	17.81± 0.923a	**118.46± 24.061a**
Mild (n=15)	mean± S.E. *****%Change	0.24 ± 0.065b -61.3	0.40 ± 0.059b -63.3	442.08 ± 20.855a -9.2	13.64 ± 1.366a,b -23.4	**87.27 ± 14.528a,b** **-26.3**
Moderate (n=15)	mean± S.E. *****%Change	0.20± 0.026b -67.7	0.35± 0.042b -67.9	435.56± 41.879a -10.5	12.57± 1.215b -29.4	**43.46± 2.348b** **-63.3**
Advanced (n=15)	mean± S.E. *****%Change	0.10 ± 0.025b -83.9	0.28 ± 0.042b -74.3	381.57± 25.313a -21.6	12.35 ± 1.281b -30.7	**35.03 ± 4.657b** **-70.4**

Groups with different letters have a statistically significant difference.

% Change: Percentage change from normal control.

Table 4: The mean±S.E. and P values of aqueous humor and plasma advanced glycation end products levels (µg/ml) in the control, mild, moderate and advanced stages groups.

Parameters Groups		Aqueous Humor (AGEs) (µg/ml)	Plasma (AGEs) (µg/ml)
Control	mean±S.E.	1.57±0.135c	3.75±0.328c
Mild (n=15)	mean±S.E. *****%Change	2.69±0.194b 71.33	7.23±0.350b 92.8
Moderate (n=15)	mean±S.E. *****%Change	2.73±0.161b 73.9	7.80± 0.356b 108
Advanced (n=15)	mean±S.E. *****%Change	3.53±0.216a 124.8	12.05±0.415a 221.3

Groups with different letters have a statistically significant difference.

*% Change: Percentage change from normal control.

4. Discussion

Glaucoma is the main cause of irreversible blindness worldwide. Over the last 20 years it has been evident that AGEs modification represents a major pathogenic factor in aging and in a spectrum of human diseases such as glaucoma and diabetic complication, neuro-degeneration, and atherosclerosis.

Further evidence suggests that patients with POAG exert mitochondrial abnormalities implicating that mitochondrial dysfunction is most probably a consequence of oxidative stress [12]. Oxidative stress has been implicated to be a cause of increased intraocular pressure by triggering trabecular meshwork degeneration and thus contributing to alterations in the aqueous outflow pathway [13]. This disease is characterized by apoptosis of retinal ganglion cells (RGC) and visual field loss that seems to be related to elevated intraocular pressure. Several lines of evidences have implicated the crucial role of mitochondrial dysfunction in the pathogenesis of glaucoma. Increased mitochondrial oxidative stress in RGC may underlie or contribute to susceptibility of RGC to apoptosis [14].

The current study showed an increase in the level of intra ocular pressure in all groups with the disease progression (figure1). A positive correlation between intra ocular pressure in early glaucoma (mild stage) and intra ocular pressure in advanced glaucoma ($r=0.594$, $P<0.05$) was found. The results of this study reported a direct correlation between intra ocular and progression of glaucoma as reported in [14,15].

Malondialdehyde (MDA) is a biomarker of lipid peroxidation that is widely used, sensitive, and appropriate for use in large studies. The data in our study showed highly statistical significant increase in serum MDA level in the mild, moderate and advanced glaucoma as compared to controls since the percentage showed an increase; 47.6%, 54.8%, and 62.1% respectively compared to controls. The authors in [16] reported that destruction of the drainage system in POAG is accelerated by lipid peroxidation. These results were in agreement with the findings in [17-19]. Our results showed lipid peroxidation much more likely to be involved in the pathogenesis of primary open-angle glaucoma and decreased antioxidant defense system and increased oxidative stress might play important roles in the pathogenesis of primary open-angle glaucoma. Therefore, these findings suggested the possibility that increased oxidative stress which is associated with in primary open-angle glaucoma and support the hypothesis that oxidative damage play a role in the in the pathogenesis of glaucoma.

Nitric oxide (NO) is an important intercellular messenger in the eye. The biochemical function of nitric oxide in the eye plays a vital role in the regulation of IOP, local control of ocular blood flow, loss of retinal ganglion cells by apoptosis, decrease vascular resistance and in the regulation of ocular vessel tone [20].

In the current study, there was a significant increase in plasma nitric oxide level in advanced glaucoma compared to control. The percentage increased to 39.8%, 46.7%, 81.5% in mild, moderate and advanced glaucoma respectively compared to normal control. Our data correlated to authors in [20, 21] who found that the

levels of nitric oxide may be increased in glaucoma. These results also agreed with that reported in [18] who found serum significantly higher level of nitric oxide in patients with primary open-angle glaucoma than those of controls. Our data suggested that, nitric oxide production is associated with increased oxidative stress. It is possible that patients with advanced glaucoma may stimulate more an inflammatory reaction to induce a higher nitric oxide level than mild or moderate groups.

The major function of ascorbic acid in the aqueous humor is to protect against oxidative damage, free-radical damage and acting as a cofactor for a variety of enzymes. Vitamin C neutralizes oxygen radicals. We found a highly statistical significant decrease in serum vitamin C level in mild, moderate and advanced glaucoma as compared to control. These findings were in agreement with those reported in [22] who demonstrated a significant association between a polymorphism in the ascorbic acid transporter with lower plasma concentrations of ascorbic acid in primary open-angle glaucoma. Author in [23] reported that decrease in ascorbic acid levels in plasma and aqueous humor can compromise lysosomal degradation in the outflow pathway cells with aging and contribute to the pathogenesis of glaucoma. Interestingly, our data reported that, the severity of POAG was increased with age. Similarly, our laboratory data of vitamin C level reported diminished levels with age increased (table 3). Based on the data obtained here, it is plausible that such diminished levels of ascorbic acid with aging may be contributing to the pathogenesis of glaucoma progression. We also suggested that vitamin C act as protective action against progression of of glaucoma during the increase of oxidative stress. Vitamin C is an important free radical scavenger in the eye and very important to reduce the intra ocular pressure in glaucoma patients.

The results of vitamin E in the present study showed very highly statistical significant drop with percentage changes of -63.3%,-67.9% and -74.3% in mild, and moderate and advanced glaucoma respectively as compared to control. Moreover, the results showed a significant positive correlation between vitamin E in mild and advanced glaucoma, $r = 0.572$, $P<0.05$. Also, our data showed significant negative correlation between age and vitamin E deficiency in advanced glaucoma ($r=-0.566$, $P<0.05$). The data suggested that a relation between age, vitamin E deficiency and the glaucoma progress. Another correlation was found between IOP in moderate stage and vitamin E level in advanced glaucoma. This means that IOP and the progression of glaucoma were increased with the severity of vitamin E deficiency.

Deficiency of vitamin E in glaucoma patients was reported in [24]. The protective action of vitamin E against POAG is believed to be due to the fact that, increase in oxidative stress in glaucoma patients.

A decrease in the antioxidant capacity may be the consequence of increased oxidative processes [19].

The current study showed no significant change in the activity of plasma catalase in all stages of the studied groups compared to controls. This result coincides with those obtained by Goyal and his colleagues in [24]. In spite that the catalase activity showed no significance; there was a gradual decrease in the catalase activity according to severity of the glaucoma progression. The percentage change was recorded -9.2% in the mild and -10.5% in the moderate, and -21.6% in advanced glaucoma as compared to control. The possible explanation for this non-significant decrease in catalase activity associated with glaucoma patients was discussed by authors in

[25] who suggested that nitric oxide inhibit the activity of catalase enzyme.

Our data revealed a negative correlation with non-significant change between the levels of reduced glutathione and age in advanced glaucoma (r=-0.221 P>0.05). In the current study, there was a statistical significant decrease in erythrocyte reduced glutathione concentration in moderate and advanced glaucoma compared to controls. A correlation between the level of reduced glutathione in mild and advanced glaucoma (r=0.712, P<0.01) was found. Also, the data of the percentage change showed a decrease in the levels of reduced glutathione; -23.4%, -29.4%, and -30.7% in mild, moderate and advanced glaucoma respectively compared to control group. Park & Moon in [26] found that glaucoma may occur by oxidative damage induced by an abnormal glutathione redox state. This result agreed with that reported in [27].

The laboratory data shows that reduced glutathione concentration observed in our patients in different stages of glaucoma and continued deficient reduced glutathione lead to chronic oxidative stress and cause more pathogenesis of glaucoma in our patients.

As shown in the present study data illustrated highly statistical significant decrease in the SOD activity in moderate glaucoma as compared to control. Furthermore, there was very highly statistical significant increase in SOD activity in advanced group as compared to controls. This was in agreement with that reported in [28]. A relation between intraocular pressure in our mild glaucoma and the activity of superoxide dismutase in advanced stage was observed (r = - 0.562, P<0.05)**.** The increase in intraocular pressure leading to oxidative damage may induce trabecular meshwork degeneration in patients included in this study. On the other side, data of the percentage changes were decreased to -26.3%,-63.3% and -70.4% concerning to mild, moderate and advanced glaucoma respectively as compared to controls. The decrease in superoxide dismutase activity in patients with glaucoma could be due to abnormally high level of oxidative stress affect on the trabecular meshwork and causing damage to the cellular component and could directly affect the intra ocular pressure regulation**.**

The current data showed higher plasma and aqueous humor advanced glycation end products levels in different stages in comparison to control group. The aqueous humor advanced glycation end products levels were increased from 2.69±0.194 µg/ml in mild stage to 3.53±o.216 µg/ml in advanced stage. Also, the level of advanced glycation end products was increased in plasma from 7.23±0.35 µg/ml in mild stage to 12.05±0.415 µg/ml in advanced stage. Also results in the current study show a relation between advanced glycation end products in plasma and aqueous humor of our patients. A positive correlation found in aqueous humor in mild glaucoma and plasma in moderate glaucoma (r=0.600, P<0.05). Similarly, increase in advanced glycation end products levels according to the disease severity was found.

The results in this study showed a positive correlation between intra ocular pressure in both moderate and advanced glaucoma and the levels of advanced glycation end products in plasma of the mild stage, but there was non-significant change between them (r = 0.042, 0.062, P>0.05 respectively).

There was a significant positive correlation between advanced glycation end products in aqueous and intraocular pressure in advanced stage (r =0.541, p<0.05). Upon these facts, elevation of intraocular pressure with increased

levels of advanced glycation end products in advanced group is very important risk factors for the glaucoma progression. So the oxidative stress has been implicated to cause increased intraocular pressure by triggering trabecular meshwork degeneration and thus contributing to alterations in the aqueous outflow pathway as mentioned in [29]. Similarly, as shown in our cases severity of optic nerve damage in advanced glaucoma with primary open angle glaucoma is correlated with the elevation of intraocular pressure and changes in the trabecular meshwork. High level of intraocular pressure cause recurrent reperfusion which leads to a chronic oxidative stress. These results agreed with the reports of Tezel in [30] who suggested that advanced glycation end products may contribute to the pathogenesis of optic neuropathy based on the established pivotal role of oxidative stress in glaucomatous degeneration and found a connection between advanced glycation end products and glaucoma.

5. Conclusion

We can concluded that catalase activity, reduced glutathione and superoxide dismutase act as defense enzymes to the oxidative stress in glaucoma and the increase in antioxidant activity can be the rule within the initial steps of the oxidation process. Plasma and aqueous humor levels of advanced glycation end products have been shown to be a biomarker for the severity of glaucoma independent from other well known risk factors such as elevation in IOP, MDA and NO. AGEs may be cause degeneration of trabular meshwork and lead to aqueous back up. Chronically elevated in the IOP and retinal atrophy may be induced, and also vision may be gradually lost. Advanced glycation end products act as predictors of the severity. Finally, oxidative stress is associated with the progression of primary open angle glaucoma

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Conflict of Interest

There are no conflicts of interest.

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