

Association of total antioxidants level with glaucoma type and severity

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ABSTRACT

الأهداف: هدفت الدراسة إلى مقارنة متوسط مجموع مضادات الأكسدة في ثلاثة أنواع مختلفة من داء الزرق هي: زرق التقشير الكاذب، الزرق الأولي مفتوح الزاوية إضافة إلى الزرق الأولي مغلق الزاوية. كما هدفت الدراسة إلى بحث الارتباط المحتمل بين مستوى مجموع مضادات الأكسدة ومختلف الدلائل السريرية لداء الزرق.

الطريقة: في هذه الدراسة تم الحصول على عينات البلازما المستخدمة خلال الفترة بين سبتمبر 2013 م و أكتوبر 2014 م في مستشفى الملك عبد العزيز الجامعي بالرياض. كما تم إدراج عدد 54 حالة من مرضى الزرق التقشيري، 147 حالة من مرضى الزرق مفتوح الزاوية، إضافة إلى 139 حالة من مرضى الزرق مغلق الزاوية. كما تم إدراج عدد 351 حالة في المجموعة الضابطة والتي روعي فيها أن تكون خالية تماماً من داء الزرق ومطابقة لعينة المرضى من حيث الفئة العمرية، النوع الاجتماعي، العرق، إضافة إلى 5 أمراض أخرى من الأمراض الجهازية. تم قياس مستوى مجموع مضادات الأكسدة في جميع حالات المجموعتين باستخدام أسلوب القياس اللوني. كما تم مقارنة مستويات مجموع مضادات الأكسدة بين المجموعتين.

النتائج: وجدت الدراسة أن متوسط مجموع مضادات الأكسدة في مجموعة المرضى 0.77 ± 0.32 أقل منه في المجموعة الضابطة 1.1 ± 0.22 ، حيث كان هذا الانخفاض معنوياً $p < 0.0001$. كما وجدت الدراسة أن متوسط مجموع مضادات الأكسدة كان مختلفاً بشكل معنوي بين أنواع الزرق المختلفة، حيث كان 0.86 ± 0.24 و 0.47 ± 0.32 و 0.98 ± 0.41 في كل من: داء الزرق التقشيري، داء الزرق مفتوح الزاوية و داء الزرق مغلق الزاوية على الترتيب ($p < 0.0001$). وبالإضافة إلى ذلك فقد وجدت الدراسة أن هناك ارتباط معنوي بين مستوى مجموع مضادات الأكسدة وكل من: العمر عند حدوث الإصابة ($R: 0.17, p < 0.0001$)، نسبة الكأس للقرص ($R: -0.13, p = 0.004$)، إضافة إلى عدد الأدوية المضادة للزرق المستخدمة في العلاج ($R: -0.16, p = 0.001$).

الخلاصة: خلصت نتائج هذه الدراسة إلى تقديم أدلة واضحة على أن مستويات مجموع مضادات الأكسدة يكون منخفضاً بين مرضى الزرق عنه في المعافين من المرض. كما أنه يكون أقل انخفاضاً في مرضى الزرق التقشيري ومفتوح الزاوية عنه في الزرق مغلق الزاوية. كما دعمت الدراسة الفرضية القائلة بأن انخفاض دفاع مضادات الأكسدة و/أو زيادة معدلات الأكسدة قد يلعب دوراً حاسماً في الإصابة بالزرق وتطوراته المرض.

Objectives: To compare the mean total antioxidant status (TAS) among 3 glaucoma types, namely: pseudoexfoliation glaucoma (PEG), primary open angle glaucoma (POAG), and primary angle closure glaucoma (PACG), and study its potential association with various clinical glaucoma-parameters.

Methods: In this case-control study, plasma samples were obtained between September 2013 and October 2014 from 340 glaucoma patients (PEG [n=54]; POAG [n=147]; PACG [n=139]), and 351 controls of matching age, gender, ethnicity, and 5 different systemic comorbidities from King Abdulaziz University Hospital, Riyadh, Saudi Arabia. The TAS in all samples was determined by a colorimetric-based assay.

Results: The mean±standard deviation of TAS was significantly lower among cases: 0.77 ± 0.32 than controls: 1.1 ± 0.22 , $p < 0.0001$. Moreover, the TAS levels were significantly different across the 3 types of glaucoma: 0.86 ± 0.24 in PEG, 0.47 ± 0.32 in POAG, and 0.98 ± 0.41 in PACG (all $p < 0.0001$). In addition, there was a significant correlation between TAS and age at onset (Pearson correlation coefficient [R] 0.17, $p < 0.0001$), cup/disc ratio (R: -0.13, $p = 0.004$), and number of anti-glaucoma medications (R: -0.16, $p = 0.001$).

Conclusion: Our findings provide evidence that plasma TAS levels are decreased in patients with glaucoma, more so in POAG and PEG than PACG, supporting the hypothesis that decreased antioxidative defense and/or increased oxidative stress may have a critical role in the pathogenesis of glaucoma.

Saudi Med J 2015; Vol. 36 (6): 671-677
doi: 10.15537/smj.2015.6.10697

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Received 5th November 2014. Accepted 5th April 2015.

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Glaucoma is a progressive optic neuropathy associated with optic nerve damage, and is one of the most leading cause of blindness worldwide.¹ Elevated intraocular pressure (IOP) as a result of reduction in normal aqueous outflow is a major causal risk factor that is well supported by animal studies.²⁻⁴ Although IOP is considered a major risk factor for glaucoma,^{2,3} other concomitant factors affecting the pathophysiology of glaucomatous retinal ganglion cell (RGC) death include retinal ischemia,⁵ nutritional status,⁶ and oxidative stress.⁷ There is evidence of oxidative damage in ocular diseases, such as cataract and age-related macular degeneration.⁸ In addition, significant oxidative damage has been demonstrated in human trabecular meshwork (TM) cells of patients with glaucoma,⁷ causing elevated IOP and visual field damage.⁹ Furthermore, our previous studies have documented mitochondrial abnormalities¹⁰⁻¹² (oxidative stress marker), and glutathione-S-transferase (antioxidant) gene (GST) polymorphisms to be associated with various types of glaucoma.¹³ It is clearly evident from the literature, and our own studies, that oxidative stress mechanisms play a critical role in the pathogenesis of glaucoma. Previous studies had demonstrated reduced total antioxidant capacity in aqueous humor and blood samples from patients with glaucoma.¹⁴⁻¹⁷ To evaluate the role of oxidative stress in different types of glaucoma we had previously investigated total antioxidants status (TAS) in the plasma of pseudoexfoliation glaucoma (PEG) patients,¹⁸ primary angle closure glaucoma (PACG) patients,¹⁹ and in the plasma of primary open angle glaucoma (POAG) patients.²⁰ As an extension to these studies, here, we compare the mean TAS level among these 3 glaucoma types, and study the potential association between the TAS level and various clinical parameters important to each type of glaucoma.¹⁸⁻²⁰

Methods. Search method. PubMed and Google search engines were used to locate research work published in this area. Keywords such as 'total antioxidants', 'oxidative stress', 'glaucoma' and their combinations were used for searching the literature.

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company. This study was supported by the Deanship of Scientific Research, Glaucoma Research Chair, Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Study population. This case-control cross-sectional study was conducted between September 2013 and October 2014 at King Abdulaziz University Hospital, King Saud University, Riyadh, Saudi Arabia. The study adhered to the tenets of the Declaration of Helsinki, and was approved by our institutional review board committee. Informed consents were obtained from all the participants. We recruited 340 Saudi glaucoma patients (54 with PEG, 147 with POAG, and 139 with PACG). Inclusion and exclusion criteria for each type of glaucoma have been detailed previously.¹⁸⁻²⁰ In our study, the team included 2 glaucoma consultants who carried out the measurements and reviewed the collected data. For this purpose, a standardized technique was meticulously followed. In our facility, in general, we adopt the European glaucoma society guidelines,²¹ where all assessment procedures are standardized. Information on all risk factors (smoking, diabetes mellitus [DM], hypertension, coronary artery disease [CAD] and hypercholesterolemia) was procured through their medical records. An ethnically-matched healthy control group (n=351) included individuals of age >40 years with normal IOP, open angles on gonioscopy, normal optic nerves, and free from glaucoma as described elsewhere.¹⁸ All our patients and controls declared that they were not taking any antioxidant supplements at the time when the blood was collected.

Plasma preparation and storage. Ethylene-diamine-tetraacetic acid (EDTA) blood samples were used to collect the plasma layer after centrifugation at 5,500 xg for 5 min. The plasma samples were stored at -80°C until use for TAS assay.

Plasma total antioxidant status. A colorimetric-based assay from Randox (Randox Laboratories Ltd, Antrim, UK) was used to estimate the plasma TAS levels. The method is based on detection of a reduction in the generation of the ABTS^{•+} (2, 2'-Azinobis-di [3-ethylbenzthiazoline sulphonate]) radical cations by plasma antioxidants, which is proportional to the total antioxidant concentration. The assay was performed in duplicate on an automated biochemical analyzer, ChemWell-T (Awareness Technology Inc., Palm City, FL, USA), as per the manufacturer's instructions (Randox Laboratories, Antrim, UK) and has been detailed elsewhere.¹⁸ The plasma TAS levels were expressed in mmol/L.

Statistical methods. Data were retrieved and recorded in a specific hard copy of the study form. Data were then reviewed and stored into a specifically designed database using Microsoft Access 2010[®] software (Microsoft Corporation, Redmond, WA, USA). Data

management was then performed after a process of data cleaning. Data coding and analysis was carried out using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA), and StatsDirect® statistical software, version 2.7.2 (StatsDirect Ltd., Cheshire, UK). Descriptive analysis was primarily carried out, where categorical variables were presented in the form of frequencies and percentages and continuous variables in the form of mean \pm standard deviation (SD) and range (minimum - maximum). Alternatively, Chi-squared test was conducted to detect the association between types of glaucoma and different systemic comorbidities (Fisher's exact test, when indicated). Inferential analysis was carried out in forms of comparison of means using Student's t-test, and analysis of variance (ANOVA) while comparing different glaucoma groups. A consecutive post-hoc analysis was conducted to variables that showed significant differences in the ANOVA test to identify the source of a cross-groups variation. Test of correlation was carried out using where Pearson

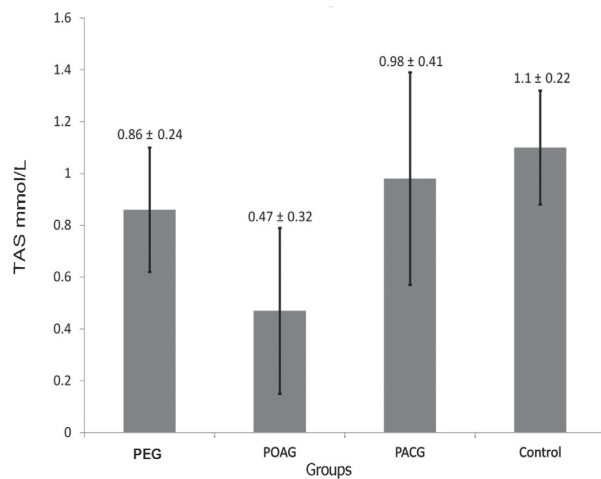


Figure 1 - Mean \pm standard deviation of total antioxidant concentration for different types of glaucoma. PEG - pseudoexfoliation glaucoma, POAG - primary open angle glaucoma, PACG - primary angle closure glaucoma, TAS - total antioxidant status

correlation coefficient (R) and the corresponding p -value were calculated, while the Spearman's test was alternatively used whenever indicated. To detect an adjusted effect size of different study variables on the TAS concentration, linear regression analysis was finally carried out including all potentially associated factors. A 95% confidence interval (CI) was calculated and $p < 0.05$ was considered statistically significant.

Results. In the current study, we recruited a total of 691 subjects. These included 340 (49.2%) cases with a confirmed diagnosis of various types of glaucoma, and 351 (50.8%) healthy individuals free from glaucoma that served as controls. Overall, the mean \pm SD TAS level was found to be significantly lower among cases as compared with controls (0.77 ± 0.32 versus 1.1 ± 0.22 ; $p < 0.0001$, 95% CI: -0.291 - -0.175). Moreover, the levels were also found to be significantly different across the 3 glaucoma types as shown in Figure 1. The demographic details of the subjects included in the study is shown in Table 1. Table 2 shows the distribution of different systemic co-morbidities, glaucoma related indices, and glaucoma specific indices including TAS levels among the 3 groups of glaucoma cases. Glaucoma awareness and age at onset were found to be significantly distributed ($p < 0.0001$) among the cases. Similarly, except for cup/disc ratio other glaucoma specific indices such as IOP ($p = 0.005$), number of anti-glaucoma medications ($p < 0.0001$), Logarithm of the minimum angle of resolution (LogMAR) visual acuity ($p = 0.034$), and TAS ($p < 0.0001$) showed significant distribution. In addition, significant distribution was noted for diabetes mellitus ($p = 0.003$) and hypertension ($p = 0.022$) among cases. However, no significant difference was seen for any of the 5 different systemic co-morbidities when total cases were compared with controls (Table 3). To identify the source of variations between groups showing significant distributions, post hoc analysis

Table 1 - Demographic characteristics of subjects included in an ophthalmology study in Saudi Arabia.

Demographic characteristics	Type of glaucoma			Control n=351	Total cases n=340
	PEG n=54	POAG n=147	PACG n=139		
<i>Age</i>					
Mean \pm standard deviation	67.6 \pm 10.4	66.8 \pm 17.9	63.1 \pm 9.6	61.8 \pm 11.7	65.8 \pm 12.6
Range, min - max	40 - 83	40 - 112	40 - 87	40 - 93	40 - 112
<i>Gender, n (%)</i>					
Male	37 (68.5)	84 (57.1)	85 (61.2)	218 (62.1)	206 (70.8)
Female	17 (31.5)	63 (42.9)	54 (38.8)	133 (37.9)	85 (29.2)
PEG - pseudoexfoliation glaucoma, POAG - primary open angle glaucoma, PACG - primary angle closure glaucoma					

Table 2 - Clinical and systemic diseases at presentation of subjects included in an ophthalmology study in Saudi Arabia.

Clinical index	PEG n=54	POAG n=147	PACG n=139	Total cases n=340	P-value across groups
<i>Systemic diseases, n (%)</i>					
Diabetes mellitus	14 (25.9)	65 (44.2)	37 (26.6)	116 (34.1)	0.003
Hypertension	14 (25.9)	58 (39.5)	35 (25.2)	107 (31.5)	0.022
Hypercholesterolemia	1 (1.9)	3 (2.0)	2 (1.4)	6 (1.8)	0.988
Coronary artery disease	0 (0.0)	1 (0.7)	1 (0.7)	2 (0.6)	0.998
Smoking	1 (1.9)	2 (1.4)	3 (2.2)	6 (1.8)	0.869
<i>Glaucoma related indices</i>					
Family history of glaucoma	3 (5.6)	16 (10.9)	12 (8.6)	31 (9.1)	0.533
Consanguinity	1 (1.9)	5 (3.4)	6 (4.3)	12 (3.5)	0.792
Awareness of having glaucoma	1 (1.9)	47 (32.0)	17 (12.2)	65 (19.1)	<0.0001
Age at onset, years*	66.4 ± 10.8	58.2 ± 13.9	62.6 ± 9.5	62.4 ± 11.4	<0.0001
Duration of disease, months*	35.4 ± 37.6	49.2 ± 70	47.1 ± 42.9	43.9 ± 50.2	0.667
<i>Glaucoma specific indices*</i>					
Intraocular pressure, mm Hg	21.75 ± 8.41	19.83 ± 8.25	18.24 ± 7.48	19.94 ± 8.05	0.005
Number of anti-glaucoma medications	1.98 ± 0.95	2.07 ± 0.99	1.55 ± 1.17	1.87 ± 1.04	<0.0001
LogMAR visual acuity	0.34 ± 0.32	0.59 ± 0.77	0.57 ± 0.74	0.50 ± 0.61	0.034
Cup/disc ratio	0.73 ± 0.22	0.72 ± 0.22	0.68 ± 0.23	0.71 ± 0.22	0.166
Total antioxidant level	0.86 ± 0.24	0.47 ± 0.32	0.98 ± 0.41	0.77 ± 0.32	<0.0001

*Values are expressed as mean ± standard deviation. PEG - pseudoexfoliation glaucoma, POAG - primary open angle glaucoma, PACG - primary angle closure glaucoma, LogMAR - Logarithm of the minimum angle of resolution

Table 3 - Comparing cases to controls in terms of systemic co-morbidity of subjects included in an ophthalmology study in Saudi Arabia.

Variable	Case n=340	Control n=351	P-value
	n (%)		
Diabetes mellitus	116 (34.1)	126 (35.9)	0.677
Hypertension	107 (31.5)	100 (28.5)	0.437
Hypercholesterolemia	6 (1.8)	9 (2.6)	0.648
Coronary artery disease	2 (0.6)	6 (1.7)	0.319
Smoking	6 (1.8)	9 (2.6)	0.648

was performed, and the results are indicated in Table 4. Importantly, post hoc analysis for TAS revealed that the source of variation was reciprocal among all different glaucoma categories (PEG versus POAG, $p < 0.0001$; PEG versus PACG, $p = 0.003$ and POAG versus PACG, $p < 0.0001$). Overall, correlation testing showed that there was significant correlation between TAS and age at onset (R: 0.17, $p < 0.0001$), cup/disc ratio (R: -0.13, $p = 0.004$), and number of anti-glaucoma medications (R: -0.16, $p = 0.001$) (Table 5). As indicated in Table 6, linear regression analysis showed that TAS levels were significantly associated with age ($p = 0.020$), diabetes ($p = 0.001$), hypertension ($p = 0.024$), awareness of having glaucoma ($p = 0.001$), type of glaucoma ($p = 0.032$), and cup/disc ratio ($p = 0.001$).

Table 4 - Post hoc analysis for pairwise mean differences among different glaucoma groups.

Variable	P-value
<i>Age at onset</i>	
PEG versus POAG	<0.0001
PEG versus PACG	0.146
<i>Intraocular pressure</i>	
PEG versus POAG	0.239
PEG versus PACG	0.007
POAG versus PACG	0.066
<i>Number of anti-glaucoma medications</i>	
PEG versus POAG	0.586
PEG versus PACG	0.009
POAG versus PACG	<0.0001
<i>LogMAR visual acuity</i>	
PEG versus POAG	0.029
PEG versus PACG	0.066
POAG versus PACG	0.988
<i>Cup/disc ratio</i>	
PEG versus POAG	0.988
PEG versus PACG	0.535
POAG versus PACG	0.247
<i>Total antioxidant level</i>	
PEG versus POAG	<0.0001
PEG versus PACG	0.002
POAG versus PACG	<0.0001

PEG - pseudoexfoliation glaucoma, POAG - primary open angle glaucoma, PACG - primary angle closure glaucoma

Table 5 - Correlation between total antioxidant concentration and different glaucoma indices among subjects included in an ophthalmology study in Saudi Arabia.

Variable	PEG		POAG		PACG		Total Cases	
	R	P-value	R	P-value	R	P-value	R	P-value
Age at onset	-0.25	0.039	0.08	0.198	0.13	0.031	0.17	<0.0001
Duration of disease	0.44	0.010	0.02	0.793	-0.21	0.268	0.043	0.543
Intraocular pressure	0.32	0.011	0.03	0.966	-0.14	0.041	-0.036	0.414
Number of anti-glaucoma medications	0.27	0.060	-0.11	0.142	0.09	0.221	-0.16	0.001
LogMAR visual acuity	0.03	0.806	-0.05	0.398	0.08	0.183	-0.016	0.695
Cup/disc ratio	0.18	0.166	-0.23	<0.0001	-0.04	0.585	-0.13	0.004

R - Pearson correlation coefficient, PEG - pseudoexfoliation glaucoma, POAG - primary open angle glaucoma, PACG - primary angle closure glaucoma, LogMAR - Logarithm of the minimum angle of resolution

Table 6 - Adjusted estimated effect size using multiple regression analysis of subjects included in an ophthalmology study in Saudi Arabia.

Variable	Adjusted effect size (β)	Standard error of estimate	P-value	95% CI, lower limit - upper limit
Constant	0.26	0.27	0.325	-0.264 - 0.0792
Age	0.18	0.00	0.020	0.001 - 0.010
Gender	-0.06	0.05	0.425	-0.150 - 0.064
Family history of glaucoma	-0.13	0.11	0.225	-0.346 - 0.082
Consanguinity	0.06	0.14	0.562	-0.195 - 0.357
Diabetes mellitus	-0.28	0.06	0.001	-0.323 - -0.357
Smoking	0.02	0.35	0.804	-0.602 - 0.776
Hypertension	0.19	0.06	0.024	0.019 - 0.261
Hypercholesterolemia	0.14	0.25	0.062	-0.024 - 0.959
Awareness of having glaucoma	0.31	0.06	0.001	0.098 - 0.350
Duration of disease	-0.12	0.00	0.181	-0.002 - 0.0
Type of glaucoma	0.16	0.06	0.032	0.11 - 0.235
Intraocular pressure	0.01	0.00	0.890	-0.007 - 0.008
Number of anti-glaucoma medications	0.12	0.03	0.138	-0.014 - 0.101
LogMAR visual acuity	-0.08	0.06	0.315	-0.167 - 0.054
Cup/disc ratio	-0.26	0.13	0.001	-0.697 - -0.178

CI - confidence interval, LogMAR - Logarithm of the minimum angle of resolution

Discussion. We obtained plasma samples from 340 cases of extensively diagnosed glaucoma patients and 351 healthy controls. Our control group was carefully selected in terms of age, gender, and ethnicity. We established that they were free of glaucoma by extensive clinical examination. Age and gender are known to affect TAS levels independent of glaucoma status; therefore, age-and-gender matched control groups were selected for this study.²² In addition, as indicated in Table 3 there was no difference in the distribution of 5 different systemic co-morbidity (DM, hypertension, hypercholesterolemia, CAD, and smoking) between patients and controls thus eliminating any other factors which may influence the TAS level and allowing us to compare TAS level as independent risk factor for glaucoma. There is strong evidence of oxidative mechanisms in POAG,¹⁰ and PEG.²³ However, optic nerve injury in PACG is mainly considered to be caused

by increased IOP as a result of anatomic changes in the anterior,²⁴ and posterior globe.^{25,26} The role of oxidative stress in PACG has perhaps therefore received limited attention, despite the fact that PACG affects 3.9 million people worldwide.¹ We previously investigated the TAS level in a PACG group of patients.¹⁹ Among the 340 glaucoma cases we had 54 PEG cases, 147 POAG, and 139 PACG cases. When every group of glaucoma was compared to controls independently, we detected the following: i) TAS levels among PEG were decreased in relation to controls;¹⁸ ii) TAS level was also decreased in POAG patients versus controls, and TAS level was associated with increased cup-to-disc ratio;^{20,27} and iii) TAS level was almost similar in PACG patients versus controls; however, there was an inverse correlation of TAS level with IOP.^{19,28} From these results, it is clear that decreased TAS level plays a bigger role in PEG and POAG compared with PACG. This may indicate that

oxidative stress represented indirectly by the TAS levels; play a bigger role in cases of POAG and PEG than for PACG.

Our current observation that oxidative stress may play a minor role in PACG development compared to other types of glaucoma is well supported by our previous findings in PACG.²⁹ It is also possible that decreased levels of TAS are more linked to particular clinical indices important for PACG rather than the disease per se. Collectively, the mean total antioxidant concentration was significantly lower among cases compared with controls. This fact confirms our previous findings in the Saudi population,¹⁸⁻²⁰ and agrees with the published literature. Gherghel et al³⁰ reported decreased levels of plasma glutathione in patients with glaucoma suggesting compromised antioxidative defense. Erdurmus et al¹⁵ reported decreased serum antioxidant capacity and increased blood oxidative stress in patients with POAG and PEG. Zanon-Moreno et al³¹ reported a significant increase in oxidative status measured as malondialdehyde-thiobarbituric acid reactive substances (MDA-TBARS), and decreased total antioxidant activity in the aqueous humor of glaucomatous eyes than the cataract eyes. In addition; however, our current study highlights the association between type and severity of glaucoma and the mean TAS level. Our findings suggest that not only the mean TAS level may be decreased due to glaucoma, but it is also more decreased in open angle than in closed angle or pseudoexfoliation types, enforcing our earlier investigations that oxidative stress may play a minor role in PACG development compared with other types of glaucoma.²⁹ Oxidative stress in the anterior chamber of glaucomatous eyes can be an ocular manifestation as a result of systemic insult. A study by Sorkhabi et al¹⁶ provided evidence for increased oxidative damage and decreased TAS in serum and aqueous humor of glaucoma patients suggesting that in glaucoma the increased oxidative burden in the anterior chamber of glaucomatous eyes may be due more to a faulty antioxidative defense and increased oxidative stress than to a systemic insult, compromising the trabecular meshwork and its functioning. Hence, we speculate that decreased levels of TAS, as a result of increased oxidative stress in the glaucomatous eyes, may at least in part play a role in occurrence and progression of POAG and PEG.

We also observed that the more reduced mean TAS level, the more acute or severe the glaucoma type. Additionally, the inheritance factors are playing a major role in this causative pathway, as our findings show that the prevalence of consanguinity and a family history

of glaucoma are much more among both POAG and PACG groups than the PEG group. Although most of our recruited patients are usually set on anti-glaucoma medications, there was a statistically significant difference across the 3 subgroups of cases in terms of both mean IOP and number of glaucoma medications levels. It is well established that chronic glaucoma patients suffer an increasing pattern of IOP until reaching the maximum tolerated level of medication and are indicated for surgical intervention. This fact was also true for the visual acuity level. Furthermore, our findings suggest that the mean TAS level may be used as an indicator of the severity of glaucoma, and possibly on the duration and compliance to treatment among chronic cases. Nevertheless, this area requires more investigation and recruitment of a large cohort of patients in a longitudinal multi-centric study to provide concrete evidence.

This study has its own limitations. There is a great debate in the literature regarding the role of cataract in glaucoma surgery. Visual acuity assessment as a glaucoma severity index used in our study could therefore be affected by other diseases common in these patients including cataract. However, among our subjects we did not have enough cases to compare vision with and without combined cataract. Reviewing our data, we found only 2 cases with combined cataract, where visual acuity was better than 20/40; so, it was not worth controlling for them. In addition, our current study used age at first diagnosis and self reported age at onset as a surrogate to the actual age at onset. The reason for that was to roughly estimate the duration of having glaucoma. We understand that this measurement would face a lot of potential bias. However, we think that in terms of age, it would be much more appropriate to use "age at onset" than using the "current patient age" where this approach is expected to minimize the estimation error. Although significant correlation has been reported between serum and aqueous levels of TAS,¹⁶ it should be noted that the results reflect the antioxidant status in the plasma samples and not aqueous fluid and hence require cautious interpretations. The study is purely descriptive in nature and provides no evidence of mechanistic conclusions. In a perfect scenario an ideal study group would include glaucoma patients without anti-glaucoma medications; however, these cannot be included for ethical reasons. Our findings do not necessarily indicate that low levels of TAS is a primary cause of glaucoma, but suggest future avenues for research.

In conclusion, our findings provide evidence that plasma TAS levels are decreased in patients with

glaucoma, more so in open-angle and pseudoexfoliation than angle-closure glaucoma. The study adds to the growing body of evidence supporting the hypothesis that decreased antioxidative defense and/or increased oxidative stress may have a critical role in the pathogenesis of glaucoma. As far as we know, this study is unique in its approach, and may open the gate for more investigations in the area of antioxidant research in glaucoma that would yield a more in-depth understanding of the association between pathogenic mechanisms of the disease and antioxidants level, and provide a new therapeutic approach to control the increasing prevalence of blinding glaucoma.

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