Biochemical changes in Oxidative stress in the Patient with primary open angle glaucoma with Diabetes mellitus

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Abstract

Background: Primary glaucoma is a group of diseases characterised by abnormally high intraocular pressure (IOP), visual dysfunction, and optic nerve head cupping, and it is one of the leading causes of irreversible blindness worldwide. Oxidative stress appears to play a role in the progressive neuronal death seen in glaucomatous optic nerve damage.

Aim: To estimate biochemical study of antioxidant enzymes in the Patient with POAG with Diabetes mellitus and without POAG Diabetes mellitus as compared to control.

Methods: The present study was carried out in the Department of Biochemistry and Central Investigation Laboratory in collaboration with the Department of Ophthalmology. 100 diabetic patient Primary open-angle glaucoma patients, 100 diabetic without Primary open-angle glaucoma of both genders attending the Ophthalmology ward of the Hospital for the last one year have been included in this study as compared to 100 healthy control groups.

Results: The results showed a mean values of FBS, HbA1c were significantly increased ($P<0.01$) in Diabetes mellitus Patients Primary open-angle glaucoma, diabetes without Primary open-angle glaucoma cases as compared to normal healthy individuals. significantly decreased levels of antioxidant enzymes activity (SOD, GR, GPx) levels in Diabetes mellitus with Primary open angle glaucoma, Diabetes without (POAG) as compared to the controls groups and significantly increased levels of MDA, Protein Corbonyl levels in Diabetes mellitus with Primary open angle glaucoma, Diabetes without (POAG) as compared to the controls groups ($p<0.01$).

Conclusion: The alteration of various antioxidant enzymes, and after this observation, we can suggest that early antioxidant enzyme investigation is a must in POAG diabetes cases. It is better to investigate antioxidant enzymes for better diagnosis and prognosis of the POAG in diabetes patients.

Keywords: FBS, HbA1c, antioxidant enzymes (MDA, SOD, CAT, GPx, Protein carbonyl) Primary open-angle glaucoma, diabetes mellitus.

Introduction

Primary open-angle glaucoma (POAG) is a chronic, age-related disease that is the leading cause of irreversible visual disability. Glaucoma is a leading cause of irreversible blindness worldwide, characterized by progressive "glaucomatous" optic neuropathy and corresponding visual field loss (1).

Primary glaucoma is a group of diseases characterized by abnormally high intraocular pressure (IOP), visual dysfunction, and optic nerve head cupping, and it is one of the leading causes of irreversible blindness worldwide (2).

Primary open-angle glaucoma (POAG) is defined as a multifactorial optic neuropathy characterized by acquired optic nerve atrophy and loss of retinal ganglion cells and their axons, as well as characteristic visual field abnormalities in the presence of open anterior chamber angles. "Diabetes mellitus occurs more frequently in patients with primary open-angle glaucoma than in the non-glaucomatous population," Backer stated in 1971. Similarly, diabetics are more likely to develop glaucoma than non-diabetics." The diabetic population appears to have a higher prevalence of chronic open-angle glaucoma (COAG) (3).

Oxidative stress appears to play a role in the progressive neuronal death seen in glaucomatous optic nerve damage. (4,5). In general, oxidative stress is caused by the formation of multiple reactive oxygen species, such as hydrogen peroxide and superoxide, which can initiate and propagate free radicals (6).

It is believed that DM and POAG have a direct relationship. Several hypotheses about the relationship between DM and POAG have been proposed. Long-term hyperglycemia combined with dyslipidemia may increase the risk of neuronal damage from oxidative stress (7).

Materials and Methods

The present study was carried out in the Department of Biochemistry and Central Investigation Laboratory in collaboration with the Department of Ophthalmology. The study was approved by the Institutional Ethical and Research Committee to use human subjects in the research study. Informed consent was taken from patient and control subjects. 100 diabetic patients with primary open-angle glaucoma patients and 100 diabetics without Primary open-angle glaucoma of both genders attending the Ophthalmology ward of the Hospital for the last year have been included in this study as compared to 100 healthy control groups.

The exception criteria included Patients with cataracts, ocular infection, Taking medication like steroids and having systemic diseases such as diabetes mellitus and hypertension, having the habit of alcohol, congenital glaucoma, and evidence of renal or hepatic diseases were excluded from this study.

Estimation of Serum Fasting blood glucose, Serum Post Prandial blood glucose was measured by Method: Glucose Oxidase and Peroxidase (GOD – POD) Method (8).

Estimation of Glycated Hemoglobin, Method: Nephelometry Method by Latex Enhanced Immuno-turbidimetric method (9).

Malondialdehyde (MDA) activity, Method: The MDA Activity in RBC lysate was estimated by the method of Placer and Cushman (1966) Method (10).

Superoxide Dismutase (SOD) Activity, Method: The SOD Activity in RBC lysate was estimated by the method of McCord and Fridovich, 1969 (11).

Glutathione Reductase Activity, Method: The GR Activity in RBC lysate was estimated by the method of Hazelton and Lang; 1995 (12).

Glutathione Peroxidase (GPx) Activity, Method: GPx activity in RBC lysate was measured by the method of Pagila and Valentine (1967) (13).

Principle: During the conversion of hydroperoxide radical into non-reactive hydroperoxides, Glutathione Peroxidase utilizes reduced Glutathione as a cofactor. In this pathway the amount of GSH utilized is a measure of enzyme activity, GSH is converted into oxidized glutathione (GSSG).

Protein carbonyls Protein oxidation was evaluated by measuring protein carbonyl content in plasma proteins according to the method of Levine et al., (1990) (14) using DNPH.

About 3-5 ml of venous blood was collected in a vacutainer using a sterile needle, from the antecubital vein. It was allowed to clot for a few minutes and was subjected to centrifugation for 10 minutes at 3000 rpm to separate the serum and kept at -20°C until analysis was carried out. By this sample estimation of serum fasting blood glucose, serum postprandial blood glucose, and antioxidant enzyme (SOD, CAT, and GPx) were done by the following methods.

Statistical analysis: Data were compiled and analyzed using by t-tests (student t-test) software package. It was expressed as mean ± S.D. (standard deviation).

Result

This study was conducted as per the guidelines of the Institutional Ethics Committee. A total 300 subjects were studied of which 100 POAG with DM, 100 DM without POAG as compared to 100 normal healthy individuals were chosen as a control group.

Table 1: Age (years) distribution in study participants:

Subjects	Mean $\pm$ SD Age years	P Value
POAG with DM	65.45 $\pm$ 8.44	$p< 0.01$
DM without POAG	59.12 $\pm$ 8.56	$p< 0.01$
Controls	49.04 $\pm$ 9.30	$p< 0.01$

Table 2: Gender wise distribution of participants:

Subjects	Male		Female	
	No.	%	No.	%
DM without POAG	64	64 %	36	36 %
POAG with DM	60	60 %	40	40 %
Controls	63	63 %	37	37 %

Table No. 3: Age and sex-wise distribution.

Age in years	Diabetes with POAG		Diabetes without POAG		Control Subjects	
	Male	Female	Male	Female	Male	Female
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
30-40	4	2	8	3	11	7
41-50	16	8	16	12	15	10
51-60	22	13	20	16	28	16
61-70	19	11	12	7	9	4
71 & above	3	2	4	2	0	0
Total	**64**	**36**	**60**	**40**	**63**	**37**

Table 4: The Mean value of blood glucose in Diabetes Mellitus with POAG, Diabetes Mellitus without (POAG), and Control POAG.

Parameter	Diabetes with POAG (n=100)	Diabetes without POAG (n=100)	Control Subjects (n=100)	P –Value
	Mean ± SD	Mean ± SD	Mean ± SD	
Fasting Blood Glucose (mg/dl)	161.38 ± 22.72	149.25 ± 22.16	92.76 ± 7.81	p< 0.01
HbA1c (%)	6.63 ± 0.88	6.30 ± 0.58	5.28 ± 0.58	p< 0.01

Table 5: Assessment of Oxidative Stress parameter in DM with POAG, DM without POAG and Healthy Controls.

Parameter	DM with POAG	DM without POAG	Healthy Control	P- value
MDA (µmol/l)	4.83 ± 0.53	2.77 ± 0.19	1.01 ± 0.10	p< 0.01
SOD (u/mg protein)	3.13 ± 1.63	4.44 ± 1.68	5.21 ± 1.94	p< 0.01
GPx (nmol NADPH oxidized/min/mg protein)	26.50 ± 4.13	34.01± 9.28	36.91 ± 11.26	p< 0.01
GR (unit/min/mg protein)	1.04 ± 0.39	1.31 ± 0.44	1.49 ± 0.75	p< 0.001
Protein Corbonyl (µmol/L)	28.43 ± 2.09	20.08 ± 1.08	9.89 ± 1.24	p< 0.01

P< 0.01 statistically significant, P< 0.0001 extremely statistically significant.

Table no- 5. MDA significantly increased with POAG Diabetic patients and without POAG Diabetic patients compared to Healthy volunteer subjects (p< 0.01). Protein Carbonyl significantly increased 28.43 ± 2.09 µmol/L with POAG Diabetic patients and 20.08 ± 1.08 µmol/L without POAG Diabetic patients compared to 9.89 ± 1.24 µmol/L Healthy volunteer subjects (p< 0.01).

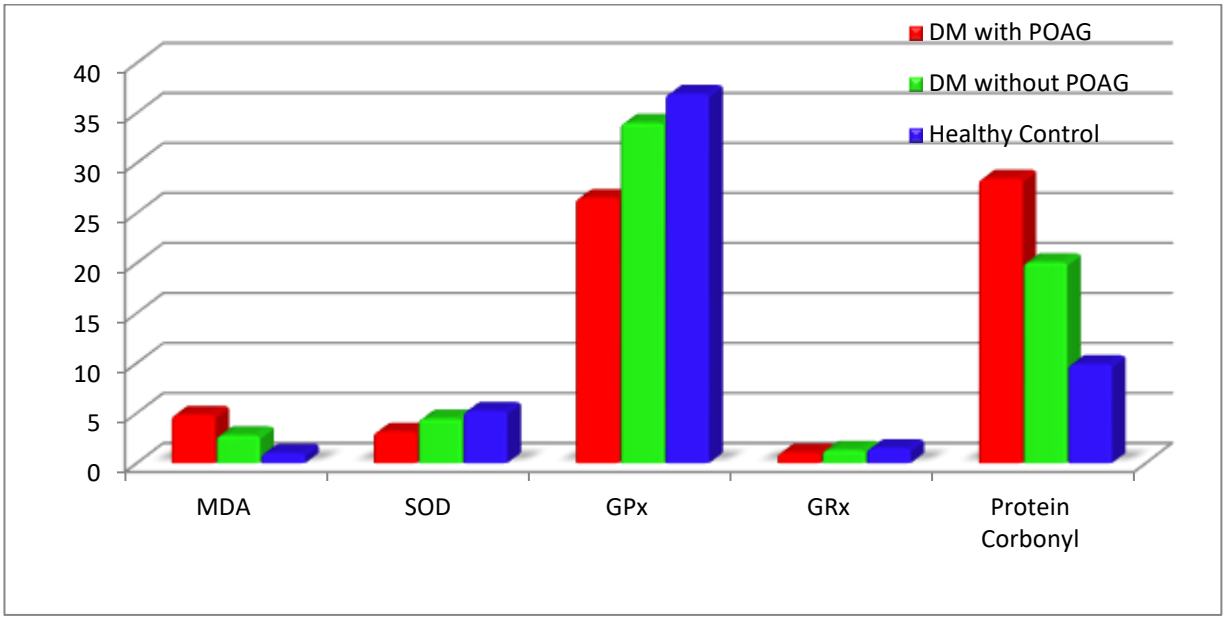

Fig. 1 Mean value Oxidative Stress parameter in DM with POAG, DM without POAG and Healthy Controls

Discussion:

Metabolic abnormalities in patients with POAG have been the focus of various studies15. Other factors, such as decreased antioxidant potential and increased free radicals, may also play an important role in the progression of glaucoma16. Primary open-angle glaucoma is a major cause of blindness. It has been suggested that metabolic diseases may play a role in the evolution of the disease. Fasting blood glucose levels were significantly high in POAG patients than control group17.

Our study reported that the Age group mean value of Diabetes Primary open-angle glaucoma, Diabetes without (POAG), 65.45 + 8.44, 59.12 + 8.56, and control 49.04 + 9.30 statistically significant ($p<0.001$). Table no.1 our study correlated well with finding shown by Hazari N.R. et al16, Channabasappa S. et al18, Desai A19 Tiwari A.K. et al., (2020)21.

The Fasting Blood Glucose and HbA1c Diabetes Primary open-angle glaucoma, Diabetes without (POAG), as compared to control elevated and statistically significant. Our result finding correlated with showed by Hazari N.R. et al16, Channabasappa S. et al18, Desai A19 Tiwari AK. et al., (2020)21.

Our study means SOD activity was 3.13 ± 1.63 U/mg protein, 4.44 ± 1.68 U/mg protein in diabetes mellitus with POAG and without POAG diabetes mellitus patients whereas it was 5.21 ± 1.94 U/mg protein in healthy controls. The SOD activity decreased significantly in diabetic patients in comparison to healthy participants ($p<0.01$). Furthermore, the activity also decreased markedly in the DM with POAG group in comparison to both diabetes mellitus without POAG and controls ($p<0.001$). Similarly, all antioxidant enzymes (GPx, and GR) were found to be significantly decreased in diabetic patients in comparison to healthy volunteers ($p<0.01$). The decrease was more marked in diabetes mellitus with POAG patients with Primary open-angle glaucoma in comparison to diabetes mellitus without POAG patients and healthy subjects ($p<0.01$). Our study correlated with Atti et al,. (2012)20, Li S,. (2020)2, Tiwari A.K. et al., (2021)22.

Diabetes Mellitus occurs more often in patients with Primary Open Angle Glaucoma than in non-glaucomatous populations. Similarly, Glaucoma is more prevalent in diabetic than in non-diabetic population23.

In our observation, the mean MDA significantly increased with POAG diabetes mellitus patients and without POAG diabetes mellitus patients as compared with healthy volunteer subjects ($p< 0.01$). Protein Carbonyl significantly increased 28.43 ± 2.09 µmol/L with POAG diabetes mellitus patients and 20.08 ± 1.08 µmol/L without POAG diabetes mellitus patients compared to 9.89 ± 1.24 µmol/L Healthy volunteer subjects ($p< 0.01$). The previous study correlated with Chang D. et al., (2011)24.

Conclusion

At last, after this, we can conclude that POAG diabetes mellitus patients and without POAG diabetes mellitus cases showed disturbed oxidative stress. Which may be responsible for the alteration of various antioxidant enzymes, and after this observation, we can suggest that early antioxidant enzyme investigation is a must in POAG diabetes cases. It is better to investigate antioxidant enzymes for better diagnosis and prognosis of the POAG in diabetes patients.

Conflicts of interest

There are no conflicts of interest.

Abbreviation:

DM	Diabetes Mellitus	BSL (F)	Blood Sugar Level (fasting).
POAG	Primary open angle glaucoma	BSL (PP)	Blood Sugar Level (Post Prandial).
SOD	Superoxide Dismutase	MDA	Malondialdehyde
GPx	Glutathione Peroxidase	GR	Glutathione Reductase

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