Assessment of Serum Paraoxonase1 Activity in Diabetic Retinopathy Patients

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ABSTRACT

Introduction: Diabetic retinopathy (DR), a major microvascular complication of diabetes mellitus comprises of characteristic group of lesions occurring in the retina leading to preventable blindness. Paraoxonase1 (PON1), an enzyme attached to High Density Lipoprotein (HDL) is known to enhance the antioxidant and anti-inflammatory role of HDL and prevents Low Density Lipoprotein (LDL) peroxidation, thus may prevent the complications of Diabetes Mellitus. The present study was undertaken to estimate serum PON1, lipid profile in diabetic retinopathy patients.

Material and Methods: Study subjects consisted of 30 clinically diagnosed cases of Diabetic retinopathy, 90 cases of type 2 Diabetes Mellitus (T2DM) and 90 age sex matched healthy controls. In all study population lipid profile were estimated on AutoQuanta 400 Merilyzer and serum basal PON1 activity and salt stimulated PON1 activity by spectrophotometric method.

Results: A significantly lower levels of basal and salt stimulated PON1 activity was seen in diabetic patients with and without DR ($p<0.0001$). Also a significant decrease in basal PON1 ($p=0.0001$) and salt stimulated PON1 activity ($p=0.0491$) was observed in DR patients compared to T2DM patients. Significant increase in total cholesterol ($p=0.016$), triglycerides ($p=0.002$), LDL ($p=0.005$) and Very Low Density Lipoprotein ($p=0.002$) was seen in diabetic patients with and without DR. There was no significant difference in lipid profile between T2DM and DR patients. In Discriminantfunction analysis, PPPG was more significantly influencing the DR (score 1.159).

Conclusion: T2DM have reduced PON1 activity which is further reduced in DR patients along with dyslipidemia which may play a significant role in pathogenesis of retinopathy. Thus estimation of PON1 may help in risk assessment of DR in T2DM.

Keywords: Dyslipidemia, Diabetic Retinopathy, Paraoxonase1.

INTRODUCTION

Diabetes mellitus (DM), a chronic metabolic disease is on epidemic rise even in developing countries like India. The International Diabetic Federation (IDF) has predicted that the prevalence of DM patients will rise from 65.1 million in 2013 to 109 million in 2035 in India.[1]

Diabetic Retinopathy (DR) is a major microvascular complication of DM comprising of characteristic lesions occurring in the retina of an individual. Based on the types of lesions DR may range from non proliferative Diabetic Retinopathy (NPDR), proliferative Diabetic Retinopathy (PDR) and diabetic macular oedema (DME).

DR has a significant impact on world health system resulting in blindness of over 10,000 people with diabetes per year.[2] In the developed countries it is the major cause of preventable blindness in working adult age group.[3]

The prevalence of DR in India has been reported variedly ranging from 10.3%[1] to as high as 34.1%.[4] The Chennai Urban Rural Epidemiology (CURES) Eye Study, a study in south India reported prevalence of 17.6% in DM patients in South India.[5]

The predominant risk factors for development and progression of DR is the duration of DM, degree of hyperglycemia and dyslipidemias.[4] Dyslipidemias potentially contributes to the development of DR. Hard exudates in retinopathy patients are associated with high levels of total cholesterol (TC) and low density lipoprotein (LDL).[2] Increase in lipid peroxides occurring due to poor glycemic control may contribute to the development of microangiopathies in DM.[6] Oxidised LDL (oxLDL) is cytotoxic to retinal capillary endothelial cells and pericytes contributing to pathogenesis of DR.[7]

High Homocysteine and homocysteine thiolactone (HCTL) causes homocysteinylation and loss of protein function leading to increase risk of development and progression of microvascular complications of DM.[8]

Paraoxonase (PON) is an aryldialkyl phosphatase (EC: 3.1.8.1) having both arylesterase and lactonase activity which hydrolyses HCTL.[7,9] It is a calcium dependent 43KDa enzyme, a transcriptional product of gene present on chromosome 7q21-22. The gene family codes for threeisoenzymes PON1, PON2 and PON3.[8] The PON1enzyme is closely associated with HDL, located on subfraction that contains Apo A1 and clusterin (Apo J).[10] The antioxidant and anti-inflammatory role of HDL is attributed to the ability of paraoxonase1(PON1) enzyme to hydrolyze the oxidized phospholipids and hydroperoxides of oxLDL.

Many studies have shown decrease in PON1 activity in DM.[11] However there are limited studies associating decreased serum paraoxonase1 levels with diabetic retinopathy. The present study was undertaken to evaluate the serum

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	Controls Group I N= 90	T2DM Group II N= 90	DR Group III N= 30	Overall P value	Significance P value		
					Group I - Group II	Group I - Group III	Group II- Group III
Age	54.49±7.23	56.44±14.07	60.53±11.12	0.060	0.177	0.0008*	0.075
Female/Male(%)	43.3/56.7	43.3/56.7	46.7/53.3	0.4353	1	0.83	0.83
FPG (mg/dl)	91.77±13.35	174.43±67.96	219.7±101.12	<0.0001	<0.001**	<0.001**	0.040*
PPPG (mg/dl)	122.43±15	236.53±86.45	341.73±94.44	<0.0001	<0.001**	<0.001**	<0.001**
TC (mg/dl)	181.03±32.66	193.33±39.41	211.3±46.42	0.016	0.460	0.012*	0.199
TG (mg/dl)	157.33±66.01	220.87±77.56	213.30±74	0.002	0.003**	0.010**	0.912
HDL (mg/dl)	44±8.82	39.47±10.78	39.7±10.93	0.160	0.204	0.239	0.996
VLDL (mg/dl)	31.1±13.15	43.63±15.37	42.26±14.87	0.002	0.003**	0.010**	0.929
LDL (mg/dl)	105.57±32.64	115.03±41.77	137.20±38.15	0.005	0.596	0.005**	0.063+

**Highly significant, *Significant, FPG: Fasting plasma glucose, PPPG: Postprandial plasma glucose, TC: Total cholesterol, TG: Triacylglycerol, HDL: High-density lipoproteins, VLDL: Very low density lipoproteins, LDL: Low density lipoproteins.

Table-1: Demographic and Biochemical parameters in the three groups

DM Duration (years)*	T2DM cases	DR cases
Recently diagnosed	0.0	3.3
1-2	26.7	0.0
3-5	26.7	23.3
5-10	40.0	56.7
>10	6.7	16.7
Mean duration of DM in yrs+	6.2±5.5	8.3±4.8

*P=0.011, Significant, Fisher exact test
+ -not significant
DM – Diabetes Mellitus, DR – Diabetic Retinopathy

Table-2: DM Duration (%)

paraoxonase1 activity and any concurrent dyslipidemia in DR patients.

MATERIAL AND METHODS

A cross sectional study was carried at Adichunchanagiri Institute of medical sciences, BG Nagara. The study population consisted of 210 subjects comprising of 90 healthy subjects on routine check up (Group I), 90 cases of Type 2 DM (T2DM)(Group II) and 30 cases of DM with retinopathy complication (DR) diagnosed clinically by fundus examination of both the eyes by direct ophthalmoscopy (Group III). Patients with chronic renal diseases, liver diseases, smokers, heart diseases, stroke, patients on lipid lowering agents, under 18 patients, pregnant women and psychiatric patients were excluded from the study. The study was approved by the institutional ethical committee.

After an overnight fast, 5ml of blood sample was withdrawn aseptically from all subjects for estimation of fasting blood sugar (FBS), lipid profile, basal PON1 activity and salt stimulated PON1 activity. 2ml of blood sample was taken 2 hours of taking the meal to estimate postprandial blood sugar (PPBG).

Plasma Glucose (Trinder's method), serum total cholesterol (TC) (CHOD-PAP method), High density lipoprotein (HDL), Low Density Lipoprotein (LDL) and Triglycerides (TG)(Glycerol phosphate oxidase method) were measured by using standard kits on Autoquanta 400 Merilyzer. Very low density lipoprotein (VLDL) was calculated by dividing TG with five (TG/5) when TG level was less than 400mg/dl.

Serum PON1 activity was measured spectrophotometrically using phenyl acetate as substrate.[12] Basal PON1 activity was measured by monitoring the rate of formation of phenol from phenyl acetate in Tris-HCl –$CaCl_2$ by PON1 at 412nm. The salt stimulated PON1 activity was measured by adding 1M NaCl to the reagents.

STATISTICAL ANALYSIS

The continuous variables are presented as mean ± SD and categorical variables as percentage. Analysis of variance (ANOVA) test was used to test the statistical difference between the multiple groups and student unpaired t test was used to assess the difference between two groups. Chi-square test and Fisher exact test were done for categorical variable. Significance is assessed at 5 % level of significance.

RESULTS

Table 1 shows the demographic, plasma glucose and lipid profile data in the three groups. Even though statistically there was no difference in the mean age, but diabetic retinopathy patients were older. There was significantly higher levels of TC, TG, VLDL and LDL levels in diabetic patients and diabetic retinopathy patients. However there was no significant change in HDL levels between the three groups as well as no significant difference in lipid profile between Diabetic patients and Diabetic patients complicated by retinopathy.

Table 2 shows duration of Diabetes in the diabetic and diabetic retinopathy groups with significantly higher number of diabetic retinopathy patients in longer DM duration (p=0.011). All the retinopathy patients were of non proliferative diabetic retinopathy grade and among them 60% had Mild NPDR, 30% had moderate NPDR and 10% had severe NPDR.

Table 3 compares the basal PON activity and Salt stimulated activity between the three groups. There was highly significant decrease in both basal and salt stimulated PON activity in diabetes and diabetic retinopathy patients. Also there was significant decrease in PON activity in retinopathy patients when compared to diabetic patients without retinopathy.

Discriminating analysis was employed to find the significant

	Controls Group I	T2DM Group II	DR Group III	p	Significance P value		
					Group I - Group II	Group I - Group III	Group II - Group III
Basal PON1	67.4±12.14	34.46±17.88	24.8±10.96	0.0001**	0.0001**	0.0001**	0.0001**
Salt Stimulated PON1	76.28±12.97	46.40±20.1	37.9±11.18	0.0001**	0.0001**	0.0001**	0.0491**
**Highly significant, PON1: Paraoxonase1							

Table-3: Comparison of PON levels in three groups studied with post-hoc test

	Wilk's Lambda	F	df1	df2	Sig.
Age	0.920	10.211	1	118	0.002
TC	0.904	12.519	1	118	0.001
TG	0.726	44.486	1	118	0.000
HDL	0.908	11.943	1	118	0.001
LDL	0.888	14.930	1	118	0.000
VLDL	0.728	44.136	1	118	0.000
FPG	0.438	151.148	1	118	0.000
PPPG	0.192	495.275	1	118	0.000
Basal PON1	0.297	279.074	1	118	0.000
Salt Stimulated PON1	0.360	210.209	1	118	0.000
FPG: Fasting plasma glucose, PPPG: Postprandial plasma glucose, TC: Total cholesterol, TG: Triacylglycerol, HDL: High-density lipoproteins, VLDL: Very low density lipoproteins, LDL: Low density lipoproteins, PON1:Paraoxonase1					

Table-4: Descriptive analysis: Test of Equality of Group means

	Function
	1
Age	0.116
TC	-0.430
TG	0.654
HDL	0.051
LDL	0.617
VLDL	-0.343
FPG	-0.564
PPPG	1.159
Basal PON1	-0.385
Salt Stimulated PON1	-0.161
FPG: Fasting plasma glucose, PPPG: Postprandial plasma glucose, TC: Total cholesterol, TG: Triacylglycerol, HDL: High-density lipoproteins, VLDL: Very low density lipoproteins, LDL: Low density lipoproteins, PON1:Paraoxonase1	

Table-5: Results of Discriminant Function analysis: Standardized Canonical Discriminant Function coefficient

factors predicting the DR, all the variables are significant (Table 4), as per Table 5, the PPPG is more significantly influencing the DR (score 1.159), followed by TG (0.654), FPG (-0.584), TC (-0.430) and basal PON with -0.385.

DISCUSSION

Diabetic retinopathy is a common complication of diabetes mellitus which occurs due to damage of microvasculature of retina of eyes. Studies have shown after 15-20 years of duration of DM, almost all type 1 DM and nearly 75% of type 2 DM would have developed DR.[4]

The pathophysiological mechanism for development of DR is multifactorial. It may be directly associated with the age of onset of DM, duration of DM and degree of control of glycemia.[6] There may also be a genetic predisposition.[2] Hyperglycemia may damage the retinal vessels by a number of mechanisms including increased polyol pathway, increased formation and damage by advanced glycation end products, activation of adverse cellular metabolic pathways via protein kinase C, increased oxidative stress etc.[6]

Oxidized LDL may play a key role in pathogenesis and progression of DR by damaging retinal capillary endothelial cells and pericytes.[7] HDL prevents LDL oxidation through its antioxidant and anti-inflammatory properties by PON1 hydrolytic activity. PON1 decreases the lipid peroxides on LDL.[7]

In our earlier study we had demonstrated decrease in PON1 activity in DM patients.[13] Further decrease in PON1 activity has been reported in diabetic patients with complication.[10,12] Hyperglycemia may have a direct role in reducing PON1 activity due to glycation and glyoxidation of both HDL and paraoxonase enzyme.[14]

Nowak M et al[9], reported significant decrease in PON1 activity in DR patients with also a significant decrease in PON1/CRP ratio. In the present study the basal and salt stimulated serum PON1 was significantly reduced ($p<0.0001$) in all diabetic patients with and without DR. Further there was significantly reduced basal and salt stimulated PON1 activities in DR patients compared to T2DM patients without retinopathy ($p<0.0001$and $p=0.0491$ respectively). Our study was in accordance to studies by Mackness B et al.[7] Similar findings were echoed in studies conducted by Hampe MH et al.[6] They also concluded that PON1 arylesterase activity as demonstrated by phenyl acetate hydrolytic activity may be more important in risk assessment of DR. Both these studies also demonstrated that subjects with PON RR phenotype [having arginine instead of glutamine (QQ type) at 192 position] are at higher risk of development of retinopathy.

Dyslipidemia is another major contributor for the development of diabetic complications. Yo-Chen Chang et al[3], in review of 19 studies on association of dyslipidemia in DR, found inconsistent results. However they also reviewed studies on lipid lowering therapies in DR cases and concluded that lipid lowering therapy may be an effective adjunct in management of DR especially in patients with diabetic macular oedema requiring laser therapy.

In the present study there was significant increase in TC ($p<0.016$), TG ($p<0.002$), LDL ($p<0.005$) and VLDL ($p<0.002$) levels in diabetic patients compared to controls although there was no significant difference between HDL lev-

els (p=0.160). But there was no significant difference in lipid parameters in DM patients with and without retinopathy.
Agroiya P et al[15] reported significant changes in LDL, HDL and TG levels in DR patients. In another study by Mathur A et al[16], diabetic patients had higher levels of TC, LDL and TG levels with lower HDL levels. They also found difference in TG level between DM and DR patients. However Ebru Nevin Cetin et al[17], found no difference in lipid profile between diabetic patients without DR and patients with various grades of DR even though they found significant correlation between mean blood glucose and HbA1c with various lipid species.

CONCLUSION

In conclusion serum PON1 activity was decreased in diabetic patients which was further reduced in DR patients thus reducing the protective role of HDL on prevention of LDL peroxidation. Added to this is diabetic patients are high risk group in terms of dyslipidemia with increased atherogenic lipids and decreased antiatherogenic lipids. Thus evaluation of PON1 may help in risk assessment and management of DR patients.
Further studies with large sample size are warranted to establish the role of PON1and its polymorphism in pathogenesis of retinopathy.

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