RESEARCH PAPER | Medical Science | Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

Serum Paraoxonase (Arylesterase) activity in Type 2 Diabetes Mellitus and diabetic nephropathy

KEYWORDS: Serum paraoxonase (Arylesterase), Diabetes mellitus, diabetic nephropathy

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ABSTRACT *Background: Serum paraoxonase-1 (PON1) prevents oxidation of low density lipoprotein cholesterol (LDL-C) delaying the development of atherosclerosis. Increased susceptibility for oxidation of LDL -C has been reported in Diabetes mellitus and nephropathy. This study was undertaken to measure serum PON - arylesterase activity and PON1 phenotype in Diabetes mellitus, diabetic nephropathy and healthy individuals. Materials and methods: The study group had 90 subjects- non-diabetic healthy controls(n = 30), diabetics without complications (group I, n=30) and diabetics with nephropathy (group II, n = 30) . PON1 activity, fasting blood sugar (FBS) levels were determined. Results: FBS (p<0.001) was significantly increased and PON1 (p<0.001) were significantly decreased in group II patients. In group I patients compared with the controls, PON1 activity did not significantly differ. Conclusion: This indicates significant physiologically active functioning of PON1 in these patients, thus protecting them from diabetes related complications.*

Introduction

Paraoxonase-1 (PON1) is a calcium dependent enzyme. PON1 has lactonase and esterase activity and thus is able to catalyze the hydrolysis of lipid peroxides and organophosphate pesticides [1]. Its physiologic function has not been fully elucidated. The PON1 enzyme attaches to high-density lipoprotein (HDL) particles in serum, and inhibits low-density lipoprotein (LDL) oxidation, and plays a preventive role in atherogenesis [2]. Increased susceptibility for oxidation of LDL -C has been reported in Diabetes mellitus and Diabetic nephropathy. Accelerated atherosclerosis and altered lipoprotein metabolism is responsible for increased cardiovascular events and cardiac death in these conditions [3][4]. It has been reported that significant reduction of serum paraoxonase activity occurs in chronic renal failure, resulting in high and premature incidence of atherosclerosis in these individuals [5]. PON1 activity exhibits a substrate-dependent activity polymorphism in human beings. Three different phenotypes are reported based on the responses of the two isoenzymes to salt concentrations. The phenotypic distribution of PON can be used to subdivide the population into three groups AA represents low, AB intermediate and BB high enzyme activity [6].

Arylesterase activity is a measure of PON activity, in which p- nitro phenyl acetate is used as a substrate. The aim of this study was to measure serum PON - arylesterase activity in patients with Diabetes mellitus, diabetic nephropathy (both on conservative management and on hemodialysis) and healthy individuals.

Material and Methods

A cross sectional study was conducted for a period of one year, starting from January 2007, at the M.S.Ramaiah Medical College & Hospitals (MSRMH), Bangalore. Informed consent was taken from all the participants. Ethical Clearance for this study was obtained from Ethical Review Board of MSRMH. Subjects were divided into three groups of 30 subjects each. All the subjects were above 45 years of age and of either sex.

1. Control group: healthy subjects, attending the Out Patient Department (OPD) of MSRMH.

2. Group I: Type 2 diabetes patients with no clinically demonstrable complications, attending the OPD or admitted in the wards. Nephropathy was ruled out by taking relevant history.

3. Group II: Type 2 diabetes patients with nephropathy, attending the OPD or admitted. Relevant clinical history was taken and clinical examination performed. The height and weight of the patient was recorded.

Patients with type 2 diabetes mellitus with complications other than nephropathy, acute coronary syndromes, cerebrovascular accidents or stroke and severe peripheral vascular artery diseases were excluded from group II whereas type 2 DM with nephropathy were excluded from the group I.

About 5 ml of blood sample was collected in the vacutainer without any anti-coagulant. The blood sample was collected after 12 hours of fasting under aseptic conditions and before hemodialysis. Blood was allowed to clot for 30 minutes and was centrifuged 3000 rpm for 15 minutes and clear serum was used for analysis. Following investigations were performed immediately- fasting blood glucose (FBS), serum creatinine & blood urea nitrogen (BUN). They were measured on an auto-analyzer – Dade Behring Dimension series RXL Max. Blood glucose was estimated by an adaptation of the hexokinase – glucose 6-phosphate dehydrogenase method [7]. Creatinine and BUN were estimated by colorimetric assays [8][9]. Remaining serum sample was stored between 0 – 4°C, to measure the arylesterase activity of PON.

Serum PON1 assay-This enzyme was estimated spectrophotometrically using 5.5 mM 4-nitrophenylacetate as the substrate in 20 mM Tris-HCl buffer at a pH of 8.0. The increase in absorbance due to the formation of the yellow 4-nitro-phenol was monitored at 412 nm for three minutes. For each sample basal PON activity as well as salt-stimulated PON activity was determined as described below [10]. Basal PON –This was estimated by using the Tris-HCl buffer containing only 1mM calcium chloride. Salt Stimulated PON - This was estimated by using the same Tris-HCl buffer which however contained 1mM calcium chloride as well as 1M NaCl. PON Activity was calculated after corrections were made for non-enzymatic hydrolysis. PON was taken to be 1 U/L when the rate of formation of the product 4-nitrophenol was 1 nmol/ml/ minute under the assay conditions. Chemicals: - 4-nitrophenol

Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

acetate was procured from Sigma Chemical Company (St. Louis, MO,USA). All other chemicals used for the assay of PON were of analytical grade.

Paraoxonase Phenotype Distribution The Pearson stimulation of PON was calculated as

SALT STIMULATED PON ACTIVITY- BASAL PON ACTIVITY
---------- X 100%
BASAL PON ACTIVITY

Individuals were classified for PON phenotype using the antimode of PON as proposed by Eckerson et al[6]. Individuals below the limit of 60% stimulation were considered AA phenotype, between 60-200% AB phenotype, and above the level 200% stimulation BB phenotype.

Statistical Analysis: The results were expressed as mean ± standard deviation. p value of < 0.05 was considered statistically significant. Statistical analysis was performed using the statistical package for social sciences (SPSS 10). Student't' test was used to compare mean values. Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients.

Results: The study included 30 subjects of Type 2 diabetes mellitus without nephropathy or any clinically demonstrable complications (Group I), 30 subjects of Type 2 diabetes mellitus with nephropathy (Group II) and 30 normal subjects as controls. Amongst Group II, 43% (n=13) of the patients were on conservative treatment and 57% (n=17) were on hemodialysis.

Table 1: Comparative study variables of groups of patients studied

Variables	Groups			p value
	Control	Diabetes mellitus Group 1	Diabetic Nephropathy Group 2	
FBS (mg/dl)	96.70±12.03	214.63±67.31	240.17±48.13	<0.001**
BUN (mg/ dl)	11.00±2.87	11.83±4.41	59.06±31.32	<0.001**
Serum Creatinine (mg/dl)	0.89±0.19	0.93±0.18	6.34±3.20	<0.001**
GFR (ml/min/1.73m2)	112.73±29.01	102.55±24.11	20.99±14.91	<0.001**

Table 1 shows the FBS, BUN, Serum Creatinine & GFR values amongst the different study groups.

Paraoxonase Phenotype distribution: Amongst controls, 16(52%) subjects belonged to AA Phenotype (low Paraoxonase activity), 13 (46%) belonged to AB Phenotype (intermediate Paraoxonase activity) and 1 to BB Phenotype (High Paraoxonase activity). In patients with Diabetes Mellitus, 16(53%) subjects belonged to AA Phenotype (low Paraoxonase activity) and 14 (47%) belonged to AB Phenotype (intermediate Paraoxonase activity). In patients of Diabetes Mellitus with nephropathy, both on conservative management and on hemodialysis, 11 out 13 patients and 14 out of 17 subjects belonged to AA Phenotype (low Paraoxonase activity) respectively.

Arylesterase Activity in the study groups is shown in Table 2.

Table 2: PON1 or Arylesterase activity in the study subjects

Basal PON1/ arylesterase activity (nmol/mL/min)		
Parameter	Mean±SD	p value*
Control	102.81±45.62	
Diabetes mellitus Group 1	109.09±21.50	0.547
Diabetic Nephropathy Group 2	81.90±28.28	0.05
Basal PON1/ arylesterase activity (nmol/mL/min)		
Parameter	Mean±SD	p value*
Control	136.08±33.17	
Diabetes mellitus Group 1	139.25±28.41	0.73
Diabetic Nephropathy Group 2	114.39±28.02	0.01*
* Student t test **Significant		

Discussion

The aim of this study was to measure serum PON1/arylesterase activity in patients with Diabetes mellitus, diabetic nephropathy (both on conservative management and on hemodialysis) and healthy individuals.

The PON1 phenotypes were determined on the basis of percent activation of enzyme activity by 1M NaCl- AA Phenotype (low Paraoxonase activity < 60% activation) and AB Phenotype (intermediate Paraoxonase activity 60-200% activation) and Phenotype BB (High Paraoxonase activity >200% activation). In patients with chronic renal failure, both on conservative management and on hemodialysis 11 out 13 patients and 14 out of 17 subjects belonged to AA Phenotype (low Paraoxonase activity) respectively. This explains the cause for accelerated atherosclerosis in chronic renal failure. In the control group and in the group with diabetes mellitus, 52% and 53% respectively belonged to AA Phenotype which also adds to iincreased incidence of atherosclerosis in the general population. In a study conducted by Patel AB et al,.[11], a similar trimodal distribution of PON 1 phenotypes amongst Gujarathi's was seen- 52% low Paraoxonase activity, 46.6% intermediate and 1.4% of the population with high enzyme activity. In our study, most individuals in all the three groups belonged to phenotype-AA (low PON1 activity) showing potential vulnerability towards atherogenesis.

Significantly high levels of FBS (p<0.001), BUN (p<0.001), Serum creatinine (p<0.001) were found in Group I Diabetes mellitus, as compared to normal controls. In group II patients, FBS (p<0.001), BUN (p<0.001), Serum creatinine (p<0.001) were found to be significantly increased, as compared to the normal controls. High levels of FBS (p<0.001) in the group II patients indicated a poor glycaemic control, which led to the increased glycation of proteins and other biomolecules. Prolonged dysglycaemia might have caused increased damage to the biomolecules and the biomembranes, thus leading to various diabetes associated complications [12]. PON1 or arylesterase activity in group II (diabetic nephropathy) was found to be significantly lower p<0.01 than in the controls. This decrease in the activity of PON1 might prevent the normal physiological function of HDL-C and its antiatherogenesis function, thereby leading to accelerated atherogenesis and its related complications [1]. There was no significant difference in arylesterase activity between group I (Diabetes mellitus) and controls. This may possibly indicate the significant physiologically active functioning of PON1 in these patients, thus protecting them from diabetes related complications. In a study by Kopprasch S et al., [13], though IGT (Impaired glucose tolerance) subjects and diabetes mellitus patients had significantly increased levels of oxLDL in the circulation, serum PON1 activity was not altered in IGT and early Diabetes Mellitus when compared with controls. This suggests that PON1 activity loss is an event occurring later in the course of diabetes mellitus and that PON1 does not affect oxidation of circulating LDL, at least in early diabetes mellitus. Our study had two major drawbacks- we did not record the duration of diabetes mellitus and we did not do a lipid profile

Volume : 3 | Issue : 4 | April 2013 | ISSN - 2249-555X

for our study subjects. Therefore, a further, prospective, well designed, clinical and pathophysiological study has to be undertaken to establish the role of PON1 in diabetes mellitus and its related complications. It can be concluded that type 2 DM patients with nephropathy have significantly decreased PON1 activity, possibly indicating their decreased biochemical roles in these patients.

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