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Original Research Paper

Study of Serum Paraoxonase Levels in Type 2 Diabetes Mellitus Patients & Suggests Risk of Cardiovascular Disease

Medical Science

KEYWORDS : *Dyslipidemia, Paraoxon as substrate, paraoxonase, Type 2 diabetes mellitus*

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ABSTRACT

Background and Objectives:

Diabetes, a lifelong progressive disease, with metabolic dysregulation, is associated with increased risk of cardiovascular complications. Paraoxonase (PON), an enzyme associated with high-density lipoprotein (HDL) is known to modulate the antioxidant and anti-inflammatory role of HDL and may have a protective role in the prevention of these complications. The present study was undertaken to evaluate serum PON, paraoxon as reacting substance and serum lipid profile in Type 2 diabetes mellitus (DM).

Materials and Methods:

The study group consists of 30 Healthy Controls, 30 patients with diabetes mellitus with CAD, 30 non-diabetic patients with CAD, 30 Patients with Diabetes mellitus without clinical Coronary artery disease. Lipid profile was estimated by enzymatic methods using analysed on Biochemistry Autoanalyser (AU480) in Central Clinical Laboratory, and serum PON by spectrophotometric method.

Results:

Total cholesterol levels are highly increased in group II & IV as compared to group I (P<0.001). Group III shows significant increase in Total cholesterol as compared to group I (P<0.05). T.G. levels are highly increased in study group (i.e. group II, group III, & group IV) as compared to group I (P<0.001). The levels of LDL are significantly increased in study groups as compared to control group (P<0.05). The mean levels of HDL significantly decreased in study group (i.e. group II, group III, & group IV) as compared to group I (P<0.05). PON1 levels is significantly decreased in study groups ((i.e. group II, group III, & group IV) as compared to control group I (P<0.001).

Conclusion:

Our study demonstrates that Type 2 DM patients have significantly lower levels of PON activity than the healthy control. Serum Paraoxonase levels were decreased in patients with diabetes & CAD.PON activity directly correlation with HDL levels. This may reduce the protective role of HDL and increases the susceptibility of cardiovascular complications.

Introduction

Type 2 diabetes mellitus (DM) is a disease of metabolic dysregulation, involving the impaired uptake and the utilization of glucose, altered lipid metabolism, the accumulation of various lipid species in the circulation and in the tissues, and the disruption of metabolic signaling pathways that regulate insulin secretion from the pancreatic beta-cells.[1]Several studies have demonstrated that the increased susceptibility of low density lipoprotein (LDL) to oxidation and higher levels of oxidized LDL in DM correlated with an increased risk of cardiovascular complications.[2]Serum PON1 is located in a subfraction of high density lipoprotein (HDL) that contains apoA-I and clusterin (apoJ). It has been suggested that, this subfraction of HDL is principally responsible for the breakdown of lipid peroxides, and that it consequently protects against lipoprotein oxidation.[3] Paraoxonase (PON) (EC 3.1.8.1), a human serum arylesterase is a polymorphic enzyme consisting of PON1, PON2, and PON3 that catalyzes the hydrolysis of organophosphates such as paraoxon and aromatic carboxylic acid esters of fatty acid. . It is produced by the liver and is associated with high-density lipoprotein (HDL) particles and is known to modulate the antioxidant and anti-inflammatory role of HDL. [4] Enzyme PON1 also plays an important role in protecting lipoproteins (LDL, HDL) and membrane cells from oxidative damage, and currently is one of the most important enzymes, anti-oxidants and Anti -atherogenic enzyme.[5] Homocysteine thiolactones, a metabolite of homocysteine causes endothelial dysfunction and vascular damage. PON1 plays a vital role in cardioprotection, as it is required for detoxification of homocysteine thiolactone.[6]

Reduced PON activity increases the oxidative stress in the patients. Hence, the present study was undertaken to evaluate serum PON levels and serum lipid profile in Type 2 DM. Monitoring the trends in cardiovascular complications via PON 1 is of critical importance in managing patients with Type 2 DM

Materials and Methods

Place of study: Department of Biochemistry and Department of Medicine. MGM Medical College & hospital, Kamothe, Navi Mumbai

Study period : February 2015 to February 2016.
Study design : Prospective, case control study.
Age Group : 25 – 70 yrs. & Study included both genders.
The subjects selected for the study were categorized into the following four groups:

- Group 1: 30 Healthy Controls.
- Group 2: 30 patients with diabetes mellitus with CAD.
- Group 3: 30 non-diabetic patients with CAD.
- Group 4: 30 Patients with Diabetes mellitus without clinical Coronary artery disease.

Written and verbal consent will be taken from patients and healthy individuals. Patients will be considered between February2015 to February 2016.

Informed consent was taken and the study was approved by the ethical committee of the

institution. Patients suffering from chronic renal disease, Chronic liver disease, Malnutrition HIV patient, Rheumatoid arthritis, Sepsis ,Asthma ,Malignancy Pregnant women. 5 ml of fasting blood sample was drawn from all subjects under the aseptic precaution and 2 ml Of blood was drawn in postprandial period. Fasting samples were analyzed for routine blood parameters, fasting plasma glucose (FPG), serum lipid profile, and PON levels.

Postprandial sample was analyzed for postprandial plasma glucose (PPPG). Plasma Glucose was measured by HK G6P-DH, serum total cholesterol (TC) by CHO – POD, HDL by CHO-POD (enzyme colour test, immuno-inhibi-

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tion) methods. Tests are perform on AU480 auto analyser Very low-density lipoprotein (VLDL) was calculated by dividing TG with five (TG/5). LDL

level were calculated using Friedwald's formula, LDL= TC - (HDL+TG/5). Serum PON was measured by spectrophotometric method using 2.2 mmol/L paraoxon (O,O-diethyl-O-pnitrophenyl Phosphate) as Substrate.

Biochemical Determinations
PON1 was estimated spectrophotometrically by the method which is described elsewhere, with minimal modifications. Briefly, the assay mixture consisted of 500 µl of 2.2 mmol/l

paraoxone substrate in 0.1 mol/l tris-HCl buffer, pH 8.0, containing 2 mmol/l CaCl2 and 50 µl of fresh serum specimen. The absorbance was monitored at 405 nm, at 25 °C. The PON

1 activity was expressed in international units (IU). One IU was defined as 1 µmol of p-nitrophenol which was formed/min/L at 25 °C. [7]

Statistical analysis
Results are represented as mean ± standard deviation. Statistical analysis was done using

Student's t-test, and statistical significance was compared between the cases and the controls.

Pearson correlation between the study variables was performed to establish the relationship. Probability value (P) of <0.05 was considered as statistically significant. Statistical analysis was done using the Statistical Software: SPSS-16.

Results
Table 1: Comparison of FBS, PPBS and HbA1c in Control (Gr-I) & Study Group (Gr-II, III, IV)

Parameters	Group-I (Control) Mean± SD	Group-II (CAD with DM) Mean± SD	Group-III (CAD) Mean± SD	**Group-IV (T2DM) Mean± SD**
FBS ((mg/dl)	91.6±9.6	148.98±54.58**	96.7±9.42*	140.7±41.8**
PPBS (mm/dl)	129.4±10.6	257.1±56.3**	139.5±11.1**	234.9±62.4**
HbA1c (%)	5.5±0.4	8.1±1.0**	6.0±0.6**	7.8±0.8**

**p ≤ 0.05 significant, **p ≤ 0.001 highly significant & #p ≥ 0.05 non-significant.*

Table 2: Comparison of Lipid Profile in Control (Gr-I) & Study Group (Gr-II, III, IV)

Parameters	Group-I (Control) Mean± SD	Group-II (CAD with DM) Mean± SD	Group-III (CAD) Mean± SD	Group-IV (T2DM) Mean± SD
Total Cholesterol (mg/dl)	176.6±26.2	207.4±20.5**	200.3±30.4*	192.3±11.2**
Triglyceride (mg/dl)	144.0±29.6	207.1±46.0**	193±41.7**	182.7±41.9**
HDL-C (mg/dl)	36.2±5.6	32.1±5.7*	32.8±7.2*	32.6±5.1*

p ≤ 0.05 significant,p ≤ 0.001 highly significant & #p ≥ 0.05 non-significant*

Table 3: Serum PON1 in (Gr-I) & Study Group (Gr-II, III, IV)

Parameters	Group-I (Control) Mean± SD	Group-II (CAD with DM) Mean± SD	Group-III (CAD) Mean± SD	Group-IV (T2DM) Mean± SD
PON1(IU/L)	325.4±21.6	227.4±27.2**	243.5±42.7**	293.5±18.5**

p ≤ 0.05 significant,p ≤ 0.001 highly significant & #p ≥ 0.05 non-significant*

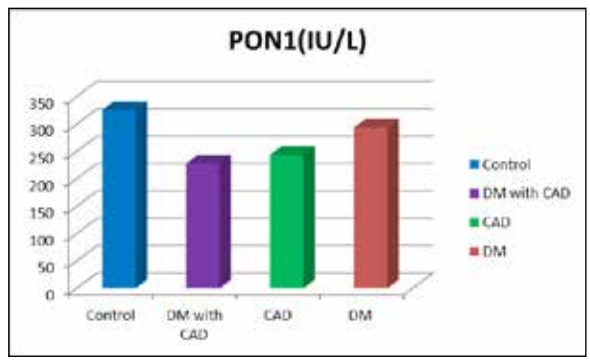

Fig 1: Shows serum PON1 levels in control & study groups

Discussion
Significantly high levels of FPG (p<0.001) in the group II &group IV patients indicated a poor glycaemic control in these patients, which led to the increased glycation of proteins and other biomolecules. Prolonged dysglycaemia in these patients might have caused increased damage to the biomolecules and the biomembranes, thus leading to various diabetes associated complications.[8] Diabetic status was assessed by estimating FBS in study & control group. Table 1 shows mean levels of fasting plasma sugar; were significantly increase in group II & groups IV (P<0.001) as compared to the control group. Table 1 shows significantly increase Postprandial plasma sugar level in group II & IV (P<0.001) as compared to the control group. There was slightly increased value in group III as compared to group I (P< 0.05).

Table 2 shows the mean levels of lipid profile in group I & study group (i.e. group II, group III, & group IV). Total cholesterol levels are highly increased in group II & IV as compared to group I(P<0.001). Group III shows significant increase in Total cholesterol as compared to group I (P<0.05).

T.G. levels are highly increased in study group (i.e. group II, group III, & group IV) as compared to group I (P<0.001). The levels of LDL are significantly increased in study groups as compared to control group (P<0.05)

Table 2 shows the mean levels of HDL significantly decreased in study group (i.e. group II, group III, & group IV) as compared to group I (P<0.05).

Ahmed I et al 2013 showed the high prevalence of dyslipidemia in subjects with type 2 diabetes mellitus and also stated, hypertriglyceridemia is more common than hypercholesterolemia in diabetic subjects. [9]

Prabodh VS et al. 2012 show that frequencies of the high TC, high TG and high LDL-C levels were higher in the diabetic group, thus indicating that diabetic patients were more prone for dyslipidemia, which could cause cardiovascular disorders. [10]

Our study also shows same finding in DM patients that suggest that they can cause cardiovascular disorder.

In our study we estimated serum paraoxonase (PON1) levels & found that PON1 levels is significantly decreased in study groups ((i.e. group II, group III, & group IV) as compared to control group I (P<0.001) table 3. However the level of PON1 is highly decreased in group II as compared to group I fig 1.

M Prakash et al 2011 shows that Type 2 DM patients with complications have significantly decreased HDL-C levels and PON1 activity, possibly indicating their decreased biochemical roles in these patients. [11]

Our results for paraoxonase are supported by M Prakash et al & suggest greater risk of atherosclerosis and cardiovascular disease.

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