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Lack of Correlation of Paraoxonase (PON1) Activity with Smoking among the South Indians and Risk of Cardiovascular Disease

[1]Mahadesh Prasad A.J., [1]K. Kemparaju, [1]Elizabeth A. Frank, [1]Priscilla Jaichander, [2]Anjali Arun, [3]C.Raju and [1]Cletus J.M.D'Souza

[1]Department of Biochemistry, University of Mysore, Mysore 570006, India
[2]Vikram Hospital, Yadavagiri, Mysore 570013, India
[3]JSS Institutions, Manasagangotri, Mysore 570006, India

Abstract: paraoxonase is an antioxidant enzyme associated with HDL. Anti atherogenic properties of HDL are attributed to this Enzyme. We determined the PON1 activity of 288 young individuals in the age group of 20 to 39 years of both sexes. The PON1 activity was measured as aryl esterase using phenyl acetate as substrate. PON1 did not correlate with age, sex or even smoking. The PON1 activity was negatively skewed. Our results suggest that PON activities even in the young individuals are already sufficiently modified so that smoking does not affect the activity further.

Key words: PON1 · Aryl esterase · Smoking

INTRODUCTION

High density lipoprotein (HDL) is an antiatherogenic molecule.HDL-Cholesterol is the only negative risk factor for cardiovascular diseases [1]. The biochemical basis for its cardio protective action is through its involvement in reverse cholesterol transport [2]. The anti atherogenic property of HDL is also because of the HDL-associated proteins like Paraoxonase. (PON1). Paraoxonase is tightly associated with HDL and confers antioxidant property to HDL [3]. The cardio protective function of HDL is also explained on the basis of the substrate specificities of PON1. For example it has been shown to reduce lipid hydro peroxides [4, 5]. It can prevent LDL oxidation [6, 7] and is also believed to protect HDL from oxidative modification. In recent years it is becoming increasingly clear that HDL and HDL -associated proteins are also subject to modification, resulting in a loss of function without affecting their relative quantities [8]. Thus HDL-Cholesterol measurement may not truly indicate the functional status of HDL.Oxidative stress, a major risk factor of cardiovascular disease, is a predominant factor which inactivates HDL- associated molecules. Cigarette smoking is one of the four major risk factors positively correlated with risk of cardiovascular disease [9]. Although the Molecular Mechanism of reduction in function of HDL by Cigarette smoking is not known, *in vitro* studies have shown that it may be by increasing the Oxidative stress. Smoking is also shown to reduce thiols and anti oxidant enzymes [10].

Asians in general and Indians in particular have a high risk of cardiovascular disease. [11] While changing life style may be an important factor, genetic factors may also play a major role in risk of cardiovascular disease. In order to find out whether the HDL of young Individuals itself has modified level of PON1 in serum, we chose healthy individuals of young age (25-39years) for this case study. Here we Report that even in the young adults, the PON1 is modified.

MATERIALS AND METHODS

Materials: Tris buffer, EDTA, SDS and Ethylene diamine tetraacetic acid (EDTA) purchased from SRL chemicals (Mumbai, INDIA) Ethyl alcohol, Acetic acid Were purchased from ranbaxy chemicals (New Delhi).The affinity matrix Cibacron Blue Agaraose used was purchased from Sigma - Aldrich USA. Other chemicals, acid, bases, solvents and salts used for the study were of analytical grade.

Blood was collected from normal healthy volunteers of age group 20-39 years of both sexes, in accordance to guidelines mentioned by the Institutional Ethics committee for biomedical research. Kits for the assay SGPT, creatinine and blood glucose were purchased from Mediclone Biotech Pvt.Ltd Chennai, India.

Corresponding Author: Cletus J.M.D'Souza, Department of Biochemistry, University of Mysore, Mysore - 570 006, India

METHODS

Synthesis of Phenyl Acetate: 20 ml of distilled phenol was taken in a round bottom flask; to this 20 ml of Acetyl chloride was added drop by drop and mixed. The mixture was then heated at 70° C for 1 hour. The content of the flask were cooled and 200 ml of Ice cold water was added. The free acid was neutralized by adding alkali (NaOH). The oily layer was dissolved in petroleum ether and separated by using a separating funnel. Petroleum ether was evaporated to yield Phenyl acetate. Phenyl acetate was dissolved in Isopropyl alcohol and used as substrate.

Blood Analysis: Whole blood was collected from the vein (5ml) and allowed to clot. The serum was separated by centrifugation. An aliquot was frozen at -20°C. The remaining serum was used for analysis of SGPT, Random blood sugar and creatinine using clinical assay procedures described by Mediclone kits [12].

PON 1 / Arylestrase Activity of Human Serum Samples: PON1 activity towards phenyl acetate was measured spectrophotometrically at 270nm in an automated Shimadzu UV-1601, UV visible Spectrophotometer [13]. Buffer substrate was prepared by adding 50 mM Tris-HCl buffer pH 8.0 containing 2 mM Calcium. Phenyl acetate in Isopropyl alcohol was added such that the Phenyl acetate concentration was 2 mM and the isopropyl alcohol concentration was less than one percent. To 2.99 ml of buffered substrate 10 micro liters of the serum was added and the optical density was measured at 270 nm continuously at intervals of 30 sec for 3 min. PON1 activity was calculated from the linear part of the curve by calculating the change in OD per min. One unit of activity was defined as that amount of enzyme which produced 1 micromole of phenol per min. The molar extinction coefficient of phenol was 1310 M^{-1} cm^{-1}.

Inhibition with EDTA: PON activity in the serum samples was confirmed by inhibition by EDTA. The assay was carried out as described above but without added calcium and in the presence of 1mM EDTA solution.The arylesterase activity under these conditions was zero.

Heat Inactivation: PON 1 activity in serum samples was also confirmed by the heat inactivation of PON1. Selected serum samples were heated at 80°C for 15 prior to determining PON 1 activity as described above. There was no precipitation by this treatment and the activity was essentially zero.

RESULTS AND DISCUSSION

Asians in general and Indians in particular are predisposed to cardio vascular diseases. [14] Many reasons have been attributed to this fact. However the two main factors that may be responsible are genetic basis and change in lifestyle. [15]. Indians in particular are moving towards a sedentary lifestyle, while the fat intake has increased particularly through convenience foods to suit this life style. As a result, there is a shift towards increased lipid load and decreased exercise in routine life, compounded by the stress, shifting the population towards diabetes and cardiovascular diseases. Thus Genetic predisposition and changing lifestyle among the Asians has been attributed to the high risk of cardiovascular disease.

The main objective of this study was to evaluate whether the only cardioprotective molecule in the blood namely HDL is in any way affected even in the young individuals. To assess the functionality of HDL we have used PON 1 levels as an index, since PON 1 is susceptible to oxidative inactivation [16]. Assay of HDL associated enzymes provide a convenient method for evaluation of function of HDL. We have used the activity of PON as an index for functional HDL. The characteristics of the sample in our study group are shown in Table 1.

Age showed a normal distribution both with respect to males and females as well as the total sample. In this sample there was none diabetic but 6 were having some heart diseases(CHP) and were taking heart disease related medication. 5 had taken some medication, two for thyroid related problems and three for pain, none of the subjects taking medications was a smoker.

Table 1: Characteristics of the study sample

	Mean±SD	Range
Age (Yrs)	25.4±3.1	(20-39)
Sex	50F 238M	
SGPT Units /dl)	22.3±12.4	(5-90)
RBS (mg/dl)	76.9±8.7	(66-105)
Creatinine (Units/dl)	0.90±0.15	(0.5-1.3)
Smoking	13	(F2 M11)
Diabetes	nil	
Heart Disease	6	
Medication	5	
PON 1 Activity (Units/L)	53426±16504	(8397-78244)

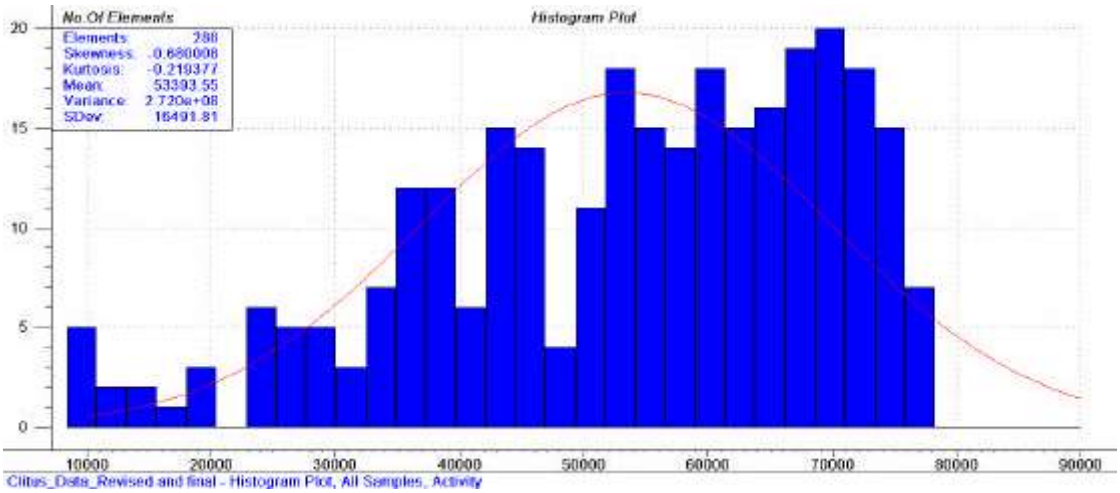

Fig. 1: Distribution of PON 1 activities. Histogram of distribution of PON 1 activity is shown in FIG 1

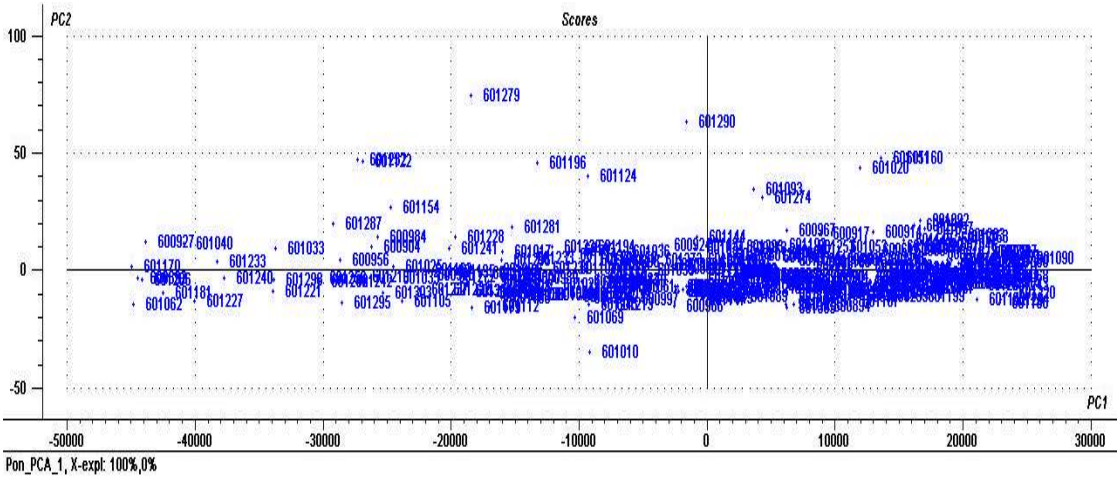

Fig. 2: Scatter plot of PON1 activities

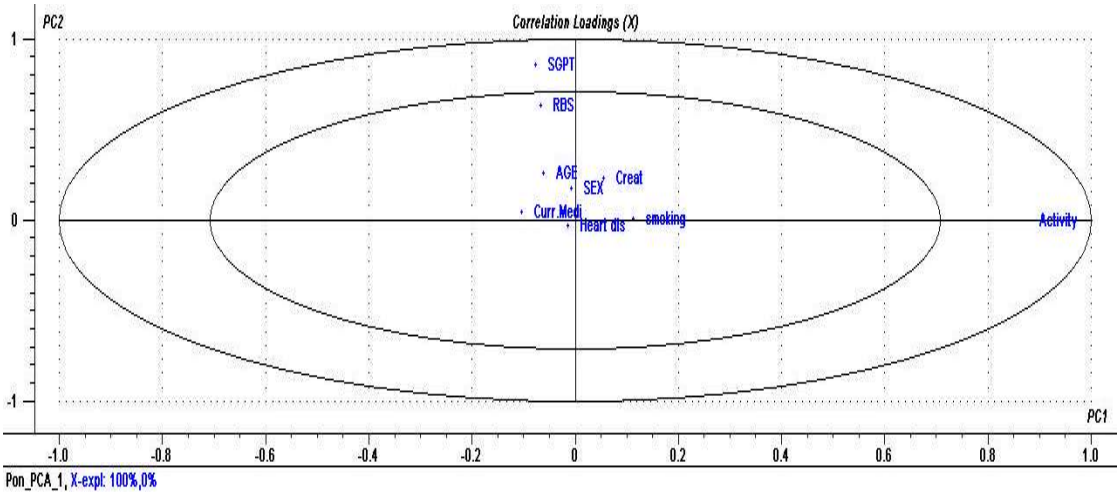

Fig. 3: Principal component analysis

Distribution of PON1: The PON activity of the sample population was similar to that reported in literature [17] however the sample distribution showed negative skewness. This indicated that the sample with lower values was more. In fact 127(44%) samples were below the mean.

The values of PON1 activity did not show any clustering. The X-Y plot of Principal component analysis is shown in FIG 3.

From these Figures, The PON 1 was found not to correlate with Age, Sex, or even smoking. Although this study was not designed to evaluate the effect of smoking on PON1 activity, he PON1 activity of smokers in the sample did not correlate with PON1.Although the sample size is small, it is interesting that these values did not cluster together. Smoking is one of the major risk factors for CVD, [18] and even though smoking has been shown to increase oxidative stress, it is interesting that it did not correlate with PON1. It is possible that the other stress factors, mainly oxidative stress would have affected the PON1 activity to such an extent that smoking did not affect it any further. This is consistent with the observation that the PON1 activity was negatively skewed.

Thus our results suggest that even at a young age, the PON1 activity among south Indians is already modified.

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