Original article:

Paraoxonase1 (pon1) and it's correlation with lipid ratios of atherogenicity in patients with type2 diabetes mellitus: a cross sectional study

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Abstract

Introduction: Both macrovascular and microvascular complications due to lipid peroxide-tion and atherogenesis cause significant morbidity and mortality among diabetic subjects. Paraoxonase1(PON1), a HDL-c associated glycoprotein, has been implicated in LDL-c peroxidation by it's peroxidase activitiy, by preventing homocysteinylation of Apo-B100 of LDL-c and by detoxification of the homocysteinethiolactone by it's thiolactonase activity. Therefore decreased levels of PON1 activity have been found in patients with type2 diabetes mellitus(T2DM) in studies related to both microvascular and macrovascular complications.

Methods: PON1 and fasting lipid profile were estimated and lipid ratios of atherogenicity, ie Atherogenic index of plasma(AIP), Castelli's risk index I and II(CRI-I,CRI-II) and Atherogenic coefficient(AC) were calculated in 30 T2DM patients without complications, 30 T2DM patients with complications, above 40 years of age and were compared with 30 age and sex matched controls after applying exclusion criteria. Paraoxonase1 and fasting lipid profile were estimated using BIO-RAD 680 ELISA microplate reader and MERCK microlab 300 Semiautoanalyser respectively considering p value **<0.05** as significant.

Results and observations: Patients with both T2DM with and without complications had significantly decreased levels of Paraoxonase1 (PON1) and significantly increased levels of AIP, CRI-I, CRI-II and AC compared to controls **(P<0.0001)**. Further, PON1 showed significant negative correlation with all the ratios of atherogenicity (**P<0.01**) in both T2DM patients with and without complications.

Conclusion: Hence low PON1 concentration could be an early sensitive marker of atherogenesis implicated in various microvascular and macrovascular complications associated in patients with T2DM and also in diabetic patients susceptible for having vascular complications in future.

Key Words- PON1, Fasting lipid profile, Atherogenic index of plasma(AIP), Castelli's risk index I and II(CRI-I & CRI-II), Atherogenic coefficient(AC)

Introduction-

The worldwide prevalence of Diabetes mellitus has risen dramatically over the past two decades presumably because of increasing obesity, reduced activity levels as countries become more industrialized, and the aging of the population [(1)]. Both macrovascular and microvascular complications, commonly due to atherogenesis, cause significant morbidity and mortality among diabetic subjects [(2)]. The prevalence of neuropathy was the most common complication (24.6%) followed by cardiovascular (23.6%), renal (21.1%), eye (16.6%) complications and foot ulcer (5.1%) in a recent study [(3)]. Several mechanisms have been proposed for the explanation of the antiatherogenic properties of high density lipoproteins (HDL-c) including enzymatic removal of lipid peroxide [(4)].

Among them paraoxonase1 (PON1) has raised a special interest.

Human serum Paraoxonase1 (PON1, EC3.1.8.1) a 43-kDa glycoprotein, synthesized primarily from liver has organophosphatase, arylesterase & lactonase activities [5]. Experiments have shown that PON 1 is closely ass-ociated with HDL subspecies that contains apo-A1 and clusterin [6]. **Kinumi T *et al*** [7] reported that the enzyme decreases accumulation of the lipid peroxides in low density lipoprotein (LDL-c) due to its ability to reduce hydroperoxides and attenuates biological effects of mildly oxidized LDL-c. The ability of PON1 to hydrolyze toxic peroxides in oxidized lipids of both LDL-c and HDL-c can thus reverse potential atherogenic effects [5].

Thus, Paraoxonase1, a glycoprotein associated with HDL-c, has been implicated in LDL-peroxidation by it's peroxidase activitiy, by preventing homocysteinylation of Apo-B100 of LDL-c [8] and by detoxification of the homocysteinethiolactone by it's thiolactonase activity [9].

Indices for atherogenesis-

Atherogenic index of plasma (AIP) [10], Castelli Risk Index-I (CRI-I) [11], Castelli Risk Index-II (CRI-II) [11] and Atherogenic Coefficient (AC) [12] are the four ratios commonly studied in predicting the risk of atherogenicity and CAD. Atherogenic Index of Plasma (AIP) is calculated by using two important parameters, triglyceride and HDL-c, both of which are independent risk factors for CAD [10]. Castelli Risk Index-I (CRI-I) calculated as (TC/HDL-c) [11], while Castelli Risk Index-II (CRI-II) as (LDL-c/HDL-c) [11], is another fraction which involves independent risk factors for CAD. Atherogenic Coefficient (AC) calculated as {(TC-HDLc)/HDLc} is yet another ratio relying on the significance of HDL-c in predicting the risk of CAD [12]. These are the calculated fractions which can be used in the clinical setting for early assessment of risk of atherogenicity and cardio-vascular disease beyond the routinely done fasting lipid profile.

Aims and objectives

The present study was done-

1) To determine serum Paraoxonase1 level and fasting lipid profile in patients with type2 diabetes mellitus with complications, type2 diabetes mellitus without complications and in healthy controls.
2) To determine lipid ratios of atherogenicity (atherogenic index of plasma, castelli's risk index-I, castelli's risk index-II and atherogenic coefficient) in patients with type2 diabetes mellitus with complications, type2 diabetes mellitus without complications and in healthy controls.
3) To analyze the results for a possible correlation between Serum Paraoxonase1 and lipid ratios of atherogenicity.

Materials and methods-

An analytical cross-sectional study was conducted on subjects of age above 40 yrs attending outpatient depart-ment (OPD) of Internal medicine and endocrinology department, Gauhati Medical College & Hospital after taking approval from institutional ethics committee. Total no of 60 diagnosed diabetic patients as per WHO and ADA criteria [13] were enrolled in the study and compared with 30 age matched normal normoglycemic controls. Informed consent duly signed by each of the participants was taken. Subjects were divided into three groups, control group, case group I and case group II.

1. **Control group** – This group contained age and sex-matched controls drawn from healthy population and attendents of the patients.
2. **Case group I** – This group includes patients with type 2 diabetes mellitus with duration < 8 years with HbA1c < 7%; on life style

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modifications and oral hypoglycemic drugs and free from clinical evidence of any complications.

3. **Case group II**– This group includes patients with type 2diabetes mellitus with duration > 8 years, on life style modifications, oral hypoglycemic drugs, insulin or combination of all three and associated with one or more microvascular or macrovascular complications (Diabetic nephropathy, Diabetic retinopathy, cardiovascular disease, diabetic neuropathy etc.) with HbA1c > 7% .

Biochemical parameters of fasting lipid profile (Total cholesterol, Triglyceride, HDL-c, LDL-C, VLDL-c and TC/HDL-c) and Paraoxonase1 were estimated in both case group I and case groupII and compared with healthy controls. Lipid ratios of atherogenicity were calculated as-

Atherogenic Index of Plasma (AIP) = log TG/HDLc [(10)]

Castelli's Risk Index (CRI-I) = TC/HDLc [(11)]

Castelli's Risk Index (CRI-II) = LDLc/HDLc [(11)]

Atherogenic Coefficient (AC) = (TC–HDLc)/HDLc [(12)]

Patients with type 1 Diabetes Mellitus, Myocardial infarction, Renal disease (except diabetic nephropathy), Car-diovascular disease (not associated with type2 diabetes mellitus), Hepatic disease, Infectious disease, Alcoh-olics and Smokers were excluded from the study group. Taking aseptic and antiseptic precautions, about 6 ml of fasting blood was collected by venous puncture and transferred to appropriate sterile vials. For estimation of glycated hemoglobin and fasting plasma glucose, blood was collected in EDTA and fluoride vials respective-ly while for estimation of Paraoxonase1 and fasting lipid profile, blood was collected without any anticoagulant. The serum/plasma was seperated by centrifugation at 3000 r.p.m. for 5 minutes in a centrifuge machine. The supernatant serum/plasma was used for the investigations or transferred to clean dry vials for storage at -80^{o} C if the estimations were not done at the same sitting.

Estimations of Serum Paraoxonase1 was done by using BIO-RAD 680 ELISA microplate reader version 1.7 while Fasting plasma glucose, Glycated hemoglobin, and fasting lipid profile were done using MERCK micro-lab 300 Semiautoanalyser. Serum Paraoxonase1 was measured quantitatively by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) using QAYEE-BIO human paraoxonase1 ELISA kit [(14)], Fasting plasma glucose based on Trinder's GOD/POD method by using Glucose kit of Coral Clinical Systems [(15)], Glycated haemoglobin based on cation exchange resin method by using the glycated hemoglobin kit of MEDS-OURCE OZONE BIOCHEMICAL PVT. LTD. [(16)], cholesterol by CHOD- PAP method as derived by **Allain *et al*** [(17)], triglyceride according to GPO-PAP method as derived by Bucolo & David [(18)] and Plasma HDL-c was estimated by the HDL-Cholesterol precipitation kit of AUTOPAK SIEMENS LTD based on the Phosphot-ungstate method [(19)]. Calculation of serum LDL-c (mg/dl) and VLDL-c (mg/dl) were done using Freidewalds Formula[(20)].

Statistical analysis-

Data were expressed as **mean ± SD**. ANOVA tests were used to analyze differences in baseline characteristics and biochemical parameters between the control and the test groups. Correlations were observed by using Pears-on's correlation coefficient and probability (p value) **< 0.05** was considered significant. Statistical analysis was done using GraphPad InStat version 3.00. All the statistical graphs were prepared using Microsoft Excel 2007.

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Observations and results

On comparing the biochemical characteristics, the decrease of mean levels of Paraoxonase1 (**PON1**) and high density lipoprotein cholesterol (**HDL-c)** were found to be highly significant statistically **(p<0.0001)** while increase of mean levels of Fasting Plasma Glucose (**FPG**), Glycated haemoglobin (**HbA1c**), Triglyceride (**TG**), Total cholesterol (**TC**), Low density lipoprotein cholesterol (**LDL-c**), Very low density lipoprotein cholesterol (**VLDL-c)** and **TC/HDL-c** ratio were also found to be highly signifcant statistically in both Case group I and Case group II compared to controls **(p<0.0001)** (**Table 1**). On calculating and comparing the lipid ratios of athe-rogenicity, we found that levels of Atherogenic index of plasma (AIP), Castelli's risk index I (CRI-I), Cast-elli's risk index II (CRI-II) and Athrogenic coefficient (AC) were significantly increased statistically in both diabetic patients with and without complications (Case group II and Case group I respectively) compared to control group (**P<0.0001**) (**Table 2, Figure 3-5**). Further, on applying pearson's correlation, it was found that PON1 had statistically significant negative correlation with all the calculated ratios of atherogenicity (AIP, CRI-I, CRI-II, AC) of both case group I and case group II (**P<0.01**) (**Table 6, Figure 7-10**).

Discussion

From our study, we found a highly significant decrease of mean level of PON1 in both the diabetic groups (case group I and case group II) compared to healthy controls (**p<0.0001**). This findings were in corroboration with the findings of **El-Lebedy *et al.*[21],** and **Suvarna R *et al* [22].,** who observed decreased level of PON1 or its dec-reased activity or both in patients with type2 DM and type2 DM associated with complications compared to healthy controls. It was also observed that mean levels of high density lipoprotein cholesterol (**HDL-c)** were found to be decreased significantly (**p<0.0001**) while all other components of fasting lipid profile were signifc-antly increased in both Case group I and Case group II compared to controls (**p<0.0001**). The findings of our study probably signifies dyslipidemia and in corroboration with other studies that showed inverse relationship between TG and HDL-c and that high TG to HDL-c ratio is a strong predictor of infarction [23]**.** Atherosclerosis is the major threat to the macrovasculature for patients with and without diabetes and dyslipidemia is highly correlated with atherosclerosis [24]**.**

In the present study, the lipid ratios of atherogenicity (AIP, CRI-I, CRI-II and AC) were found to be higher in both case groupI and case groupII compared to healthy controls **(p<0.001)**. Also a significant correlation existed between levels of PON1 and the above mentioned lipid ratios in both case groupI and case groupII.The findings of our study corroborates with the findings of **Patil M *et al.*[25]**who found a significant increase of these lipid indices in type2 DM with CAD when compared with healthy controls. **Patra S.K *et al* .[26]** also found that PON1 was significantly correlated with the ratios of components of lipid profile.

AIP is being used by some practitioners as a significant predictor of atherosclerosis. AIP values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high Cardiovascular risk [27]**.** Studies have shown the association of TC/HDL-c ratio with coronary plaques formation [28]**.** CRI-I in our both case groups were > 4 and CRI-II was also found to be above the upper limit for normal range i.e. >3 in case group II as observed in other studies [29]**.** Atherogenic Coefficient (AC), calculated as {(Non-HDL-c)/HDL-c} or {(TC-HDL-c)/HDL-c} and

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studies have shown Non-HDL-c being similar to Apo-B in assessing atherogenic choles-terol and lipoprotein burden [30].

Therefore, significantly increased levels of lipid indices in case group II compared to case group I and healthy controls and it's significant negative correlation with PON1 might reflect impairment of anti-atherogenetic mechanism mediated by HDL-c in patients with type2 DM with complications and might establish the role of atherogenesis as the major contributing factor in the development of chronic complications related to diabetes. Also, statistically significant negative correlation of PON1 concentration with all the calculated lipid ratios of atherogenicity might establish decrease PON1 concentration as an early sensitive marker of atherogenesis in type2 diabetic patients. Interestingly, increased levels of all these ratios of atherogenicity in type2 diabetic patients without complications (case group I) compared to controls and their statistically significant negative correlation with level of PON1 might reflect their susceptibility to have micro or macrovascular complications in near future.

Limitations of the study: Small sample size was the limitation of our study.

Conclusion

Diagnosis of complications associated with diabetes in the early stages is very important to decrease the morbidity and mortality associated with type2 diabetes mellitus. PON1 in our study was found to be decreased significantly in both diabetic patients with and without complications compared to normal healthy controls. Hence low PON1 concentration could be an early sensitive marker of atherogenesis implicated in various microvascular and macrovascular complications associated in patients with type2 DM and also in diabetic patients susceptible for having vascular complications in future. However a more elaborate study with large sample size is desirable to precisely establish the role of PON1 as an early sensitive biomarker of atherogenesis in type2 diabetics in future.

Table 1: Showing comparisons between the biochemical parameters of the studied groups.

Parameters	**Control Group** (mean± SD)	**Case group I** (mean± SD)	**Case group II** (mean± SD)	**P value**
FPG(mg/dL)	90.43± 9.86	149.26± 11.97	246.53± 77.48	<0.0001**
HbA_1c (%)	5.11± 0.28	6.73± 0.16	10.31± 2.18	<0.0001**
PON1(ng/ml)	283.93± 86.66	138.86± 17.67	109.72± 16.13	<0.0001**
HDL-c (mg/dL)	49.16± 8.36	39.66± 6.71	34.03± 4.78	<0.0001**
TG (mg/dL)	84.13± 14.68	143.23± 21.92	172.1± 29.41	<0.0001**
TC(mg/dL)	139.30±13.79	177.30±12.53	240.63±44.62	<0.0001**
LDL-c (mg/dL)	73.23± 13.85	102.03± 16.48	165.43± 40.18	<0.0001**
VLDL-c (mg/Dl)	16.86± 2.95	28.30± 4.77	35.13± 5.29	<0.0001**
TC/HDL-c	2.88±0.50	4.49±0.96	7.10±2.26	<0.0001**

ANOVA test were used for comparison of means between the three groups. **FPG**- Fasting plasma glucose, **PON1**- paraoxonase1, **HbA_1c**-glycated hemoglobin **HDL-c-** High density lipoprotein cholesterol, **TG-** Triglyceride, **TC**- Total cholesterol, **LDL-c-** Low density lipoprotein cholesterol, **VLDL-c**- Very low density lipoprotein cholesterol.

** signifies highly significant p value .

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Table 2: Showing comparison between the ratios of biochemical parameters of fasting lipid profile of case group I and case group II.

Lipid ratios	Control group (mean ±SD)	Case group I(Mean ±SD)	Case group II(mean ±SD)	P Value
AIP	-0.05±0.089	0.26±0.108	0.40±0.11	<0.0001**
CRI-I	2.88±0.50	4.49±0.96	7.10±2.26	<0.0001**
CRI-II	1.55± 0.46	2.69± 0.81	4.99± 1.63	<0.0001**
AC	1.89±0.51	3.47±0.96	6.05±1.82	<0.0001**

ANOVA test were used for comparison of means between the three groups. ** denotes highly significant p value when three groups were compared.

AIP- Atherogenic index of plasma**, CRI-I-** Castelli' risk index-I, **CRI-II-** Castelli's risk index –II, **AC-** Atherogenic co-efficient.

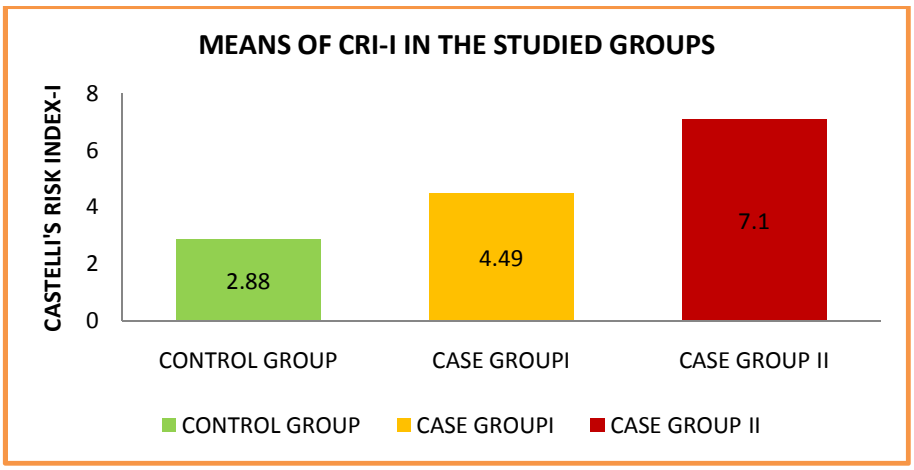

Figure 3: Showing CRI-I means in the control and case groups

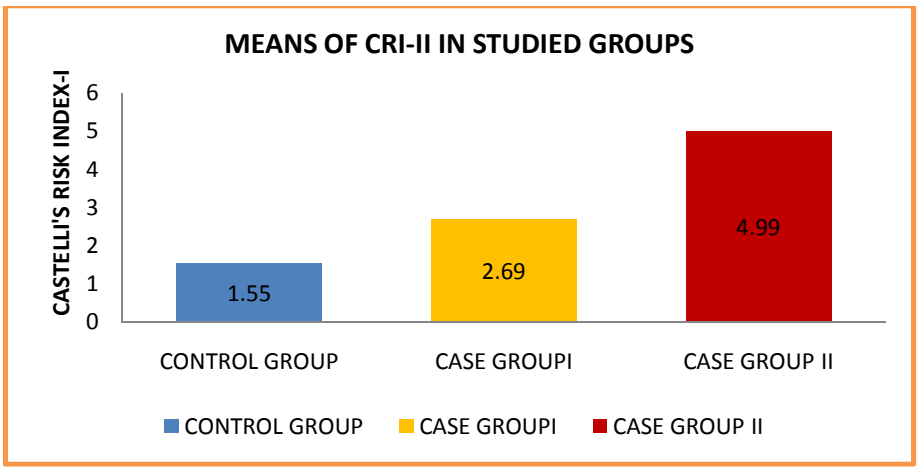

Figure 4: Showing CRI-II means in the control and case groups

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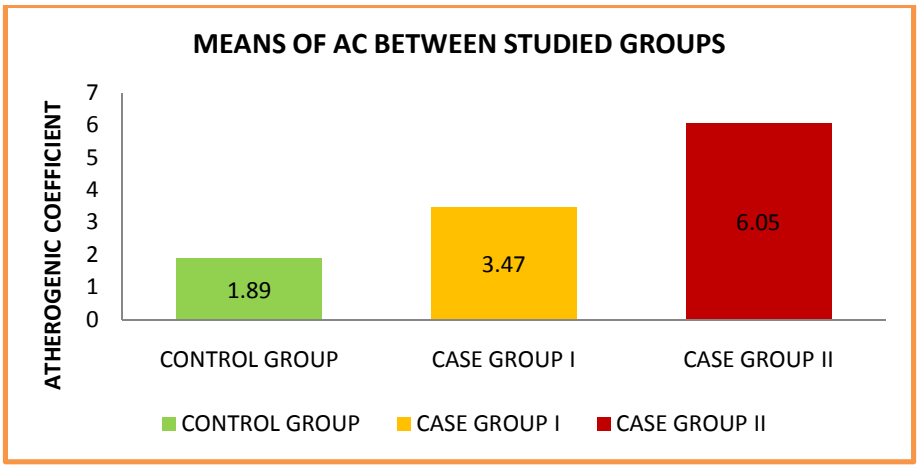

Figure 5: Showing AC means in the control and case groups

Table 6: Showing Pearson's correlation of Paraoxonase1 (PON1) with various other biochemical indices of atherosclerosis in the study groups.

	PARAMETERS	CASE GROUP I		CASE GROUP II	
PON1	AIP	r= -0.56	p=0.0012*	r=-0.54	P=0.002**
	CRI-I	r= -0.62	P=0.0002**	r=-0.48	P=0.007*
	CR-II	r=-0.63	P=0.0002**	r=-0.53	P=0.002*
	AC	r=-0.61	P=0.0003**	r=-0.56	P=0.001*

AIP-atherogenic index of plasma**, CRI-I**- castelli's risk index-I, **CR-II-**castelli's risk index-II **AC-** atherogenic coefficient ***** denotes Significant correlation and ** denotes highly significant correlation between Paraoxonase1 and measured parameters in the studied groups.

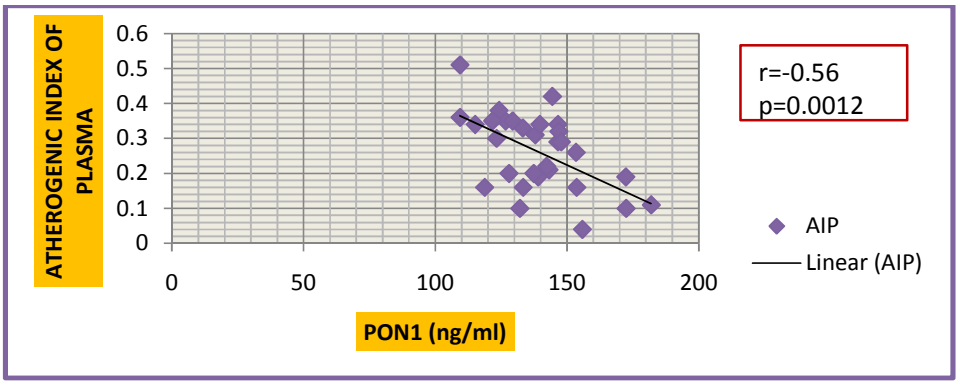

Figure 7: Showing correlation of PON1 with AIP in diabetic patients without complications (case group I)

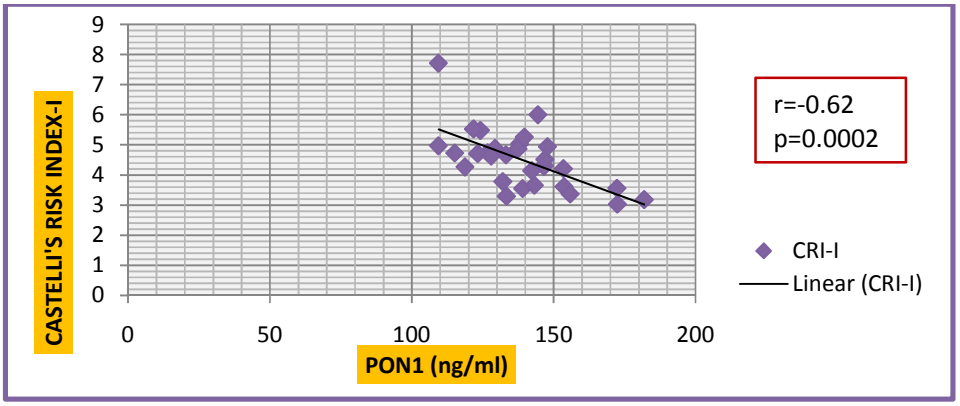

Figure 8: Showing correlation of PON1 with CRI-I in diabetic patients without complications (case groupI)

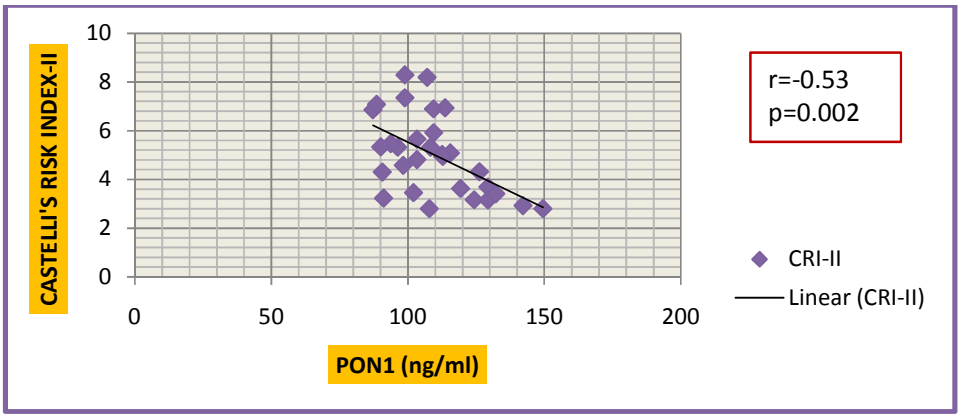

Figure 9: Showing correlation of PON1 with CRI-II in diabetic patients with complications (case group II)

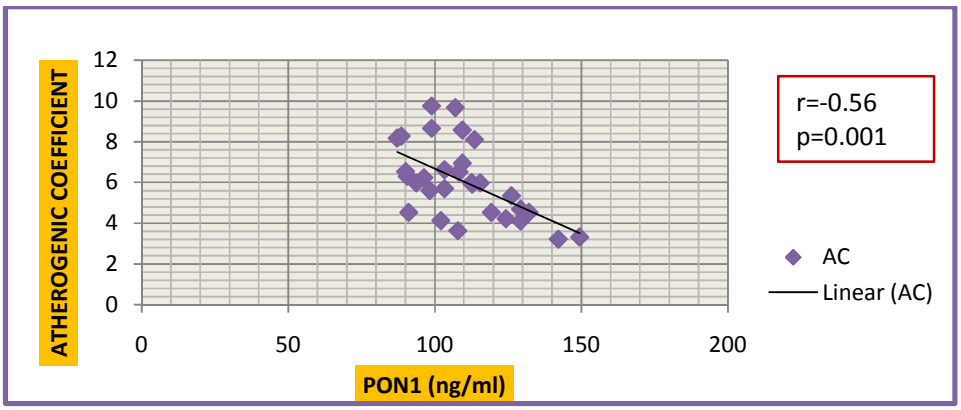

Figure 10: Showing correlation of PON1 with AC in diabetic patients with complications (case group II)

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