

A study of Paraoxonase-1 levels and other related parameters in sera of Iraqi non diabetic male with Hyperlipidemia

Dr. Tariq M.Ali*, Dr. Zeinab M. M. Al-Rubaei*,
Ban mahmood Shaker Al-joda**

*Chemistry Department, College of Education For Pure Science /Ibn Al-Haitham ,Baghdad University.

** Chemistry Department, College of medicine , Babylon University .

Abstract: The aim of the present study is to evaluate the levels of paraoxonase-1 in sera of male with hyperlipidemia divided according to Fredrecson's classification and to find the correlation of paraoxonase-1 levels with lipid profile in these patients. The study included (22) healthy Iraqi male as control group (G1) and (62) male diagnosed with hyperlipidemia which divided into three groups according to Fredrecson's classification as follows :Group (2) consist of (20) male with hypercholesterolemia [Type I] , group(3) consist of (20) male with hypertriglyceridemia [Type Iia]and Group (4):consist of (22)male with hypercholesterolemia and hypertriglyceridemia [Type Iib] .The age of all studied groups ranged between (21-50)years and BMIwith (19.8-24.3) Kg/m².Serum was used in determination of FBS, lipid profile , and paraoxonase-1. The results revealed no significant elevation in FBG levels were seen in patients groups when comparing to healthy control .The results indicates a significant elevation in TC and LDL in G2 and G4 comparing to G1. Also , there are significant elevation in G3 comparing to G 2 and G4 comparing to G3 ,while no significant elevation was found in TC and LDL in G3 with G1and G4 with G2.The results ,also, illustrated significant elevationin TG and VLDL in G3 and G4 when comparing to G1, and in G3,G4 comparing to G2 . No significant elevation was noticed in G2 comparing to G1and G4 comparing to G3 . HDL levels showed significant decrease in G2,G3 and G4comparing to G1. Also ,the results showed significant elevation in paraoxonase-1 levels in G2 , G3,G4 when comparing to G1,while no significant elevation was observed in G4 comparing to G2,G3 .A significant positive correlation was found between paraoxonase-1 and TC, in G1 while highly significant positive correlation was noticed in G2 between paraoxonase-1 and TC, also significant negative correlation was observed between paraoxonase-1 and TC in G3 but highly significant positive correlation was found in G4. A significant positive correlation was found in G1,G2 and G3 between paraoxonase-1 and TG while significant negative correlation was seen in G4 between paraoxonase-1 and TG .The results ,also found significant positive correlation between paraoxonase-1 and HDL in G1 and G3 while highly significant positive correlation was observed in G2 and G4. A significant negative correlation was found between paraoxonase-1and LDL in G1 ,G2 and G3 while significant positive correlation was seen in G4.Also the results revealed significant positive correlation between paraoxonase-1 and VLDL in G1,G2 and G3 while significant negative correlation was found in G4 between paraoxonase-1 and VLDL .

The conclusion from this study indicate that G2 which cholesterol is high in this group has the highest value for paraoxonase-1 . Also , the study found correlation between and lipid profile in all groups of hyperlipoproteinemia.

Keywords: Paraoxonase-1 and hyperlipoproteinemia

I. Introduction

Paraoxonase (aryldialkylphosphatase) is an enzyme having both paraoxonase and aryl esterase activity⁽¹⁾. It hydrolyzes aromatic carboxylic acid esters and certain organophosphorous pesticides, especially paraoxon and nerve gas⁽²⁾. Plasma paraoxonase (PON) activity in human population demonstrates polymorphic distribution due to an amino acid substitution in the active site of the enzyme, giving rise to low-, intermediate-, or high-activity isoenzymes⁽³⁾.

Paraoxonases-1 were originally discovered as enzymes hydrolyzing exogenous toxic organophosphate compounds such as insecticide paraoxon. There are three members of paraoxonases-1 family currently known: Paraoxonase-1 (PON1), Paraoxonase-2 (PON2), and Paraoxonase-3(PON3), which are encoded by three separate genes on the same chromosome-7 (human) or chromosome -6 (mouse) . PON1 protein All three human members of the family are (70%) identical at nucleotide level and(60%) identical at the amino acid level. PON-1 (EC 3.1.1.2) which consists of (354)amino acids with molecular mass(43 k Da) is a calcium-dependent glycoprotein that is present bound to HDL particles. Investigators have attempted to demonstrate that serum paraoxonase-1 decreases the risk of coronary artery disease by destroying proinflammatory molecules involved in the initiation and progression of atherosclerotic lesions^(4,5). The antiatherogenic potential of paraoxonase-1 is

derived from its capacity to hydrolyze oxidized lipids and phospholipids, thus preventing them from accumulating in LDL particles . Mutations in the human PON1 gene have been associated with aging and diseases of the cardiovascular , nervous , endocrine and gastrointestinal systems ⁽⁶⁻⁸⁾. A possible mechanism for these phenotypes may be related to HDL-dependent and independent antioxidant properties of PON1 ^(9,10) and its effects on levels of hydroperoxides and platelet activating factor, which may also affect oxidative stress in tissues ^(11, 12). PON1 can also hydrolyze acyl -homoserine lactones used by quorum –sensing bacteria to regulate multiple virulence factors during colonization of new environments ⁽¹³⁾ .

The present study aimed to determined the levels of paraoxonase-1 in male with hyperlipidemia in all patients groups and compare it with healthy control subject with aged ranged (21-50) years and BMI with (19.8- 24.3) Kg/m² .Also, to find the correlation between paraoxonase-1 and lipid profile in all the patients groups .

II. Material & Methods

This study included eighty four male with aged ranged (21-50) years and BMI ranged between (19.8-24.3) Kg/m². Subjects were divided into three groups : group(1) consist of (22) male as healthy control . Patient divided into (3) groups according to Fredrecsons classification : according to Fredrecsons classification : Group (2): consist of (20) non diabetic male with hypercholesterolemia [Type I] . Group (3): consist of (20) non diabetic male with hypertriglyceridemia [Type IIa] . Group(4): consist of (22) non diabetic male with hypercholesterolemia and hypertriglyceridemia [Type IIb] .

The patients attended the Ibn –Al Naphes hospital in Baghdad and Al-Hilla General Teaching Hospital in Babylon for the period from January 2014 to August 2014. Ten milliliters of blood were collected after an overnight fasting from all subjects by venipuncture . A licate of (0.5 ml) of whole blood was used in determination of HbA1C . The other part was left in 37C° for (15min) to clot then centrifuged at (3000 rpm) for (25min). The serum which obtained was freezed until analysis of lipid profile and paraoxonase-1 .

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (GOD) ⁽¹⁴⁾. Total serum cholesterol determined by utilizing a kit based on the enzymatic hydrolysis ⁽¹⁵⁾. The absorbance was recorded for the quinonimine (red complex) at 500 nm .

The determination of TG based on the enzymatic hydrolysis . The intensity of the color formed is proportional to the triglycerides concentration in the sample .

The Chylomicrons and lipoproteins of VLDL, and LDL contained in the serum sample were precipitates by the addition by the addition of (4%) phosphotungstic acid solution, which contain (10%) magnesium chloride (PH 6.2) . The supernatant obtained after centrifugation contains the HDL, from which the cholesterol can be determined by complementary kit used in determination of total serum cholesterol as described in reference ⁽¹⁶⁾ .

LDL-cholesterol and VLDL were estimated indirectly by using Friedewald formula ⁽¹⁷⁾:

$$\text{LDL-c} = \text{Total Cholesterol} - (\text{HDL-c} + \text{VLDL-c})$$

$$\text{VLDL-c (mg/dl)} = \text{TG}/5$$

PON-1 levels were determined by using ELISA technique . Antibody specific for PON-1 has been pre-coated onto a micro plate . Standards and samples are pipetted into the wells and any PON-1 present is bound by the immobilized antibody . After removing any unbound substances, a biotin–conjugated antibody specific for PON-1 is added to the wells . After washing , avidin conjugated Horseradish peroxidase (HRP) is added to the wells . Following a wash to remove any unbound avidin–enzyme reagent, a substrate solution is added to the wells and colors develops in proportion to the amount of PON-1 bound in the initial step . The color development is stopped and the intensity of the color is measured ⁽¹⁸⁾ .

All parameters were expressed as (mean±SD). T-test was used for comparison among the four studied groups . The P-values < 0.05 and < 0.001 were considered statistically significant and high significant, respectively.

III. The Results :

Analytical parameters :

The mean ± SD and T-test of descriptive parameters for male in all studied groups are presented in table (1) .

Table(1) : Descriptive parameters for all studied groups

Groups Parameters	G1 n=22	G2 n=20	G3 n=20	G4 n=22	T-test G1vs G2	T-test G1vs G3	T-test G1vs G4	T-test G2vs G3	T-test G2vs G4	T-test G3vs G4
FBS (mg/dl)	88.18 ± 10.5	93.25 ± 7.34	90.7 ± 8.36	91.27 ± 10.5	NS	NS	NS	NS	NS	NS

TC (mg/dl)	135.36± 29.88	223 ± 10.53	175 ± 25.73	231 ± 26.75	NS	NS	NS	NS	NS	NS
TG (mg/dl)	109 ± 17.8	130 ± 14.7	240 ± 41.7	302 ± 87.2	NS	S	S	S	S	NS
HDL (mg/dl)	51.59 ± 8.8	43.7 ± 10.5	33.5 ± 5.32	40.45 ± 8.36	S	S	S	NS	NS	NS
LDL (mg/dl)	92.2 ± 25.4	157.1 ± 20	94.32 ± 31.7	130.0 ± 20.2	S	NS	S	S	NS	S
VLDL (mg/dl)	21.8 ± 7.13	26.01 ± 5.84	48.1 ± 10.71	60.5 ± 14.9	NS	S	S	S	S	NS
Paraoxonase1 (ng /dl)	118.4 ±27.7	247.2 ±12.4	159.5 ±12.9	209.9 ±51.6	S	S	S	NS	NS	NS

*(S) significant differences , (NS) no significant differences .

The results revealed no significant elevation in FBG levels was found in patients groups when comparing to healthy control . The results indicate significant elevation in TC and LDL in G2 and G4 comparing to G1. Also , there are significant elevation in G3comparing to G2 and G4 comparing to G3,while no significant elevation was found in TC and LDL in G3 with G1and G4 with G2 .The results , also, illustrated significant elevation in TG and VLDL in G3 and G4 when comparing to G1, and in G3,G4 comparing to G2 . No significant elevation was noticed in G2 comparing to G1and G4 comparing to G3. HDL levels showed significant decrease in G2,G4comparing to G1, but HDL levels showed significant decrease inG3comparing to G1.No significant increase was seen in G3 and G4 comparing to G2 and in G4 comparing to G3.

The investigation revealed that level of circulating HDL cholesterol as a disease risk marker, genetic mechanisms that influence HDL level and disease incidence, or the impact of an intervention to increase HDL on clinical outcome, it remains uncertain whether HDL cholesterol directly impacts atherosclerosis and the risk of cardiovascular events. From a mechanistic perspective , HDL classically functions in reverse cholesterol transport , removing cholesterol from peripheral tissues and cells such as macrophages, and delivering it to the liver and steroid genic organs by binding of the major HDL Apo lipoprotein , Apo lipoprotein (Apo) A-I, to the high-affinity HDL receptor scavenger receptor, class B, type I ^(19,20) .The antiatherogenic function of HDL particles that is most commonly appreciated relates to its ability to promote the efflux of cholesterol from cells, a capacity that is generally attributed to apoA-1⁽²¹⁾ .

Oxidized low-density lipoprotein(ox-LDL) has a wide range of biological properties including up regulation of inflammatory genes, increased expression of adhesion molecules on endothelial cells , monocyte chemotaxis and destabilization of plaques ⁽²²⁾ .Ox-LDL is also associated with LDL aggregation in vivo and coronary artery cell toxicity in vitro ⁽²³⁾ as shown in figure (1) . High-density lipoprotein plays an important role as an antioxidant by both inhibiting phospholipid oxidation within and reducing the activity of minimally modified LDL ⁽²⁴⁾ .

The results also showed significant elevation in paraoxonase-1 levels in G2,G3,G4 when comparing to G1,while no significant elevation was observed in G4 with G2,G3 and in G3 when comparing to G2 . Paraoxonase-1 is an esterase and lactonase that is found in the circulation bound to high-density lipoproteins (HDL) . The original function of PON1 was that of a lactonase ; lipophylic lactones constituting its primary substrates . PON1 is thought to degrade oxidized phospholipids in lipoproteins and play an important role in the organism's antioxidant system ⁽²⁵⁻²⁷⁾ . It has crucial roles in protecting LDL against oxidation and detoxification of highly toxic substances ^(100,101) .Paraoxonase-1 shows its effect by suppressing the receipt of the oxidized low-density lipoprotein with macrophages, preventing the oxidation of the lipid peroxides , providing the increase of flow of the cholesterol out of the cell , and by preventing foam cell formation ⁽³⁰⁻³²⁾ . Both in vitro and in vivo , the effect of VLDL , or of pure triglycerides , on high-density lipoprotein HDL-associated paraoxonase-1catalytic activities ⁽³³⁾ . Recent studies demonstrated that PON-1 may have a protective role against atherosclerosis by virtue of its action on hydrolyzing lipid peroxides and preventing accumulation of phospholipids in oxidized low-density lipoprotein (LDL) ⁽³⁴⁾ .

Other study explained that there are three major players regulate atherosclerosis development ;paraoxonase-1(PON1),antioxidants , and HDL^(35,36) . PON1 protects against macrophage-mediated LDL oxidation , and increases HDL binding to macrophages which stimulates HDL ability to promote cholesterol efflux . These two major anti-atherogenic properties of HDL (and of PON1) require macrophage binding sites for HDL-associated PON1 . PON1 in addition to HDL-associated PON1 , specifically binds to macrophage PON1 binding sites may thus be a target for future cardio protection therapy . Studying the interaction among PON1 ,antioxidant ,and macrophages can thus assist in achieving appropriate treatment and prevention of atherosclerosis ^(37, 38) .

IV. Relationship between paraoxonase-1 and lipid profile :

Table (2) illustrated correlation coefficient and P-value for paraoxonase-1 and lipid profile for all studied groups .

Table (2) : Correlation coefficient and T-test between paraoxonase-1 levels and other parameters for all studied groups

groups Parameters	G1		G2		G3		G4	
	r	T-test	r	T-test	r	T-test	r	T-test
Paraoxonase-1 vs TC	0.018	S	0.389	HS	-0.0712	S	0.030	HS
Paraoxonase-1 vs TG	0.132	S	0.302	S	0.435	S	- 0.069	S
Paraoxonase-1 vs HDL	-0.282	S	-0.032	HS	-0.442	S	0.256	HS
Paraoxonase-1 vs LDL	-0.029	S	-0.235	S	- 0.328	S	0.0182	S
Paraoxonase-1 vs VLDL	0.132	S	0.302	S	0.435	S	- 0.069	S

*(S) Significant differences , (HS) highly significant differences .

A significant positive correlation was found between paraoxonase-1 and TC in G1($r_1 = 0.0182$) as shown in figure (1 A), while highly significant positive correlation was noticed in G2 between paraoxonase-1 and TC ($r_2 = 0.389$) as shown in figure (1 B), also significant negative correlation was observed between paraoxonase-1 and TC in G3($r_3 = -0.071$) as shown in figure (1 C) , but highly significant positive correlation was found in G4 between paraoxonase-1 and TC ($r_4 = 0.0309$) as shown in figure (1 D) .

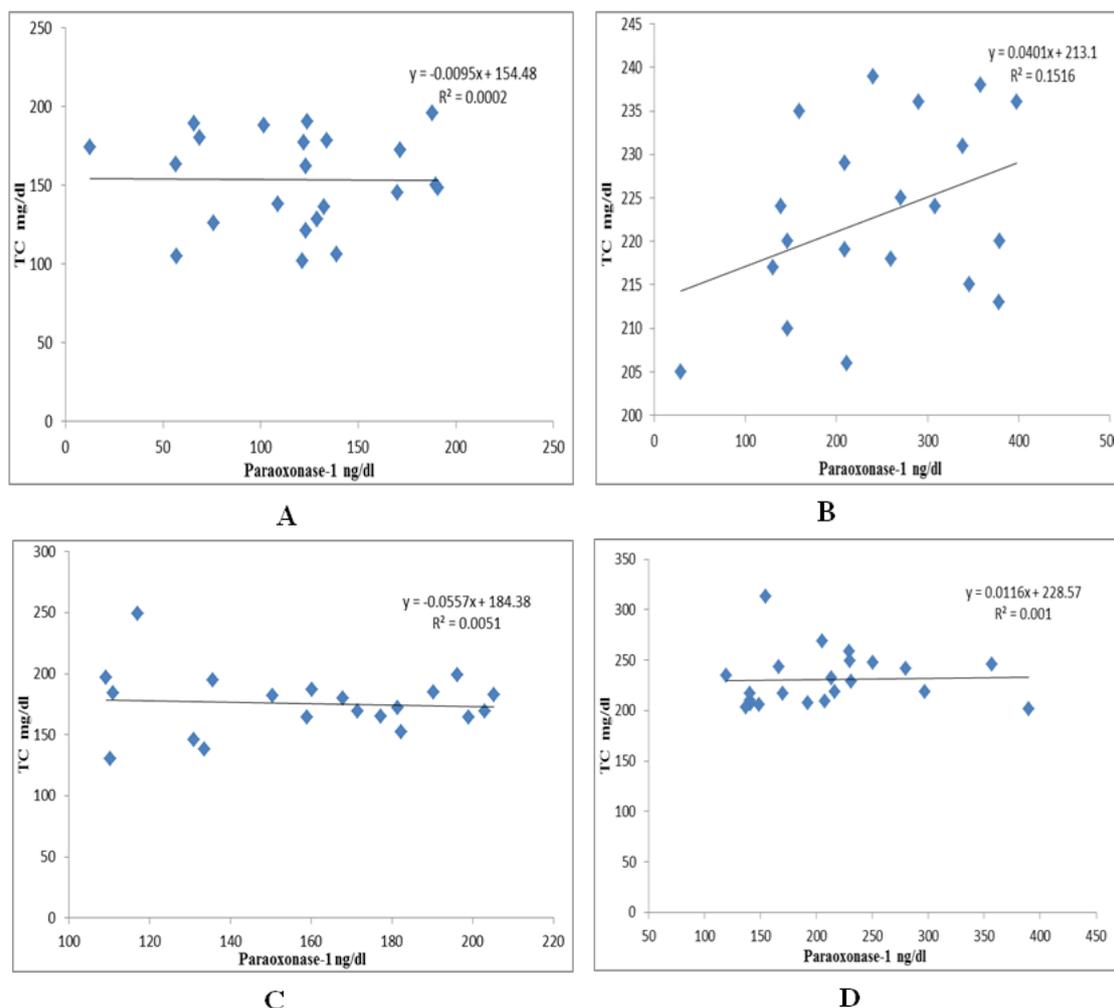


Figure (1) Correlation between paraoxonase-1 and TC in G1(A) , G2(B) , G3(C) , G4(D)

A significant positive correlation was found in G1,G2 and G3 between paraoxonase-1 and TG ($r_1 = 0.1322$, $r_2 = 0.302$ and $r_3 = 0.435$) respectively, as shown figures (2 A,B and C) ,while significant negative correlation was seen in G4 between paraoxonase-1 and TG ($r_4 = - 0.0695$) as shown in figure (2 D) .

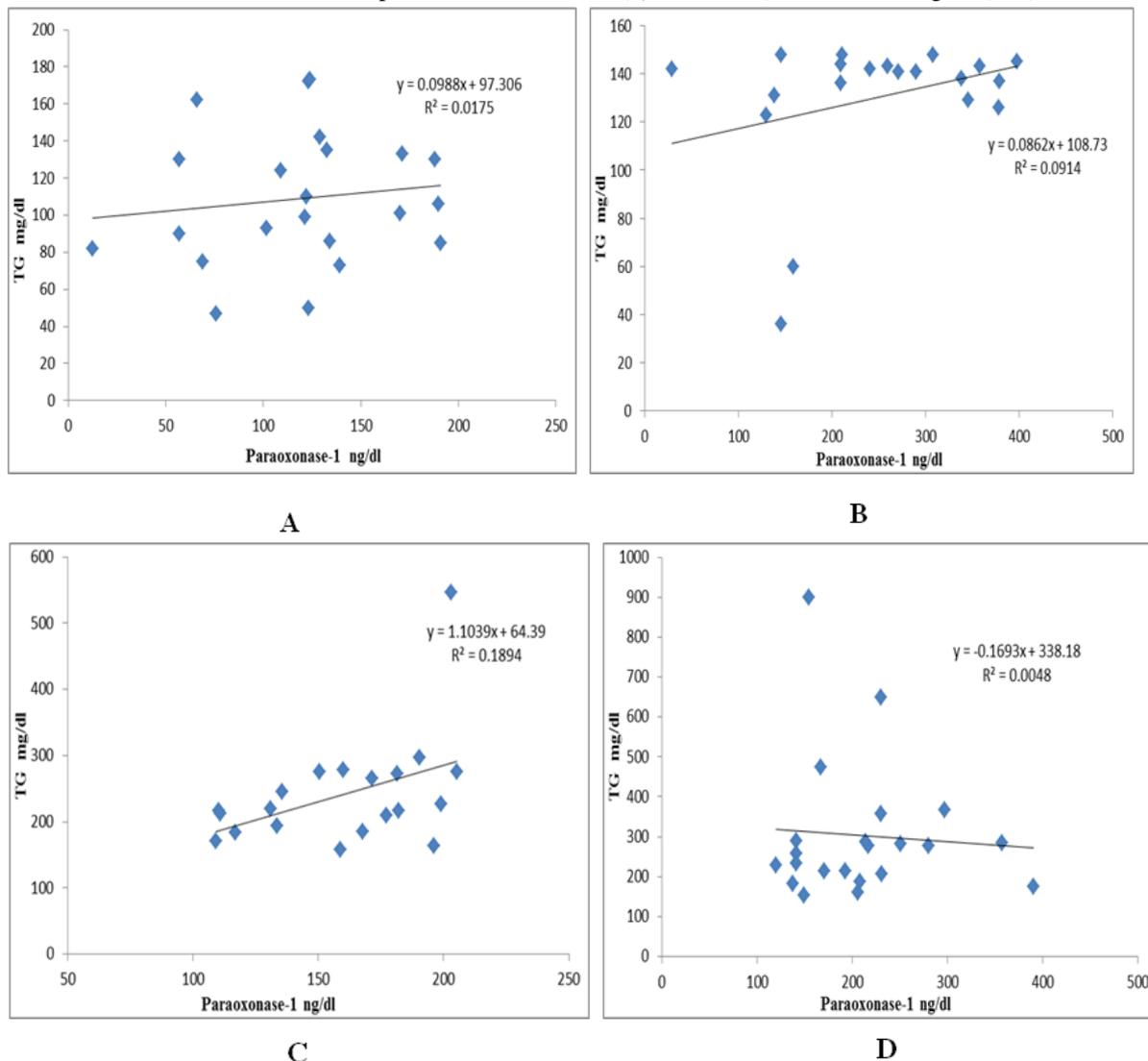


Figure (2) Correlation between paraoxonase-1 and TG in G1(A) , G2(B) , G3(C) , G4(D)

The results ,also, showed significant positive correlation between paraoxonase-1 and HDL in G1and G3 ($r_1= 0.0263$, $r_3 =0.1667$) respectively, as shown in figures (3A,C) , while highly significant positive correlation was observed between paraoxonase-1 and HDL in G2 and G4 ($r_2 = 0.363$, $r_4 = 0.345$) respectively ,as shown in figures (3 B,D) .

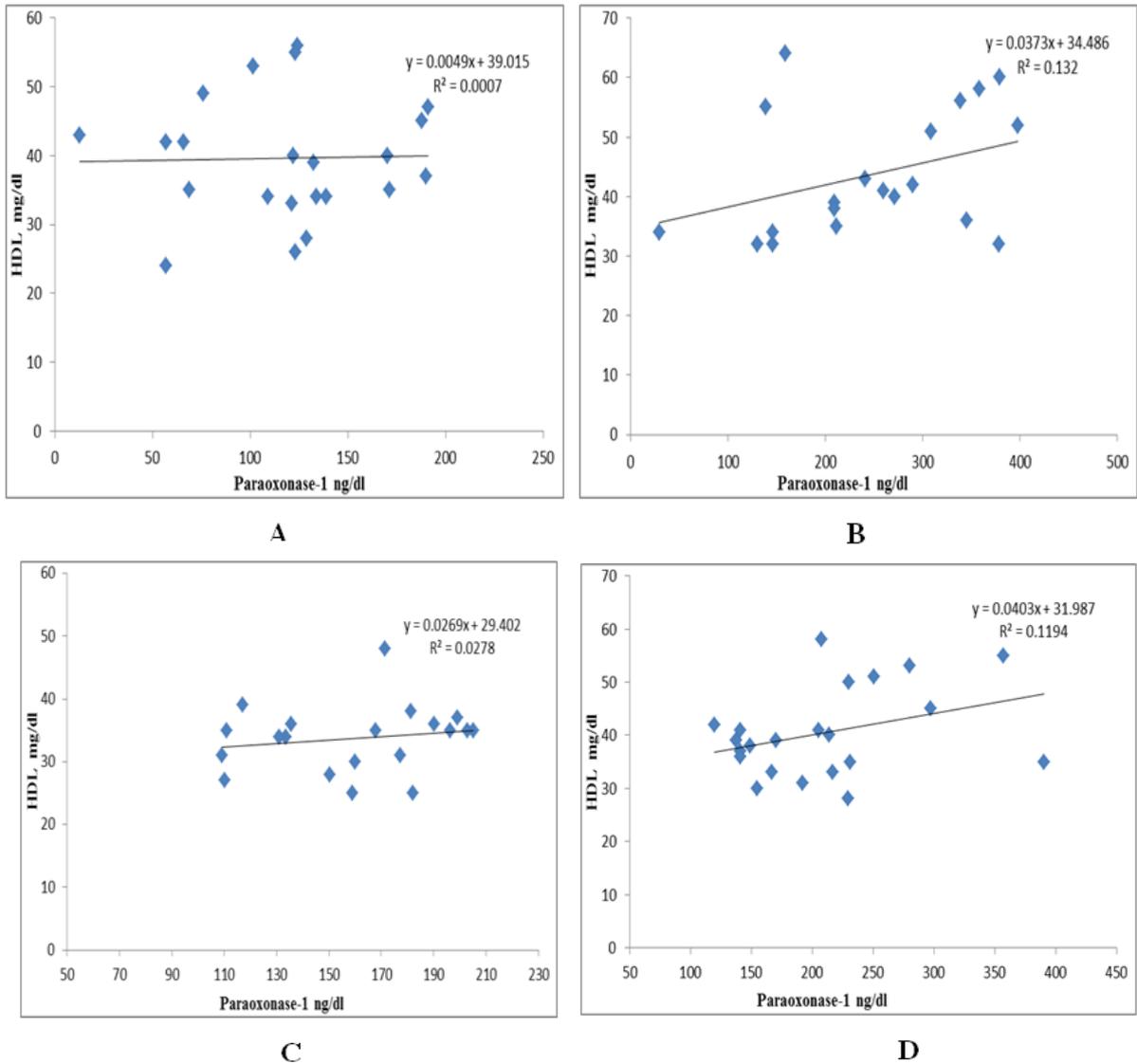
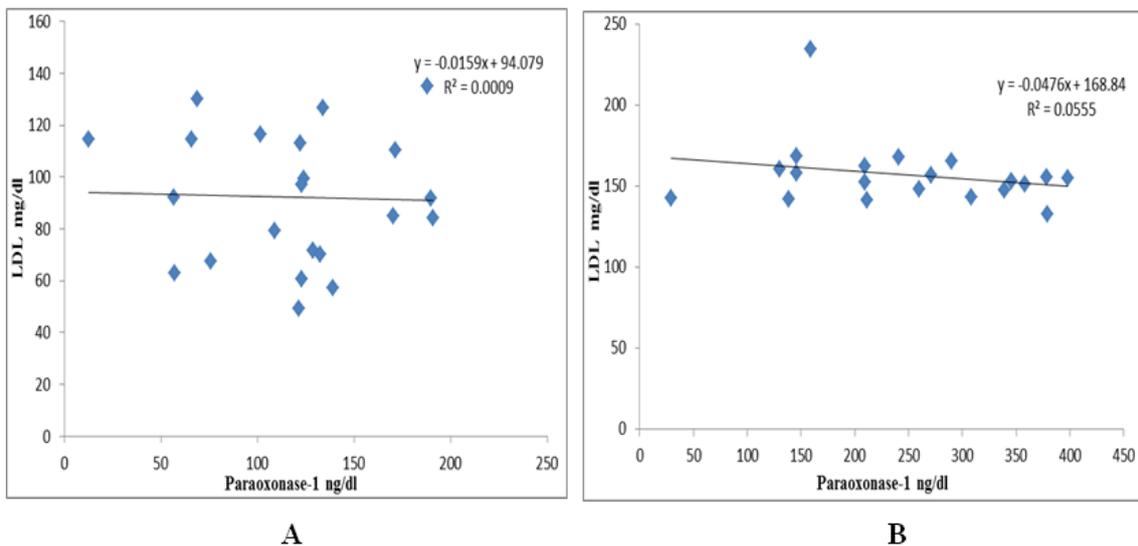


Figure (3) Correlation between paraoxonase-1 and HDL in G1(A) , G2(B) , G3(C) , G4(D)



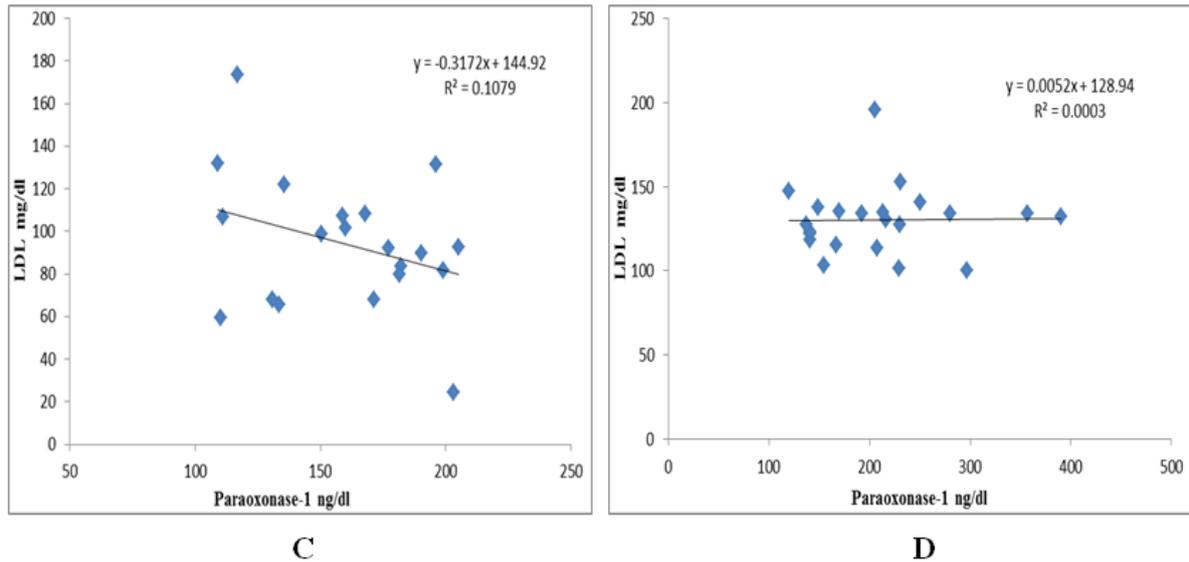


Figure (4) Correlation between paraoxonase-1 and LDL in G1(A) , G2(B) , G3(C) , G4(D)

A significant negative correlation was found between paraoxonase-1 and LDL G1,G2 and G3($r_1 = -0.0297$, $r_2 = -0.235$ and $r_3 = -0.328$) respectively , as shown in figures (4 A,B and C) ,while significant positive correlation was seen in G4 between paraoxonase-1 and LDL ($r_4 = 0.0182$) , as shown in figure (4 D) .

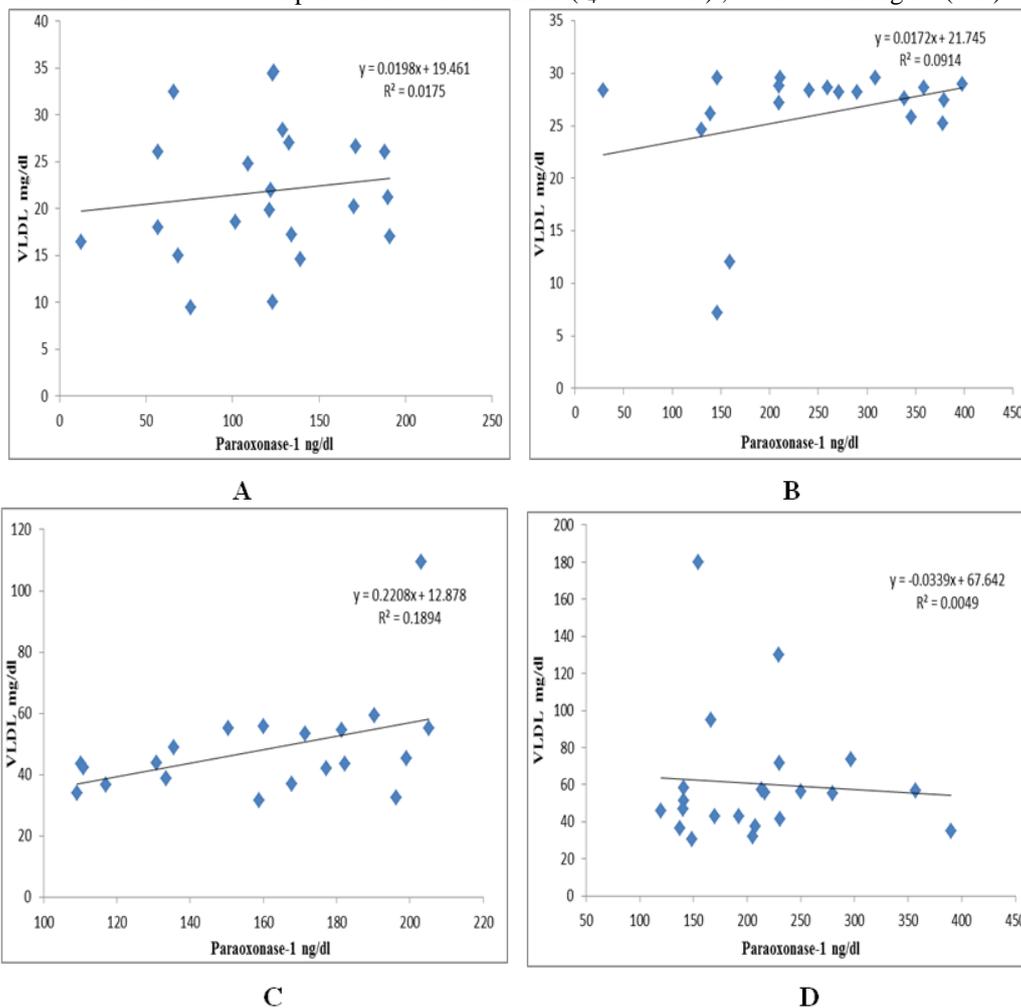


Figure (5) Correlation between paraoxonase-1 and VLDL in G1(A) , G2(B) , G3(C) , G4(D)

Also the results revealed significant positive correlation between paraoxonase-1 and VLDL in G1, G2 and G3 ($r_1 = 0.132$, $r_2 = 0.302$ and $r_3 = 0.435$) respectively, as shown in figures (5 A,B and C) while significant negative correlation was found in G4 between paraoxonase-1 and VLDL ($r_4 = -0.0696$), as shown in figures (5 D).

The conclusion from this study indicates that G2 which cholesterol is high in this group has the highest value for paraoxonase-1. Also, the study found correlation between and lipid profile in all groups of hyperlipoproteinemia. As far as to our knowledge this is the first study determined the levels of paraoxonase-1 in young male with normal BMI and find its relation with lipid profile in patients with hyperlipoproteinemia which divided according to Fredrecons classification.

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