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Correlation between paraoxonase-1 arylesterase activities and clopidogrel drug in patients with coronary artery disease

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***Abstract*---**Background: Coronary artery disease (CAD) is one of the leading causes of death worldwide. Clopidogrel, antiplatelet drug, has been widely used for management of CAD. Arylesterase, the activity of Paraoxonase-1 (PON-1), is mainly contributed in the biotransformation of clopidogrel to its active thiol form. Aim: The purpose of this study was to investigate the effect of receiving clopidogrel drug on the arylesterase activities in CAD patients. The effect of receiving clopidogrel drug on the antioxidant activity of arylesterase was also monitored by determination of malondialdehyde (MDA) level. Methods: One hundred CAD patients, who were followed-up for 5 days after reciving clopidogrel, and 50 healthy volunteers were included in our study. The activities of arylesterase using two (phenyl acetate and p-nitrophenyl acetate) substrates, lipid profile, MDA, and total protein concentration (Tp) were determined spectrophotometrically. Results: PON-1 arylesterase activities, HDL-C, and Tp were significantly decreased ($P<0.0001$) in patients with CAD before receiving clopidogrel drug (CAD-pre-Clp) compared to baseline (non-CAD) and the levels of these parameters were returned to baseline ($P>0.05$) after 5 days of receiving clopidogrel (CAD-post-Clp). However, other parameters include total cholesterol (TC), triglyceride (TG), LDL-C, VLDL, and MDA levels were markedly increased ($P<0.0001$) in CAD-pre-Clp group and returned to baseline in CAD-post-Clp group ($P>0.05$). Significant positive correlations ($P<0.0001$) were found between arylesterase activities and each of HDL-C, and TP, while the enzyme activity was appeared significant negative correlations ($P<0.0001$) with TC, TG, LDL-C, VLDL, and MDA. Receiver operating characteristic curve

showed that arylesterase activities gave a higher sensitivity (72-80%) and specificity (37-81%) as indicator for monitoring of CAD progression. Conclusion: PON-1 arylesterase activities associated with HDL-C were significantly increased as a response of receiving clopidogrel which is helpful to reduce the risk of CAD through the activation of clopidogrel as antiplatelet and preventing or diminishing the lipid peroxidation progression.

***Keywords*---**coronary artery disease, paraoxonase - 1 arylesterase activities, clopidogrel, malondialdehyde, lipid peroxidation.

Introduction

Coronary artery disease (CAD) is the most frequent cause of mortality in worldwide [1]. It is also called coronary heart disease, ischemic heart disease and heart disease. Based on large population studies, it is reported that low concentration of cholesterol transported with HDL fraction is one of the most important risk factors of CAD and high serum HDL concentration plays a protective role [2]. The way in which HDL protect arteries against the damages induced by free radicals and prevent the development of atherosclerotic plaques has been intensively investigated in recent years. An enhanced oxidative stress is regarded as a factor influencing development of atherosclerosis. An important role in antioxidant capacity is attributed to specific enzymes connected to HDL particle such as paraoxonase (PON-1), glutathione peroxidase (GPX), or platelet activating factor acetyl hydrolase [3-5]. PON-1 (EC 3.1.8.1, formerly EC 3.1.1.2) is a glycoprotein consists of 354 amino acids with a molecular weight of (43-45) kDa [5]. PON-1 is one of three enzymes (PON1, PON2, and PON3), coded by a family of genes localized in chromosom 7 [6]. PON-1 is an enzyme that has many enzymatic activities, such as lactonase, thiolactonase, arylesterase, and aryldialkylphosphatase (commonly known as paraoxonase). PON1 is principally complexed with HDL in human serum [7] and has been demonstrated to be implicated in the protection of LDL and HDL from oxidation induced by copper ions as well as by other free radical generators [8]. Epidemiological data have suggested that a decreased level of antioxidants and increased oxidants are associated with CAD [9, 10]. However, the relationship between the extent of CAD and the level of oxidant and anti-oxidant markers is not well known. Although most studies have stated that PON1 activity was decreased in patients with CAD, the studies on the relationship between PON1 activity and the extent of coronary stenosis are very limited [11]. PON-1 exerts several anti-atherogenic activities, in vitro and in vivo, thereby significantly contributing to the HDL cardioprotective effects. Antiplatelet drugs represent an essential intervention in the prevention of acute coronary syndromes [12].

Clopidogrel is a widely used antiplatelet agent that irreversibly binds to the P2Y12 purinergic receptor of platelets and prevents platelet activation by adenosine diphosphate (ADP). The response of platelets to clopidogrel therapy is highly variable. Clinical, cellular, and genetic factors have been associated with variation in response to clopidogrel, however, the majority of this difference can be attributed to variation in the concentrations of the active metabolite. The

decreased response of platelets to clopidogrel, regardless of the mechanisms underlying it, leads to a higher incidence of arterial thrombosis [12]. Recently, PON-1 was proposed as an important contributor to the biotransformation of clopidogrel into the active thiol metabolite [13]. The aim of this study is to investigate the effect of clopidogrel on PON-1 arylesterase activities as well as study their correlations with MDA levels and some biochemical parameters of coronary artery patients in Iraq.

Methods

Study population

The study was conducted on a total of 150 subjects and divided in three groups, 100 patients diagnosed with CAD before treatment with clopidogrel (50 males and 50 females), aged (50.04 ± 8.97 years male, 49.49 ± 8.65 years female) (CAD- pre-Clp group). These patients were received 75 mg per day of clopidogrel dose for 5 days (CAD-post-Clp group). They were diagnosed with CAD based on previous medical reports, laboratory tests and clinical examination by a consultant cardiologist and registered from the Iraqi Heart Center in the Medical City and Ibn Al-Bitar Hospitals, from the period of September 2021 to January 2022. The Ethics Committee of the College of Science, University of Baghdad, approved the study protocol. Fifty volunteers (25 males and 25 females), whose age spanned
49.96 ± 10.44 years males and 46.92 ±8.87 years females, were considered healthy according to their history, without prior CAD, were enrolled (non-CAD group). Demographic and clinical data were collected for patients and control groups. Patients who take of alcohol, smoking, clopidogrel therapy before admission, and/or medication were excluded. Patients who have a history of chronic diseases, CAD before admission were also excluded from this study..

Determination PON-1 arylesterase activity (using phenyl acetate substrate)

Arylesterase activity was determined according to Shen et al. 2014, using phenyl acetate substrate [14] .PON-1 activity towards was measured in 100 mM Tris-HCl buffer (pH 8.0) containing 4 mM substrate and 2 mM CaCl2. The absorbance was monitored spectrophotometrically at 270 nm. Enzyme activity was calculated with a molar extinction coefficient of 1310 M−1 cm−1. One unit of arylesterase activity hydrolyzed 1 μmol of phenyl acetate per minute.

Determination PON-1 arylesterase activity (using p-phenyl acetate substrate)

PON-1 arylesterase activity towards p-nitrophenyl acetate was measured with the method described by Tvarijonaviciute *et al*. 2012 [15] using (50 mM Tris-HCl buffer (pH 8.0) containing 2.5 mM substrate and 1 mM $CaCl_2$) by measuring the increase in the absorbance at 405 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 14 000 $M^{-1}\,cm^{-1}$.

Determination of malondialdehyde levels in serum

Serum malondialdehyde (MDA) level was measured by precipitation method using thiobarbituric acid (TBA), as described by Weitner T. *et al*. 2016 [16]. The

absorbance of the supernatant was measured at 532nm and the result of MDA concentration expressed as a (nmol/ml).

Determination of total protein concentration

Total protein concentration (Tp) in serum was determined using AGAPPE kit. Assay principle: Colorimetric determination of Tp based on the principle of the Biuret reaction (copper salt in an alkaline medium). The Tp in plasma or serum sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the Tp as detected at 550 nm.

Determination of Lipid profile

Lipid profile: Total cholesterol (TC) ; Triglyceride (TG) and High density lipoprotein-cholesterol (HDL-C) levels were estimated by using commercial kit (Linear chemicals, Spain), while Low density lipoprotein-cholesterol (LDL-C) and the levels of very Low density lipoprotein (VLDL) were determined by using Friedwald's formula.

Statistical analysis

The statistical software GraphPad Prism 8 version 2020 was used in the statistical calculations. The results in this study were reported as the mean value ± standard deviation (SD) using the t-test to compare the correlation of the mean and Pearson. The differences were considered significant ($p \leq 0.05$).

Results

The demographic and some biochemical parameters for non-CAD, CAD-pre-clp, and CAD-post-clp groups are listed in Table 1. The results showed that there were no significant differences in age and BMI of male and female between non-CAD and patients groups. Both male and female showed that Tp and HDL-C were significantly decreased in CAD-pre-clp group compared to non-CAD and these parameters were increased again, to reach their levels in non-CAD group, after treated with Clopidogrel CAD-post-clp group. In contrast, TC, TG, LDL-C, and VLDL levels as well as atherogenic ratio-1, Atherogenic ratio-2, and atherogenic index were dramatically increased in CAD-pre-clp group and returned to decrease in CAD-post-clp group with no significant differences compared to non-CAD group. Table 2 presents the arylesterase activities and specific activities of PON-1 using phenyl acetate and p-phenyl acetate substrate and MDA levels among studied groups.

In Table No.(2) Arylesterase Activity and Specific Activity Arylesterase, activity of PON1 was measured in sera of the studied groups using two substrate phenylacetate and 4-nitrophenyl acetate. In comparison to non-CAD group,

arylesterase activities of PON-1 showed a significant decrease in male and female of CAD-pre-clp group before returned to increase with no significant differences in CAD-post-clp group. Conversely, MDA level was statistically increased in CAD- pre-clp group and decreased with no significant differences in CAD-post-clp group compared to non-CAD group. The correlations between PON-1 arylesterase activities and studied parameters are shown in Table 3

In Table No. (3) Pearson correlation analysisand Linear regression of Arylesterase (phenylacetate) and Arylesterase (4-nitrophenyl acetate) with other parameters including age, blood lipid level such as TC Con, LDL-C, HDL-C, TG Con ware evaluated, VLDL, Tp, MDA. Under effect of receiving clopidogrel, it was found that there were strong positive correlations between PON-1 arylesterase activities and each of HDL-C and Tp. However, arylesterase activities of PON-1 displayed significant negative correlations with MDA, TC, TG, LDL-C, VLDL, atherogenic ratio-1, atherogenic ratio-2, and atherogenic index. Linear regression equations in Table 3 assist to predict the levels of studied parameters for CAD patients who treated with clopidogrel therapy. Furthermore, receiver operating characteristic (ROC) curves were used to assess the validity of measuring of arylesterase activities as a diagnostic method for testing the efficiency clopidogrel in treatment of CAD patients, Figure 1. ROC analysis indicated that arylesterse activities gave high diagnostic accuracy in predicting of CAD progression before and after treating with clopidogrel drug, Table 4.

Table1. Demographic and some biochemical parameters of non-CAD, CAD-pre-clp, and CAD-post-clp groups

	Male				**Female**			
Parameters	non-CAD	CAD-pre-clp	CAD-post-clp	P-value	non-CAD	CAD-pre-clp	CAD-post-clp	P-value
Age (year)	49.96±10.44 50 (35-70)	50.04±8.97 52 (36-72)	---	$0.973^{a,b}$	46.92±8.87 48 (35-54)	49.94±8.65 49 (36-70)	---	$0.162^{a,b}$
Gender; n (%)	25 (33.3)	50 (66.7)	---	---	25 (33.3)	50 (66.7)	---	---
BMI(Kg/m^2)	26.41±3.34 26.06 (18.98-28.93)	28.72±3.32 25.38 (19.40-29.85)	---	$0.37^{a,b}$	25.78±3.93 24.68 (18.21-27.52)	28.97±2.88 26.39 (22.75-29.37)	---	$0.31^{a,b}$
Tp (g/dL)	7.24±0.47 7.2 (6.7-8.2)	5.94±0.29 6 (5.1-6.3)	7.31±0.47 7.5 (6.5-8)	$<0.0001^{a,b}$ $0.5707^{a,c}$ $<0.0001^{b,c}$	7.09±0.41 7.1 (6.7-6)	5.91±0.35 6 (5.1-6.5)	7.2±0.37 7.2 (6.6-8.1)	$<0.0001^{a,b}$ $0.6359^{a,c}$ $<0.0001^{b,c}$
TC (g/dL)	155.68±19.9 160 (103-108)	181.28±30.51 187 (108-264)	166.95±24.99 172 (110-200)	$0.0003^{a,b}$ $0.0539^{a,c}$ $0.0077^{b,c}$	167.08±34.27 170 (105-190)	189.58±25.97 191 (97-195)	160.88±23.49 162.5 (100-195)	$0.0002^{a,b}$ $0.1424^{a,c}$ $0.0003^{b,c}$
TG (g/dL)	120.96±21.9 120 (80-180)	146.46±27.06 140 (105-230)	134.16±21.68 132 (90-130)	$0.0002^{a,b}$ $0.0122^{a,c}$ $0.0009^{b,c}$	121.92±27.81 120 (78-102)	146.6±21.74 147 (105-180)	137.7±20.12 140 (100-180)	$<0.0001^{a,b}$ $0.0064^{a,c}$ $<0.0001^{b,c}$
HDL-C (g/dL)	54.13±4.41 55 (46-60)	45.51±3.45 45 (39-55)	53.06±3.28 54 (42-59)	$<0.0001^{a,b}$ $0.241^{a,c}$ $<0.0001^{b,c}$	54.57±3.31 54 (50-53)	43±5.09 44 (32-52)	53.2±2.65 53 (48-59)	$<0.0001^{a,b}$ $0.0557^{a,c}$ $<0.0001^{b,c}$
LDL-C (g/dL)	86.88±20.62 89 (32.4-115)	107.92±8.215 106 (100-142)	87.06±26.38 90 (26.2-127)	<0.0001 0.586 <0.0001	88.12±36.14 102 (11.4-31.6)	106.53±9.71 105.8 (82.2-128)	80.14±24.3 81.2 (20.6-117)	$0.0011^{a,b}$ $0.2606^{a,c}$ $<0.0001^{b,c}$
VLDL (g/dl)	24.192±4.381 89 (11-115)	29.293±5.413 28 (21-46)	27.004±4.373 26.4 (18-36)	$<0.0001^{a,b}$ $0.0109^{a,c}$ $0.0056^{b,c}$	24.38±5.562 24 (15.6-20.4)	29.32±4.34 29.4 (21-36)	27.54±4.025 (20-36)	$<0.0001^{a,b}$ $0.0064^{a,c}$ $<0.0001^{b,c}$
Atherogenic ratio-1	2.89±0.45	4.01±0.77	2.27±0.14	$<0.0001^{a,b}$ $0.815^{a,c}$ $<0.0001^{b,c}$	3.07± 0.6 5	3.73±0.84	3.03±0.48	$0.0001^{a,b}$ $0.162\ 9^{a,c}$ $<0.0001^{b,c}$
Atherogenic ratio-2	1.56±0.58	2.38±0.23	0.76±0.14	$<0.0001^{a,b}$ $0.570^{a,c}$ $<0.0001^{b,c}$	1.62±0.67	2.51±0.38	1.51±0.48	$0.0001^{a,b}$ $0.960^{a,c}$ $<0.0001^{b,c}$
Atherogenic index	0.34±0.06	0.50±0.08	0.31±0.07	$<0.0001^{a,b}$ $0\ 809^{a,c}$ $0.0001^{b,c}$	0.33±0.09	0.53±0.09	0.40±0.06	$<0.0001^{b,c}$ $0.109^{a,c}$ $<0.0001^{b,c}$

Data are presented as mean ± SD, median (min–max), a: non-CAD, b: CAD-pre-clp, c: CAD-post-clp, * p-value ≤0.05 is considered to be statistically significant, *** p-value <0.001, **** p-value <0.0001.

Table 2. The arylesterase activities and specific activities of PON-1 using phenyl acetate and p-nitrophenyl acetate as substrate and MDA levels in non-CAD, CAD- pre-clp, and CAD-post-clp groups

	Male				**Female**			
Parameters	non-CAD	CAD-pre-clp	CAD-post-clp	P-value	non-CAD	CAD-pre-clp	CAD-post-clp	P-value
Arylesterase (phenyl acetate) (KU/L)	451.14±81.35 455.36 (301.79-585.71)	380.51±52.83 360.17 (308.93-530.36)	413.27±64.52 407.14 (301.79-553.5)	<0.0001[a,b] 0. 323[a,c] <0.0048[b,c]	404.35±49.07 410.71 (312.5-496.42)	370.17±32.61 359.40 (312.50-483.93)	401.68±50.97 383.93 (312.50-483.93)	0.0006[a,b] 0.1634[a,c] 0.0009[b,c]
Arylesterase (phenyl acetate) (KU/gm)	6.23±1.22 6.36 (4.09-8.74)	6.43±0.97 6.08 (5.15-9.03)	5.67±0.94 5.56 (4.03-8.19)	0.0934[a,b] 0.9837[a,c] 0.0772[b,c]	5.65±0.68 5.70 (4.56-7.06)	6.27±0.57 6.17 (5.12-8.07)	5.39±0.75 5.19 (4.23-7.12)	0.4637[a,b] 0.4445[a,c] 0.0667[b,c]
Arylesterase (p-nitrophenyl acetate) (KU/L)	415.14±85.33 410.71 (253.57-567.86)	359.91±82.57 359.08 (3.57-530.36)	409.48±105.10 407.14 (119.64-716.07)	<0.0001[a,b] 0. 991[a,c] 0.0004[b,c]	420.36±47.96 358.04 (232.14-483.92)	356.14±0.35 358.04 (232.14-483.92)	405.07±91.2 387.5 (155.36-642)	<0.0001[a,b] 0.482[a,c] 0.0007[b,c]
Arylesterase (p-nitrophenyl acetate) (KU/gm)	7.24±0.47 7.2 (6.7-8.2)	5.94±0.29 6 (5.1-6.3)	5.64±1.51 5.90 (1.53-9.18)	0.33[a,b] 0.7836[a,c] 0.1059[b,c]	5.87±1.07 6.02 (3.17-7.65)	6.04±0.82 6.10 (3.8-8.06)	5.64±1.33 5.29 (2.16-9.59)	0.167[a,b] 0.7435[a,c] 0.0946[b,c]
(MDA) (nmol/ml)	5.74±1.28 5.65 (3.29-8.38)	9.62±3.47 8.24 (0.06-10.4)	7.08±0.78 6.96 (5.16-8.75)	0.0003[a,b] 0.425[a,c] <0.0001[b,c]	6.83±0.61 6.82 (5.70-8.17)	11.08±3.98 11.3 4.31-18.80	7.24±1.09 6.93 (5.65-11.53)	<0.0001[a,b] 0.870[a,c] <0.0001[b,c]

Data are presented as mean ± SD, median (min–max), a: non-CAD, b: CAD-pre-clp, c: CAD-post-clp, * p-value ≤0.05 is considered to be statistically significant, *** p-value <0.001, **** p-value <0.0001.

Table 3. Correlations and linear regression of arylesterase activities with age, lipid profile, Tp and MDAparameters as well as atherogenic ratio-1, atherogenic ratio-2, atherogenic index.

Male

Parameters	Arylesterase (phenyl acetate)					Arylesterase (p-nitrophenyl acetate)				
	Correlation			Linear regression		Correlation			Linear regression	
	r	p-value	Slop	R^2	Equation	r	p-value	Slop	R^2	Equation
Age	0.096	0.288	0.718 ± 0.67	0.0092	Y = 0.718 *X + 371.6	0.2344	0.091	0.435 ±0.91	0.054	Y = 0.435 *X + 269.1
Tp (g/dL)	0.291	0.001	35.99±10.75	0.0847	Y = 35.99 *X + 164.6	0.205	0.022	25.34± 3.66	0.042	Y = 25.34 *X + 219.7
MDA (nmol/ml)	-0.437	<0.0001	-22.06±4.128	0.191	Y = -22.06 *X + 558.1	-0.152	0.093	-5.607±3.314	0.02	Y = -5.607 *X + 436
TC (g/dL)	-0.172	0.057	-0.635±0.33	0.029	Y = -0.635 *X + 477.9	-0.056	0.531	-0.391±0.624	0.003	Y = -0.391 *X + 498.8
TG (g/dL)	-0.186	0.038	-0.817±0.390	0.0349	Y = -0.817 *X + 481.8	-0.001	0.993	-0.003±0.367	5.913	Y = -0.003 *X + 391.3
HDL-C (g/dL)	0.342	0.0001	6.697±1.670	0.117	Y = -6.697 *X + 32.92	0.222	0.013	4.011±1.602	0.049	Y = -4.011 *X +189.3
LDL-C (g/dL)	-0.037	0.680	-0.151±0.367	0.004	Y = -0.151 *X +422.2	-0.228	0.011	-0.919± 0.625	0.052	Y = -0.919 *X + 478.5
VLDL (g/dL)	-0.006	0.939	0.118±1.559	4.782	Y = 0.118 *X + 404.6	-0.064	0.048	-0.297 ± 0.417	0.0041	Y = -0.297* X+87.47

Atherogenic ratio-1	-0.243	0.0031	-315.2±89.3	0.059	Y= -315.2*X +36.1	-0.334	0.008	-532.9±56.27	0.11	Y =-532.9*X + 31
Atherogenic ratio-2	-0.321	0.0040	-415.2±53.9	0.103	Y = -415.2*X +22.2	-0.324	0.003	-466.8±63.26	0.10	Y = -466.8*X + 42
Atherogenic index	-0.232	0.0046	-305.2±115.9	0.053	Y = -305.2*X +32.1	-0.246	0.005	-476.7±34.42	0.06	Y = -476.7*X + 22

Female

Age	0.057	0.526	0.600± 0.945	0.0032	Y =0.600 *X+358.8	0.084	0.355	1.854 ±1.999	0.007	Y = 1.854 *X+340.0
Tp (g/dL)	0.260	0.0035	32.64±10.95	0.067	Y = 32.64 *X+170.5	0.343	0.0001	37.33± 9.206	0.117	Y = 37.33 *X+139.3
MDA (nmol/l)	-0.272	0.0022	-8.654±2.771	0.074	Y = -8.654 *X+ 446.1	-0.206	0.021	-5.007±2.14	0.042	Y = -5.007 *X + 432.1
TC (g/dL)	-0.096	0.288	0.327±0.33	0.009	Y= 0.327 *X+336.0	-0.0677	0.455	0.199±0.27	0.004	Y = 0.199 *X + 356.7
TG (g/dL)	-0.104	0.250	-0.395±0.342	0.0108	Y=-0.395 *X+442.9	-0.101	0.260	0.336±0.3	0.010	Y = -0.336 *X + 435.1
HDL-C (g/dL)	0.239	0.007	3.327±1.220	0.057	Y = 3.327 *X+224.1	0.291	0.001	3.512±1.04	0.084	Y = 3.512 *X +215.4
LDL-C (g/Dl)	-0.005	0.95	0.020±0.32	3.215	Y= 0.020 *X+368.6	-0.069	0.0447	-0.518± 0.608	0.004	Y =- 0.518 *X+383.6
VLDL (g/dl)	-0.117	0.190	-2.255±1.713	0.0138	Y = -2.255 *X+ 449.5	-0.104	0.244	-1.722 ± 1.472	0.011	Y = -1.722 *X + 436.1
Atherogenic ratio-1	-0.442	0.004	-305.2±93.5	0.195	Y = -305..2*X +32.2	-0.425	0.026	426.7±38.4	0.180	Y =-426.7*X + 42
Atherogenic ratio-2	-0.352	0.0032	-337.4±102.9	0.123	Y = -337.4*X +22.2	-0.452	0.004	-521.9±45.2	0.204	Y = -521.9*X + 38
Atherogenic index	-0.312	0.0031	-334.2±98.9	0.100	Y = -334.2*X +42.1	-0.231	0.025	-456.8±65.3	0.53	Y = -456.7*X + 32

Table4 Analysis data of ROC curve shows the percentage of sensitivity and specificity at best cut off in control and CAD patients and the area under curve for serum Arylesterase (phenylacetate) and Arylesterase (4-nitrophenyl acetate) activities

	Arylesterase (phenylacetate)		Arylesterase (4-nitrophenyl acetate)	
	Male	Female	Male	Female
dSensitivity%	81.63	74.00	73.47	78.00
Specificity%	80.00	72.00	72.00	76.00
AUC	0.761±0.071	0.703±0.072	0.684 ± 0742	0.776 ± 0.066
Cut-off (KU/L)	<398.2	<379.5	<349.5	<379.5
p-value	0.0002	0.0042	0.0010	<0.0001

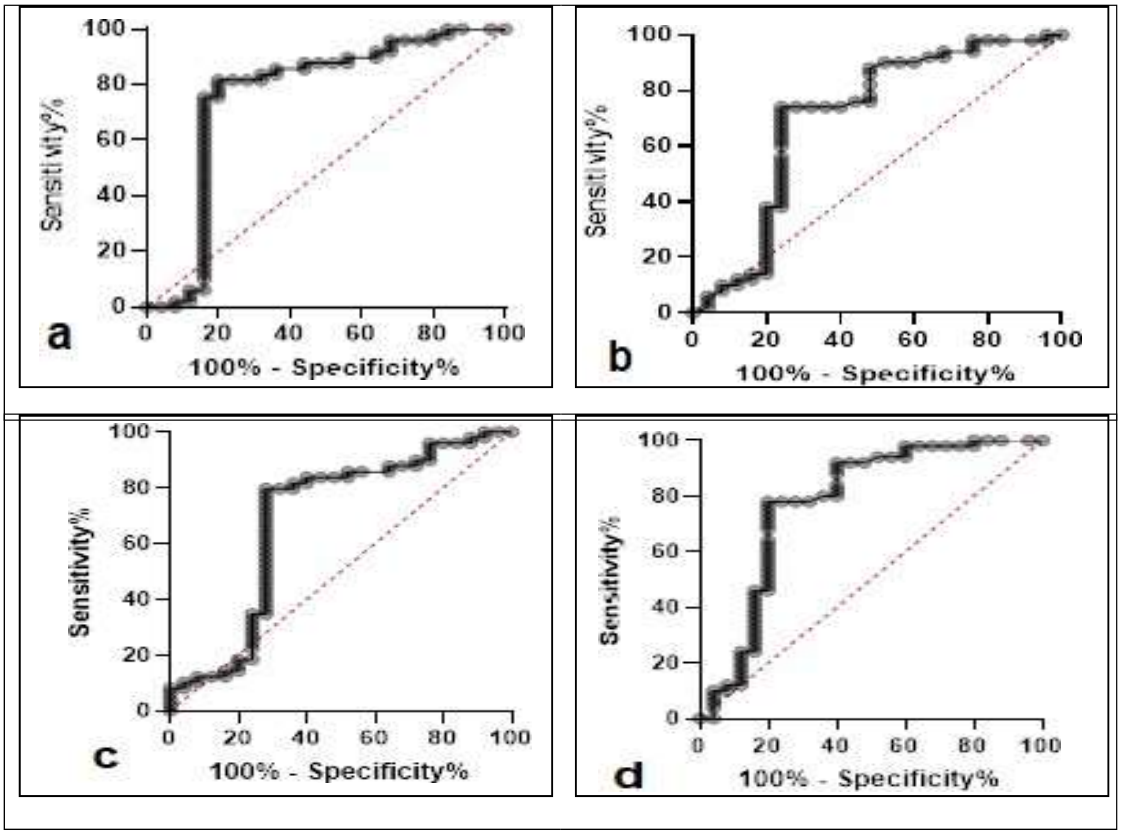

Figure1. The ROC curve in control and CAD patients for serum Arylesterase (phenylacetate) activity with (a) male and (b) female and serum (4-nitrophenyl acetate) activity with (c) male and (d) female.

Discussion

PON-1 is one of the most bioactive HDL constituents that significantly contribute to the HDL anti-atherogenic and cardioprotective potency [12]. The results of the present study indicated that the arylesterase activities associated with HDL-C were significantly decreased in CAD-pre-clp group compared to non-CAD group. This finding is in agreement with several studies reported that HDL displays cardioprotective properties through its anti-oxidant activity, that is mainly maintained by PON1[17, 18]. Human epidemiological studies have showed a strong correlation between decreased of PON1 levels and an increased of CAD risk [19, 20]. Another study reported that HDL-C activity is associated with PON1 arylesterase, but not paraoxonase activity [2]. In contrast, strong negative correlations between arylesterase activities and TC, TG, LDL-C, VLDL, atherogenic ratio-1, atherogenic ratio-2, and atherogenic index were observed. Several studies conducting on CAD patients were also reported that TC, TG, LDL-C, VLDL are mainly contributed in CAD incidence [21, 22]. MDA level was also increased in CAD patients that is usually formed as a result of oxidative stress (OS) developed in CAD. A study conducted by Varadhan, S *et al.*(2022) described that MDA level was significantly higher in CAD patients, compared to healthy individuals [22]. MDA is known as the main parameter showing the level of lipid peroxidation process induced by oxidative stress. Antioxidant enzymes play a critical role against lipid peroxidation processes. Several studies reported that the decrease in the activity of antioxidants is linked with many diseases that lead to cause of OS [7, 23-28]. Thus the decrease in the arylestease activity can contributes in the increasing of MDA.

The main findings of the current study showed that receiving of clopidogrel therapy was strongly contributed to increase arylesterase activities and HDL-C in patients with CAD to reach their levels in non-CAD group. In contrast, TC, TG, LDL-C, VLDL, MDA, atherogenic ratio-1, atherogenic ratio-2, and atherogenic index have been decreased after treated with clopidogrel to reach their corresponding levels in non-CAD group. These results are in agreement with a study conducted by Tselepis A. et al.(2011) who explained that there was a negative association between platelet activation parameters and paraoxonase activity in CAD patients adequately responding to clopidogrel [12]. In addition to the role of clopidogrel as anti-platelet agent, these results illustrated the beneficial role of treated with clopidogrel in the (1) activation of arlyesterase associated with HDL-C against the platelet formation, (2) protection from the harmful effect of LDL-C, TC, TG, VLDL and atherogenic index through reduction of their levels, (3) promotes the antioxidant activity of PON-1 arylesterase in the prevention of LDL- C oxidation and MDA formation and this was observed through the decrease in MDA level. Furthermore, ROC analysis showed that PON-1 arylesterase activities gave a higher percentage of sensitivity and specificity against clopidogrel treatment. It is therefore arylesterase activities present a useful method for monitoring the effect of treated with clopidogrel on the CAD progression.

Conclusion

From our results we can conclude that clopidogrel drug has a significant role in the alleviation of CAD progression. This can be observed from the activation of anti-platelet and antioxidant activities of PON-1 arylesterase associated with HDL-C as well as contributed in the reduction of MDA, LDL-C, LDL-C, TC, TG, VLDL levels that are associated with an increased risk of CAD.

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