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Correlation between oxidative stress and clopidogrel drug in Iraqi patients with coronary artery disease

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***Abstract*---**Background: Coronary Artery Disease (CAD) is one of the largest causes of mortality worldwide. Clopidogrel, antiplatelet drug, has been widely used for management of CAD. The current study aimed to investigate the effect of clopidogrel on the oxidative stress in CAD patients. Methods: One hundred CAD patients, who were followed-up for 5 days after receiving clopidogrel, and 50 healthy volunteers were included in this study. Parameters include catalase (CAT), total antioxidant capacity (TAC), total oxidant capacity (TOC), total protein, albumin, and globulins were determined before and after treatment with clopidogrel. Results: CAT, TAC, and Tp were significantly decreased (P<0.0001) in CAD patients compared to healthy control and the levels of these parameters were returned to increase after receiving clopidogrel. However, TOC level was markedly increased (P<0.0001) in CAD patients and returned to decrease after treating with clopidogrel. Receiver operating characteristic curve showed that CAT gave a higher sensitivity (68-73%) and specificity (72%) as an indicator for monitoring of CAD progression. Conclusion: CAT activity and TAC were significantly increased as a response of receiving clopidogrel which is helpful to reduce the risk of CAD through scavenging of oxidants formation. These results also present CAT as a predictor variable of clopidogrel action.

***Keywords*---**catalase, coronary artery disease, oxidative stress, total antioxidant capacity, total oxidant capacity.

1. Introduction

Coronary artery disease (CAD) is the most frequent cause of mortality in worldwide. It is also called coronary heart disease, ischemic heart disease and atherosclerotic cardiovascular disease [1]. Based on large population studies, an enhanced oxidative stress is regarded as a factor influencing development of atherosclerosis [2]. Oxidative stress (OS) is a state of unbalanced tissue oxidation refers to a condition in which cells are subjected to excessive levels of molecular oxygen or its chemical derivatives called reactive oxygen species (ROS) such as: hydrogen peroxide (H_2O_2) superoxide anions ($O_2^{\cdot-}$), and hydroxyl radicals ($OH^{\cdot}$). ROS is a highly reactive intermediates of the oxygen metabolism, that being generated and destroyed constantly [3]. Therefore, OS has been involved in aging process and a wide assortment of diseases such as: cardiovascular disease, neurodegenerative disorders, diabetes, and cancer [4]. Remarkably, different defense mechanisms are controlled *in vivo* the overproduction and accumulation of ROS, including: antioxidant enzyme catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD) as well as non-enzymatic antioxidants glutathione (GSH), vitamins (C, E and A) and bilirubin [5]. Catalase (CAT) (EC 1.11.1.6; oxidoreductase) is one of the most important antioxidant enzymes. It is present in almost all aerobic organisms [6]. CAT has a prime role in regulating the cellular level of hydrogen peroxide, and its hydrogen peroxide catabolism protects the cells from oxidative assault, for example, by securing the pancreatic β cells from hydrogen peroxide injury [5, 7]. CAT deficiency or malfunctioning is associated with many diseases such as diabetes mellitus, vitiligo, cardiovascular diseases, Wilson disease, hypertension, anemia, some dermatological disorders, Alzheimer's disease, bipolar disorder, and schizophrenia [8]. CAT has a prime role in regulating the cellular level of hydrogen peroxide, and its hydrogen peroxide catabolism protects the cells from oxidative assault, for example, by securing the pancreatic β cells from hydrogen peroxide injury [9-11]. Low CAT activities have been reported in schizophrenic patients such as also in patients with atherosclerosis [12]. Previously, studies on mice with acute myocardial infarction demonstrated that clopidogrel preserved the CAT activity and significantly reduced OS [13, 14]. Clopidogrel, an antiplatelet agent, is a treatment for CAD that irreversibly binds to the P2Y12 purinergic receptor of platelets and prevents platelet activation by adenosine diphosphate (ADP) [15]. It is widely used to prevent heart attacks and strokes in people who have had heart disease (recent heart attack), recent stroke, or circulatory disease (peripheral vascular disease) [16, 17], It is also used with aspirin to treat new/worsening chest pain (new heart attack, unstable angina) and to keep blood vessels open and prevent blood clots after certain procedures (such as cardiac stenting) [18]. To the best of our knowledge, no study has been conducted on the correlation among clopidogrel, CAT, and OS in human CAD patients. Therefore, the present study aimed to investigate the effect of clopidogrel on CAT and the oxidant/antioxidant status in sera of Iraqi patients with CAD.

2. Methods

2.1. 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, 94.44 ± 8.65 years female) (group, GII). These patients were received 75 mg per day of clopidogrel dose for 5 days (group, GIII). 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 (group, GI). 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.

2.2. Determination CAT activity

The CAT activity was determined using spectrophotometric method that based on the reaction of hydrogen peroxide with ammonium molybdate to produce a yellowish color, which was determined using (UV-1800 UV-Vis spectrophotometer, Shimadzu, Japan) at 374 nm. The method is characterized by adding a correction factor to exclude the interference that arises from the presence of amino acids and proteins in serum (109).

2.3. Total oxidant capacity assay

The total oxidant capacity (TOC) was determined in serum of all studied groups as described by Erel (2005). The assay was performed using colorimetric method at a wavelength of 560 nm and results were expressed as a term of micromolar hydrogen peroxide equivalent per liter (µmol H2O2 Eq. /L).

2.4. Total antioxidant status assay

The total antioxidant capacity (TAC) was determined based on the method described previously by Erel (2004). The assay was performed using colorimetric method at a wavelength of 444 nm and results were expressed as a term of millimolar uric acid equivalent per liter (mmol uric acid Eq. /L).

2.5. Oxidative stress index calculation

The value of oxidative stress index (OSI) was calculated according to the following equation (Karahan et al., 2013):

$$OSI = TOS\ (\mu mol\ H_2O_2\ Eq./L) / TAS\ (\mu mol\ uric\ acid\ Eq./L)$$

2.6. Determination of total protein concentration

Total protein concentration (Tp) in serum was determined using AGAPPE kit. Assay based on the principle of the Biuret reaction where 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 was detected at 550 nm.

2.6.1 Determination of Serum Albumin

In acidic medium, Albumin (Alb) reacts with of bromocresol green to change a color from yellow-green to green-blue. The intensity of the resulted color is proportional to the Alb concentration in the sample was measured at 530 nm [113].

2.6.2 Determination of serum Globulins

The serum Globulins (Glo) concentration was calculated by the following equation [114].

Glo conc. (g/dl) = TP conc. (g/dl) – Alb conc. (g/dl)

2.7 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 and one-way analysis of variance (ANOVA) test to compare the correlation of the mean and Pearson. The differences were considered significant ($P \leq 0.05$).

3. Results

The demographic and some biochemical parameters for GI, GII, and GIII groups are listed in Table 1.

Table1. Demographic and some biochemical parameters of GI, GII, and GIII groups

	Male				Female			
Parameters	GI	GII	GIII	*P*-value	GI	GII	GIII	*P*-value
Age (year)	49.96±10.4 50 (35-70)	50.04±8.97 52 (36-72)		0.973	46.92±8.87 48 (35-54)	49.94±8.65 49 (36-70)		0.162
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	25.78±3.93 24.68 (18.21-27.52)	28.97±2.88 26.39 (22.75-29.37)		0.31
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,c] 0.5707[b]	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,c] 0.6359[b]
Alb (g/dl)	3.3±0.29 3.3 (2.8-4.2)	4.2 ±0.32 4.1 (3.8-5.1)	4.3±0.37 4.4 (3.5-5.1)	0.0003[a] 0.0005[b] 0.776 [c]	3.2 ±0.29 3.25 (2.8 -4.2)	4±0.27 4.1 (97-195)	4.2±0.51 4.1 (3.3-5.1)	0.0002[a] 0.0003[b] 0.673 [c]
Glo (g/dl)	3.04±0.51 3 (2.1-3.9)	2.64±0.38 2.7 (1.8-3.4)	2.9±0.54 3 (2-4.2)	0.0002[a] 0.0009[b] 0.465[c]	3.6±0.46 2.8 (2-3.8)	2.6±0.46 2.8 (1.1 -3.3)	2.8 ±0.58 3.1 (1.6-4.4)	<0.0001[a] 0.0006 [b] 0.543[c]
Alb/Glo ratio	1.4 8±0.31 1.4 (0.8-2.3)	1.52 ±0.31 1.18 (0.7 -2)	1.54±0.37 1.51 (0.8-2.2)	<0.0001[a] <0.0001[b] 0.241[c]	1.45±0.3 1.3 (0.90-3.6)	1.55 ±0.3 1.24 (0.90-3.6)	1.57±0.75 1.32 (0.75-3.12)	<0.0001[a] <0.0001[b] 0.557[c]

Data are presented as mean ± SD, median (min–max), *P*-value ≤0.05 is considered to be statistically significant, in comparison of a (between GI and GII), b (between GI and GIII), c (between GII and GIII).

The results showed that there were no significant differences (P >0.05) in age and BMI for males and females between patients and control groups. The results also showed that Tp and Glo were significantly decreased (P <0.0001) in CAD patients compared to control. In contrast, Alb and the Alb/Glo ratio were increased in CAD patients compared to healthy persons. After receiving of clopidogrel, it was observed that Tp and Glo were significantly increased, while Alb and the Alb/Glo were dramatically decreased compared to their levels before treatment with clopidogrel.

Table 2, which lists the levels of the main parameters in this study, shows that TOC and OSI were markedly increased in patients with CAD compared to control. However, the CAT activity and TAC level were significantly decreased in those patients compared to control. It was also observed that TOC and OSI were statistically decreased while, the CAT activity and TAC level were significantly increased in those patients after treatment with clopidogrel. The correlations between CAT activity and studied parameters are listed in Table 3.

Table 2. The activity and specific activities of CAT and the levels of TOC, TAC, and OSI in studied groups

	Male				Female			
Parameters	GI	GII	GIII	*P*-value	GI	GII	GIII	*P*-value
CAT (U/L)	326.3±34.3 327.4 (268.0-383.1)	266.0±81.3 239.3 (169.0-436.2)	323.0±30.2 326.9 (149.9 - 550.5)	0.0007[a] 0. 874[b] 0.0018[c]	338.2±68.4 321.15 (284.2-521.1)	270.98±81.3 250.96 (176.9-445.0)	336.92±96.04 333.81 (157.7-5551.8)	0.0006[a] 0.1634[b] 0.0009[c]
CAT specific activity (U/mg)	4.51±0.62 4.40 (3.52-5.63)	4.47±1.36 4.05 (2.81-7.37)	4.44±1.45 4.89 (1.97-7.43)	0.9053[a] 0. 8146[b] 0.8875[c]	4.73±0.99 4.39 (3.56-7.55)	4.58±1.37 4.26 (2.92-8.03)	4.68±1.33 4.81 (2.16-7.86)	0.6403[a] 0.8615[b] 0.716[c]
TOC (µmol/L)	21.6 ±7.81 23 (13-34)	38.91±16.35 43 (8-78)	24.32±11.4 23 (3-53)	<0.0001[a] 0.290[b] <0.0001[c]	22.2±4.74 18 (8-38)	41.2±14.76 43 (8-78)	26.8±11.49 33 (3 -43)	<0.0001[a] 0.08 26[b] <0.0001[c]
TAC (µmol/L)	4.51±0.26 4.23 (3.3-4.5)	3.28±0.36 3.32 (2.3-3.9)	4.13±0.30 5.71 (3.03-5.43)	<0.0001[a] 0.9197[b] <0.0001[c]	4.12±0.45 4.08 (3.4-5.3)	3.31±0.22 3.30 (2.8-3.7)	3.97±0.67 3.89 (3.02 -6.96)	<0.0001[a] 0.9628[b] <0.0001[c]
OSI	518.9±175.7	771.65±401	602.7±301.2	<0.0001[a] 0.1978 [b] <0.0001[c]	544.5±224.3	734.7±349	691.5±322.0	<0.0001[a] 0.4460[b] <0.0001[c]

Data are presented as mean ± SD, median (min–max), P-value ≤0.05 is considered to be statistically significant, in comparison of a (between GI and GII), b (between GI and GIII), c (between GII and GIII).

Table 3. Correlations and linear regression of CAT activity with studied parameters

Parameters	Male					Female				
	Correlation			Linear regression		Correlation			Linear regression	
	r	*P*-value	Slop	R^2	Equation	r	*P*-value	Slop	R^2	Equation
Age	-0.045	0.614	-0.457 ± 0.67	0.002	Y = -0.457 *X + 324.7	-0.065	0.789	-0.474 ±0.91	0.004	Y = -0.789 *X +30 2.1
TOC µmol/L	-0.077	0.394	-0.453±0.530	0.0059	Y = -0.435*X + 314.3	-0.274	0.0019	-1.690±0.533	0.075	Y = -1.690*X + 364.3
TAC µmol/L	0.184	0.040	24.64±11.93	0.0340	Y = 24.69*X + 207.2	0.259	0.0035	38.51±12.94	0.067	Y = -38.51*X + 166.7
OSI	-0.187	0.056	-0.0650±0.07	0.043	Y = -*0.0650X + 320.3	-0.179	0.046	-0.0360±0.071	0.032	Y = -0.0360*X + 337.9
Tp (g/dL)	0.075	0.407	9.252±11.13	0.005	Y = 9.252*X + 243.9	0.0 38	0.674	5.000±11.9	0.001	Y = 5.00*X + 272.6
Alb (g/dl)	0.097	0.285	15.35±14.32	0.009	Y = 15.35*X + 296.0	0.052	0.562	8.480±14.6	0.002	Y = 8.480*X +122
Glo (g/dl)	0.067	0.980	34.65±15.23	0.004	Y = 34.65*X + 123.1	0.032	0.999	37.66±16.4	0.001	Y =37.66 *X +120.3
Alb/Glo ratio	0.065	0.655	13.35±29.73	0.005	Y = 13.35*X + 223.7	0.060	0.504	12.37± 18.48	0.003	Y =12.37*X +228.3

The results shows under effect of receiving clopidogrel, it was found that there were strong positive correlations between CAT activity and each of TAS, Tp, Alb, Glo and Alb/Glo ratio. However, CAT activity displayed significant negative correlations with TOC, OSI and age in CAD patients after receiving of clopidogrel. 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 CAT activity as a marker for monitoring of clopidogrel efficiency in CAD patients, Figure 1. ROC analysis indicated that CAT activity gave high diagnostic accuracy in predicting of CAD progression under effect of clopidogrel, Table 4.

Table 4. 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 CAT activity

	Male	Female
Sensitivity%	73.47	68.00
Specificity%	72.00	72.00
AUC	0.746±0.058	0.75±0.055
Cut-off (KU/L)	<296.0	<292.5
P-value	0.0006	0.0003

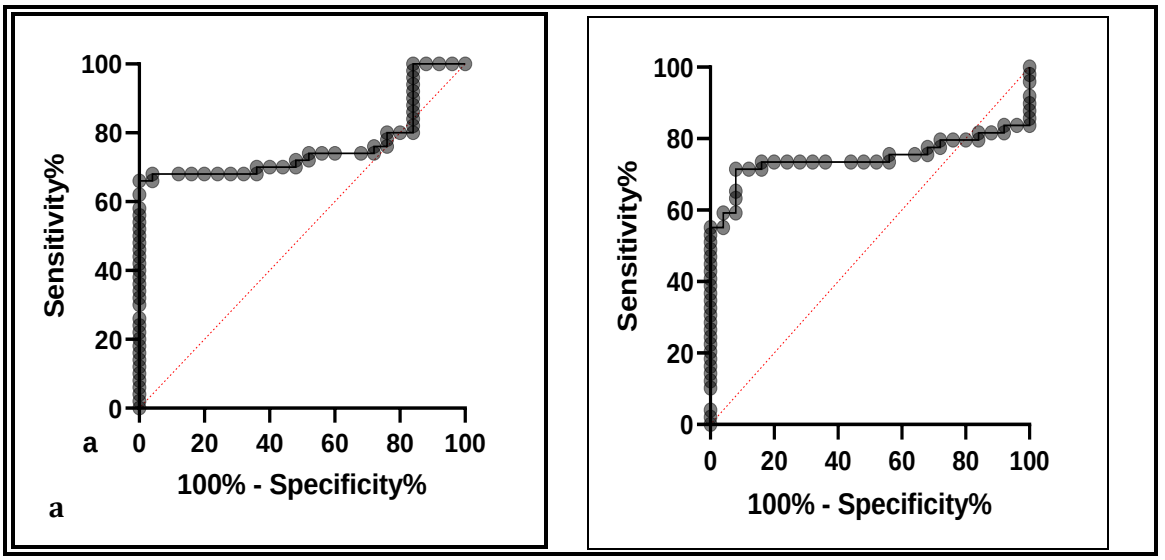

Figure1: The ROC curve in control and CAD patients for serum CAT activity with (a) male and (b) female

4. Discussion

ROS are generated from both endogenous and exogenous sources. There are many systems inside a cell that is responsible for endogenous production of free radicals [19, 20]. Interestingly, different types of cardiovascular diseases (CVD) have been shown to be associated with an excess production of ROS, which plays a critical role in the development of these diseases, such as: atherosclerosis and Heart Failure [2, 21]. Antioxidants are considered defensive mechanisms against the negative effects of ROS [22-28].

According to our results, this study suggested that the clopidogrel is mainly contributed in the reduction of TOC level and improvement of TAC generation. Experimental and clinical studies point to a pivotal role of ROS in the mechanism of platelet activation and its effect on clopidogrel [29]. ROS participate in platelet activation by inactivating nitric oxide and platelet release agonists and proatherogenic molecules [30, 31]. Therefore, this study suggested that clopidogrel may potentially prevent damage from oxidative stress through an inhibition of platelet activation that leads to reduced ROS and proinflammatory cytokines production and increased nitric oxide bioavailability, and a production of antioxidant compounds. CAT, which is an antioxidant enzyme, was found to be significantly decreased in CAD patients. This results in agreement with Nesim *et al*. who are found that the CAT activity was significantly elevated in patients with myocardial infarction [32]. This study was also found that CAT activity was increased in CAD patients after treatment with clopidogrel. To the best of our knowledge, no study has been conducted on the effect of clopidogrel on CAT in human CAD patients. Our study suggested that the increase in CAT activity is due to the inhibition of platelet activation and stimulated of antioxidants production by clopidogrel. It was also found that Tp level was markedly increased after receiving of clopidogrel. It is well known that several proteins have an antioxidant activity such as antioxidant enzymes and Alb. Therefore, we supposedthat the increase in protein concentrations is a part of antioxidant enhancement by clopidogrel.

Furthermore, the results of the present study indicated that under effect of clopidogrel there are strong positive correlations between CAT activity and each of TAC, Tp, Alb, Glo and Alb/Glo ratio. However, strong negative correlations were observed between CAT activity and each of TOC and OSI. Based on forgoing we can conclude that beside its function as anti-platelet agent, clopidogrel drug may mainly contribute in the suppressed of TOC and induction of TAC generation. ROC analysis was used to assess the validity of measuring of CAT activity as a diagnostic method for testing the efficiency clopidogrel drug in treatment of CAD patients, Figure 1. The result indicated that CAT activity gave high diagnostic accuracy in predicting of CAD progression before and after treating with Clp drug, Table 4. Thus, this study presents the CAT as a suitable marker for diagnosis and monitoring of CAD patients before and after treating with clopidogrel drug.

5. Conclusion

From our results we can conclude that in addition to anti-platelet activity, clopidogrel is mainly contributed in the suppressed of TOC and induction of TAC generation. Also, this study suggested that CAT is a potential marker for diagnosis and monitoring of CAD patients before and after treating withclopidogrel drug.

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