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Effect of Treatment with Clopidogrel on Ceruloplasmin Oxidase Activity and Concentration in Patients with Ischemic Heart Disease

Ali W. Al-Ani^{1*}, Faez H. Hadi², Azhar Kamel³

¹Department of Chemistry, College of Science, University of Baghdad, Baghdad

²Baghdad Health Directorate - Al-Karkh, Iraqi Ministry of Health, Baghdad

³Supervision and Scientific Evaluation Apparatus, Ministry of Higher Education and Scientific Research, Baghdad, Iraq

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Abstract

Ischemic heart disease (IHD) is primarily driven by coronary artery stenosis or by total blockage resulting from thrombosis or atherosclerosis that threatens human health. Clopidogrel is one of the antiplatelet therapies that is widely used in the treatment of IHD. Ceruloplasmin (Cp) is a multi-copper oxidase enzyme that exerts both antioxidant and pro-oxidant activity. This study aims to investigate the short-term effect of Clopidogrel on Cp and some biochemical parameters related to IHD. This study examined oxidase activity and Cp concentration in IHD patients treated with Clopidogrel. Fifty blood samples were collected from patients who were diagnosed with IHD (pre-treatment). Those were followed up for 5 days after being treated with Clopidogrel (post-treatment). Also, 50 healthy volunteers were included in this study. All samples were collected during the period from January to April 2025. The oxidase activity and concentration of Cp were significantly higher in the same IHD patients both before and after Clopidogrel treatment compared with the control group. However, no significant differences were observed in oxidase activity or concentration of Cp when comparing the pre-treatment and post-treatment measurements within these same patients. These findings indicate that increased oxidative stress in IHD may contribute to stimulate Cp production as a defensive response.

Keywords: Antioxidant, Ceruloplasmin concentration, Ceruloplasmin oxidase activity, Clopidogrel therapy, Ischemic Heart Disease.

تأثير العلاج بالكلوبيدوغريل على نشاط وتركيز السيرولوبلازمين وأوكسيديز في مرضى القلب الإقفاري

علي وليد نعمان^{1*}, فائز حميد هادي², ازهار كامل³

¹قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد، العراق

²مديرية صحة بغداد - الكرخ، وزارة الصحة العراقية، بغداد، العراق

³جهاز الإشراف والتقييم العلمي، وزارة التعليم العالي والبحث العلمي، بغداد، العراق

*Email: ali.w@sc.uobaghdad.edu.iq



الخلاصة

مرض القلب الإقفاري ينشأ بشكل رئيسي بسبب تضيق الشريان التاجي أو الانسداد الكامل الناتج عن الجلطة أو تصلب الشرايين والذي يهدد صحة الإنسان. يُعد كلوبيدوغريل أحد العلاجات المضادة للصفائح، ويُستخدم على نطاق واسع في علاج مرض القلب الإقفاري. السيرولولوبلازمين هو إنزيم أوكسيديز متعدد النحاس، يمارس فعالية مضادة للأكسدة ومحفزة للأكسدة. يهدف هذا البحث إلى دراسة التأثير قصير المدى للكلوبيدوغريل على السيرولولوبلازمين وبعض المتغيرات الكيميائية الحيوية المتعلقة بمرض القلب الإقفاري. هذه الدراسة تختبر فعالية الأوكسيديز وتركيز السيرولولوبلازمين لدى مرضى القلب الإقفاري الذين عولجوا بالكلوبيدوغريل. جُمعت خمسون عينة دم من مرضى القلب الإقفاري (قبل العلاج). تم متابعة وجمع عينات لهؤلاء المرضى بعد خمسة أيام من العلاج بالكلوبيدوغريل (بعد العلاج). كما شملت الدراسة خمسين متطوعاً سليماً. جُمعت جميع العينات خلال الفترة من كانون الثاني إلى نيسان 2025. كان نشاط الأوكسيديز وتركيز السيرولولوبلازمين أعلى بشكل ملحوظ لدى نفس مرضى القلب الإقفاري قبل وبعد العلاج بالكلوبيدوغريل مقارنةً بالمجموعة الضابطة. ومع ذلك، لم تُلاحظ أي فروق جوهرية في نشاط الأوكسيديز أو تركيز السيرولولوبلازمين عند مقارنة قياسات ما قبل العلاج وما بعده لدى نفس المرضى. تشير هذه النتائج إلى أن زيادة الإجهاد التأكسدي في مرض القلب الإقفاري قد تساهم في تحفيز إنتاج السيرولولوبلازمين كاستجابة دفاعية.

1. Introduction

Ischemic heart disease (IHD) is primarily driven by coronary artery stenosis or by total blockage resulting from thrombosis or atherosclerosis that threatens human health.[1]. It is characterized by the presence of obstructive atherosclerotic plaques that reduce coronary blood and causes an imbalance between myocardial oxygen supply and oxygen consumption, triggering myocardial ischemia [1, 2]. The initial pathological route that leads to IHD is atherosclerosis which has developed chiefly to cause heart failure (HF) [3, 4]. Atherosclerosis is an inflammatory disease of the arteries associated with metabolic alterations and lipid deposition driven by multiple risk factors. More than 70% of the general population have various risk factors for IHD, and only 2%-7% of them have no risk factors [3].

Antiplatelet therapy diminishes the risk of serial vascular events such as stroke, myocardial infarction and vascular death in patients with different types of cardiovascular diseases (CVD) [1]. Clopidogrel is one of the antiplatelet therapies that is widely used in the treatment of IHD, cerebrovascular disease, and peripheral artery disease regardless of the acute coronary syndrome [5, 6]. Clinical studies and post-marketing data have shown that clopidogrel has a safety profile comparable to aspirin, with low incidence of adverse effects. because of its low-risk side effect profile and positive clinical data over the last two decades [5]. It is an irreversible inhibitor of adenosine diphosphate (ADP) receptor (P2Y₁₂). Clopidogrel is a prodrug, which metabolizes in the liver to an active metabolite by cytochrome complex (CYP1A2, CYP3A4, CYP2C19). The active metabolite of Clopidogrel prevents ADP from inducing platelet aggregation through binding with P2Y₁₂ receptor [7-10]. It is used as an alternative to aspirin in cases of intolerance or contraindications or as a dual antiplatelet therapy in combination with aspirin after a transient or minor ischemic attack in patients with a higher risk of recurrent stroke [11].

Recently, a growing body of research has indicated that oxidative stress plays a pathophysiological role in the early stages and the progression of atherosclerosis through the generation of reactive oxygen species (ROS), oxidation of LDL and oxidative cell damage. It has been reported that increased ROS levels and decreased antioxidant levels are linked to the development of several human diseases [12, 13]. Various antioxidants have been synthesized or extracted for mitigating the effects of ROS and Clopidogrel has been reported to enhance antioxidant effects [14]. An elevation in oxygen-derived free radicals reduces nitric oxide (NO) bioavailability, stimulates platelet activation and abnormal interactions of platelet-endothelial [15-18]. It has been reported that reducing oxidative stress through improving endothelial NO

bioavailability may be another important process by which Clopidogrel inhibits platelet aggregation in patients with IHD [15, 17, 19].

Ceruloplasmin (EC1.16.3.1) (Cp) is a multi-copper oxidase enzyme which is synthesized and secreted mainly by the hepatocytes. Its structure includes seven copper atoms which enable the oxidation of substrates by transferring of four electrons to oxygen molecules. In healthy human plasma, Cp contains 95% of the total circulating copper [20, 21]. It is enhanced in acute inflammation conditions [21, 22]. Ceruloplasmin contributes to iron metabolism through the oxidation of ferrous into ferric. It acts as an effective antioxidant by inhibiting the Fenton reaction and lipid peroxidation as well as scavenging of circulating superoxide anion radicals and protects the cells from the harmful effect of oxidants [21, 23]. However, it has been stated that Cp exerts both antioxidant and pro-oxidant (vasculopathic) activity. Oxidation of NO by Cp may likely reduce NO levels in the heart, thereby stimulating oxidative stress. Additionally, some studies suggested that increased levels of Cp are linked with augmented risks of developing heart failure [20-23]. To the best of our knowledge, there have been no studies conducted on the effect of Clopidogrel therapy on the levels of Cp in patients who are suffering from IHD. Therefore, this study aims to investigate the short-time effect of Clopidogrel on Cp, especially the Cp synthesis and the metabolic activation of Clopidogrel occur predominantly in the liver compartment.

2. Materials and Methods

The present work was conducted on 100 participants who were divided into three groups, 50 patients diagnosed with IHD before treatment with Clopidogrel aged (50.0 ± 8.8 years) (IHD group). Those patients underwent percutaneous transluminal coronary intervention (PCI) treatment and were followed up prospectively at 5 days after receiving Clopidogrel (75 mg per day) (IHDclopidogrel group). They were categorized as IHD patients by a consultant cardiologist and registered at the Heart Center in the City Hospital, during the period from January to April 2024. Fifty participants were considered healthy according to their history (control group), without prior IHD, and were matched in age to IHD patients (48.4 ± 9.7 years), Figure 1.

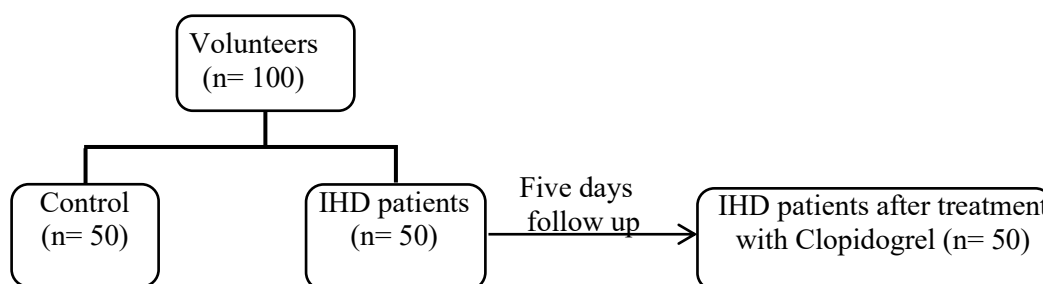


Figure 1: Volunteers' distribution included in the study. IHD: ischemic heart disease.

The following participants were excluded: severe renal or hepatic impairment, active infections, uncontrolled metabolic disorders, or recent major surgeries, and those who are smoking, drinking or taking medications that interfere with study outcomes. Informed consent was obtained from all participants before their enrolment in the study. Furthermore, the study was approved by the Consultancy of the Scientific Board (University of Baghdad, 5 May 2023, Ref. CSEC/0223/0013) which adhered to the ethical principles outlined in the Declaration of Helsinki.

Body mass index (BMI) was obtained by calculating the ratio of weight (kg) to height (m^2). Blood samples (5 mL) were obtained from the participants using a gel tube for collection serum, while an EDTA tube was used for obtaining of plasma, samples were left at room temperature for 10 minutes, then centrifuged (Jouan C4i, Thermo, France) at 1500 xg for 10 minutes and

stored at -20 °C until use. Fasting blood sugar (FBS), total protein (TP), albumin (Alb), Aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, uric acid, creatinine were measured by colorimetric methods using cobas kits (Cobas c311, Roche/Germany), while total cholesterol (TC), triglyceride (TG), High density lipoprotein-cholesterol (HDL-C), were determined using commercially available assay kits (Abbott, Chicago, Ill) with an Abbott Aeroset auto-analyzer (Chicago, IL, USA). Complete blood count (CBC) includes white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets (PLT), and lymphocytes (LYM) were measured using an auto-analyzer device (Abbott, U.S.A). Globulin value was calculated by subtracting the Alb from the TP

Ceruloplasmin activity was determined spectrophotometrically through oxidation of p-phenylenediamine (substrate) to a blue-violet colored compound (bandrowski base) as described by Rice W. [24]. All reagents were prepared freshly and pre-incubated with serum samples at 37 °C for 5 minutes. A volume of 100 µL of the serum sample was diluted with 1 mL of buffered substrate reagent (p-phenylenediamine.2HCl 5.5 mM in acetate buffer pH 5.2, 0.4 M containing 0.4 µM EDTA) and incubated at 37 °C for 15 minutes. After incubation, 3 mL of working inhibition reagent (sodium azide 3 mM and sodium chloride 18.4 mM) was added to the reaction mixture and the absorbance was measured at 540 nm. The control tube was prepared for each sample using the same procedure except that the serum was added in the last step. The oxidase activity of Cp has been expressed as the number of micromoles of Bandrowski's base formed per 15 minutes, and was calculated using the equation:

International Units = $(A_{\text{sample}} - A_{\text{control}}) \times \text{Final multiplication factor}$

The final multiplication factor is 394 and was estimated from the equation:

Final multiplication = $(1/1.91) \times (10000/15)$

Where 1.91 represents the molar absorptivity coefficient of Bandrowski's base in (L/mole.cm) and 15 represents the incubation time in (minutes). Ceruloplasmin concentration was determined by measuring the absorbance at 605 nm and calculating it using the equation:

Concentration = $(A_{\text{sample}} - A_{\text{control}}) \times 87.5$

Where 87.5 represents the Holmberg-Laurell factor [25].

GraphPad Prism 6 software was used for statistical analysis. All measurement data, expressed as mean $\pm$ standard deviation (SD), were analysed using the unpaired t-test for comparison of control with IHD and IHD treated with Clopidogrel groups, and paired t-test for comparison of IHD group with IHD treated with Clopidogrel group, and $P < 0.05$ was considered significant. The association between oxidase activity and concentration of Cp was analyzed using Pearson's correlation and the sensitivity and specificity of Clopidogrel treatment were evaluated using the receiver operating characteristic (ROC) test using receiver operating characteristic (ROC) test.

3. Results

The participants' demographic, clinical, and lifestyle characteristics are presented in Table 1. Neither age nor BMI shows significant differences between IHD patients and the control group. In the IHD group, levels of FBS, ALB/GLB, TC, TG, VLDL-C, ALT, creatinine, uric acid, RBC, HGB, MCV, MCH, and RDW were significantly higher than in the control group. However, the levels of TP, Alb, Glb, HDL-C, ALP, urea/creatinine, HCT, and MCHC were markedly decreased in the IHD group compared with the control group.

Table 1: Participant's demographic, clinical and lifestyle characteristics.

Parameters	Control (n=50)	Untreated IHD (n=50)	IHD treated with Clopidogrel	P-value
Smoking	Non		Non	-
Drinking	Non		Non	-
Chronic diseases	Non		Non	-
Take medication	Non		Non	-
Sex (male/female)	25/25	25/25		-
Age (year)	48.36±9.70	50.02±8.81		NS
BMI (kg/m ²)	26.12±3.60	28.81±3.11		NS
FBS (mg/dL)	93.38±6.64	117.21±23.20	104.60±15.22	<0.001a,b,c
TP (g/dL)	7.21±0.44	5.15±1.21	7.33±0.37	<0.001a,c
Alb (g/dL)	4.20±0.41	3.12±0.42	4.33±0.50	<0.001a,c
Glb (g/dL)	3.00±0.45	2.23±0.71	3.01±0.63	<0.001a,c
Alb/Glb	1.41±0.30	2.42±1.70	1.50±0.42	<0.001a,c
Lipid profile				
TC (mg/dL)	161.38±28.31	174.21±26.55	140.60±26.41	0.007a, <0.001b,c
TG (mg/dL)	121.44±24.75	134.01±26.20	135.92±20.92	0.006a, <0.001b
HDL-C (mg/dL)	54.41±3.90	44.21±4.46	53.11±3.02	<0.001a,c, 0.034b
LDL-C (mg/dL)	82.68±29.51	93.20±27.81	60.32±26.73	<0.001b,c
VLDL-C (mg/dL)	24.25±5.03	26.77±5.20	27.33±4.18	0.006a, <0.001b
Liver function parameters				
ALT (U/L)	14.60±3.06	15.44±1.91	15.52±1.81	0.039a, 0.025b
AST (U/L)	11.41±2.28	11.91±1.82	11.90±1.55	NS
ALT/AST	1.53±1.10	1.32±0.33	1.25±0.30	NS
ALP (IU/L)	80.21±19.16	69.01±24.85	83.43±13.07	0.006a, <0.001c
Renal function parameters				
Urea (mg/dL)	27.71±5.91	26.88±14.62	35.10±6.31	<0.001b,c
Creatinine(mg/dL)	0.80±0.12	0.92±0.20	1.01±0.59	0.003a, 0.023b
Urea/Creatinine	34.55±6.11	29.90±14.72	35.10±6.43	<0.001a,c
Uric acid (mg/dL)	4.72±0.74	5.26±1.81	4.63±0.62	0.021a, <0.001c
Complete blood count				
WBC (10 ³ /μL)	7.56±1.51	7.77±1.90	8.52±1.31	0.001b, <0.001c
LYM (%)	32.60±6.63	33.81±6.27	34.30±6.21	NS
RBC (10 ⁶ /μL)	4.55±0.54	6.40±3.87	4.61±0.43	0.002a, <0.001c
HGB (g/dL)	14.19±1.25	20.11±11.73	14.66±2.60	0.001a, <0.001c
HCT (%)	42.61±3.54	39.90±5.80	42.51±3.20	0.003a, <0.001c
MCV (μm ³)	86.03±2.40	87.22±2.58	87.01±2.73	0.007a, 0.028b
MCH (pg/cell)	28.88±1.01	30.32±2.74	29.44±1.81	0.001a, <0.001c
MCHC (g/dL)	34.11±1.10	30.14±8.03	33.77±1.02	0.001a, <0.001c
RDW (%)	12.90±0.88	15.80±5.92	13.10±0.93	0.001a, <0.001c
PLT (10 ³ /μL)	287.56±69.11	261.21±97.80	294.83±63.42	<0.001c

Data are presented as mean±SD. Significant differences (*P<0.05) are presented as (a= between control and IHD groups), (b= between control and IHDclopidogrel groups), (c= between IHD and IHDclopidogrel groups), NS: non significant. Alb: albumin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: Aspartate transaminase; BMI: Body Mass Index; FBS: fasting blood sugar; Glb: globulin; HCT: haematocrit; HDL-C: high-density lipoprotein-cholesterol; HGB: haemoglobin; IHD: ischemic heart disease; LDL-C: low-density lipoprotein-cholesterol; LYM: lymphocytes; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; PLT: platelets; RBC: red blood cells; RDW: red blood cell distribution width; TC: total cholesterol; TG: triglyceride; TP: total protein; VLDL-C: very low-density lipoprotein-cholesterol; WBC: white blood cells.

The results of other biochemical parameters showed no significant differences between the IHD and control groups. After treatment with Clopidogrel, the results for FBS, TG, VLDL-C, ALT, urea, creatinine, WBC and MCV showed a significant increase compared with the control. Nevertheless, TC, HDL-C and LDL-C decreased significantly in the IHD treated with clopidogrel group compared to the control. There were no significant differences in TP, Alb, Glb, Alb/Glb, AST, ALT/AST, ALP, uric acid, LYM, RBC, HGB, HCT, MCH, MCHC, RDW and PLT between IHDclopidogrel group and the control. These outcomes showed a significant increase in TP, Alb, Glb, HDL-C, ALP, urea, urea/creatinine, WBC, HCT, MCHC and PLT of the IHD treated with Clopidogrel compared to the IHD group. In contrast, the results of the IHD treated with Clopidogrel group showed a significant decrease in FBS, Alb/Glb, TC, LDL-C, uric acid, RBC, HGB, MCH and RDW compared to the IHD group. There were no significant differences in other biochemical parameters between the IHD and IHD treated with Clopidogrel groups.

Figure 2 shows the oxidase activity and the concentration of Cp enzyme. However, there were no significant differences in Cp oxidase activity and concentration between the IHD and IHD treated with Clopidogrel groups. In the IHD group, results indicated a significant increase in Cp oxidase activity compared to the control. This result was also observed between the IHD treated with Clopidogrel and the control group. Similarly, the concentration of Cp was significantly higher in IHD and IHD treated with Clopidogrel compared to the control. In the IHD treated with group, a strong positive correlation between oxidase activity and Cp concentration was observed. Nevertheless, no significant correlations were observed among the other studied parameters. The diagnostic accuracy for Cp activity was estimated using ROC curve cut-off values, as shown in Figure 4 and in Table 2. The results show that oxidase activity and Cp concentration have high diagnostic accuracy in predicting IHD. The oxidase activity of Cp shows promising results for monitoring Clopidogrel's effect on IHD progression.

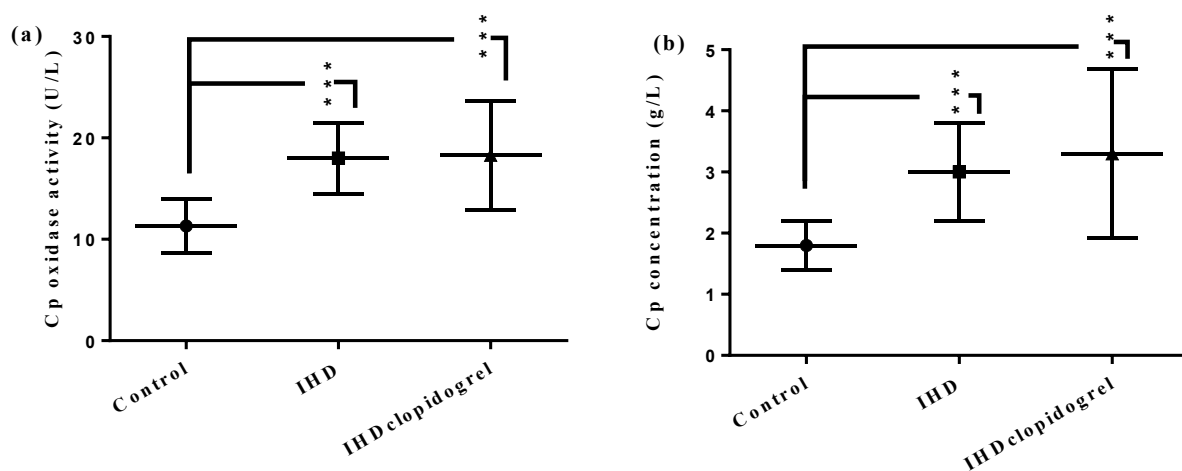


Figure 2: The oxidase activity (a) and concentration (b) of Cp enzyme in studied. IHD: ischemic heart disease; Cp: Ceruloplasmin.

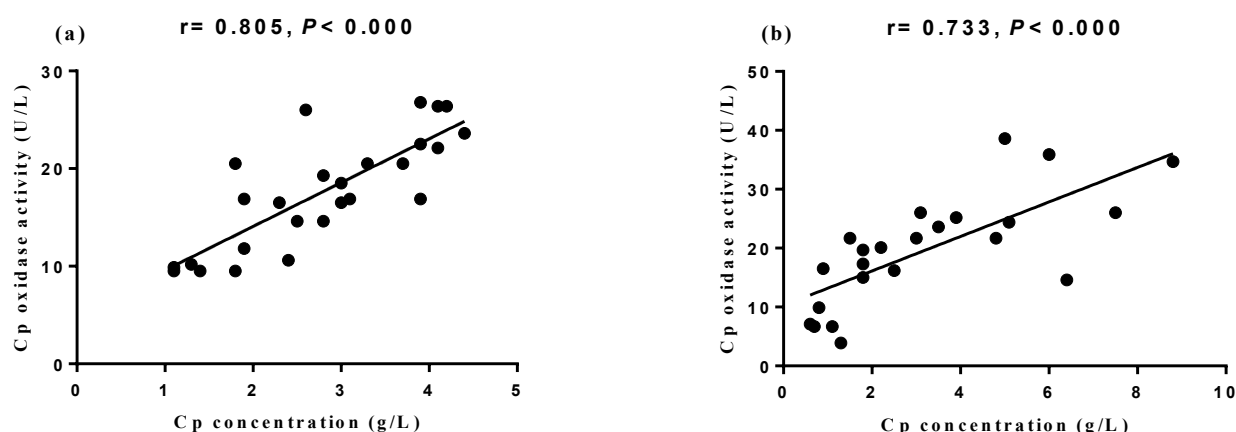


Figure 3: Pearson correlations of (a) Cp oxidase activity with its concentration in the IHD group and (b) Cp oxidase activity with its concentration in the IHD treated with Clopidogrel group. Cp: Ceruloplasmin.

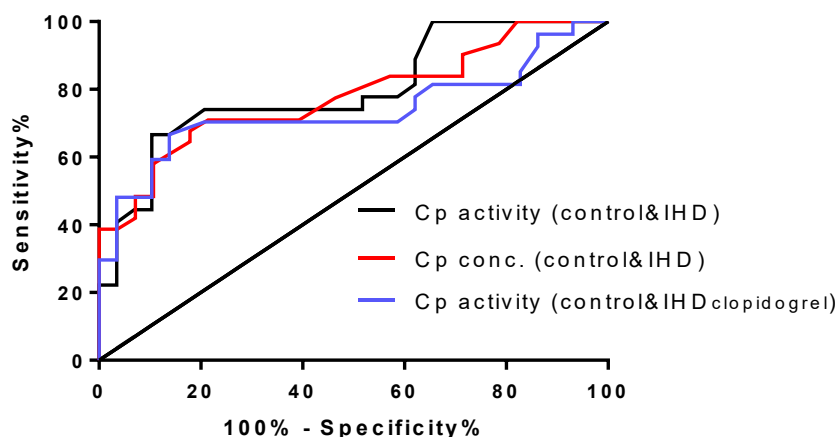


Figure 4: The ROC curve of Cp oxidase activity and concentration between Control and IHD groups and the Cp oxidase activity between control and IHD treated with Clopidogrel groups. IHD: ischemic heart disease; Cp: Ceruloplasmin.

Table 2: Analysis data of the ROC curve includes the percentage of sensitivity and specificity at best cut-off discrimination and the area under the curve.

Parameters	Cp activity (control&IHD)	Cp concentration (control&IHD)	Cp activity (control &IHD treated with Clopidogrel)
Sensitivity%	74.07	70.97	70.37
Specificity%	79.31	78.57	79.31
AUC	0.798	0.779	0.737
Cut-off (IU/L)	14.42	2.25	14.44
P-value	<0.001	<0.001	0.002

P-value <0.05 is considered significant. AUC: area under curve; Cp: Ceruloplasmin; IHD: ischemic heart disease.

4. Discussion

The main goal of the current study was to investigate the effects of Clopidogrel therapy on the Cp enzyme and several biochemical parameters in patients with IHD. The current results showed that Clopidogrel has significantly reduced the level of FBS in IHD patients. This result is consistent with a previous study [26] which reported that treatment with Clopidogrel can lower FBS levels. Total protein, Alb, and Glb were significantly increased after treatment with Clopidogrel, reaching levels similar to those in the control group. Several studies reported that CVD and IHD are negatively linked with Alb levels, and the decrease in serum Alb may contribute to some CVD deterioration such as IHD and heart failure by inducing myocardial edema [27-29]. It is therefore possible that Alb can be considered a negative acute-phase reactant, and the rise in protein level may indicate that Clopidogrel has been involved in IHD improvement.

Regarding lipid profile, treatment with Clopidogrel reduced the TC and LDL-C in IHD patients to levels lower than those in healthy individuals. However, HDL-C levels increased significantly after Clopidogrel treatment compared with the control. Neither TG nor VLDL-C levels have been affected by Clopidogrel. A study by Wadowski *et al.* [30] reported that low levels of HDL-C are connected to impaired Clopidogrel-Mediated Platelet inhibition. According to current data, Clopidogrel improves the lipid status in IHD patients. Additionally, several studies reported that treatment with Clopidogrel is associated with a 5% rise in liver enzymes and induces liver injury [6, 31]. In the current study, this was observed in ALP levels. However, Clopidogrel does not affect the levels of ALT and AST in IHD patients. Renal function results indicated that under the effect of Clopidogrel, there was a noticeable increase in urea and creatinine in IHD patients compared to the control. This study suggested that Clopidogrel which binds to the ADP site on the P2Y₁₂ receptor can prolong the adenosine half-life and raise its plasma concentration by inhibiting cellular uptake. The interaction between adenosine and the tubuloglomerular feedback system has directly influenced the tone of the renal vascular. Adenosine together with angiotensin II acts synergistically to raise renal vascular resistance and decrease renal blood flow [32]. Thus, this leads to increased levels of serum urea and creatinine. However, the urea/creatinine ratio in the IHD-treated with Clopidogrel group returned to baseline. The level of uric acid was decreased in IHD patients to reach its corresponding level in the control group. It has been reported that elevated uric acid is linked to inflammation and potentially to PLT hyper-reactivity of platelet and the level of uric acid negatively correlated with the Clopidogrel low response in patients under treatment of antiplatelet with aspirin and Clopidogrel [33]. Additionally, our finding is consistent with Zhang H. [34] who found that Clopidogrel reduced the incidence of hyperuricemia. Furthermore, treatment with Clopidogrel improved the levels of RBC, HGB, HCT, MCH, MCHC, RDW, and PLT in IHD patients. However, the WBC level was increased under the effect of Clopidogrel. These results are consistent with several studies indicating that CBC levels remained within normal limits after 3 weeks of Clopidogrel administration [35, 36].

Moreover, this study investigated variations in Cp concentration and oxidase activity in patients with IHD before and after treatment with Clopidogrel. Both Cp oxidase activity and concentration were markedly increased in IHD patients compared with healthy individuals. Except for some studies, such as Verma *et al.* [37] study which established an inverse association between coronary risk factors and Cp and Enbergs *et al.* [38] study which did not show any quantitative connection between coronary atherosclerosis and Cp through angiography, several studies were consistent with our results which suggested a high level of Cp may be connected with CVD and a higher risk of heart failure [20, 21, 39, 40]. These studies have reported that Cp is an acute-phase reactant that is increased in conditions of acute

inflammation. The mechanism by which Cp may affect CVD remains unknown. Ceruloplasmin can inhibit the Fenton reaction and lipid peroxidation, acting as a circulating scavenger of superoxide radicals and preventing the harmful effects of free radicals on cells and tissues. Nevertheless, this study revealed that Cp can exert both pro-oxidant (vasculopathy) and antioxidant (protective) properties. Cp may likely decrease NO levels in the heart through pro-oxidant function related to NO oxidase, resulting in enhanced formation of ROS and oxidative cell damage. Several studies reported that NO plays a crucial role in normal cardiac physiology and provides protection in the ischemic and failing heart [20, 21, 23]. A study by Felix and Thomas [41] indicated that imbalance between NO bioavailability and oxidative stress is involved in the injury and dysfunction of endothelial which are critical in the pathogenesis of CVD [41]. Furthermore, PLTs are well known to represent a powerful source of ROS. Release of ROS in response to PLT homotypic and/or heterotypic interactions with leukocytes may reduce vascular NO bioavailability thereby impairing endothelial function [15].

To the best of our knowledge, no study has examined on the relationship between Cp and Clopidogrel in IHD patients. Our data show that neither oxidase activity nor Cp concentration was affected by Clopidogrel treatment after 5 days of follow-up of IHD patients. Clopidogrel is a member of the thienopyridine group that acts by blocking the platelet ADP receptor, an essential phase in platelet activation [8]. Another important mechanism by which Clopidogrel prevents PLT aggregation may be through reduced oxidative stress and improved NO bioavailability [15]. Therefore, the pro-oxidant and antioxidant functions may drive the expression of the Cp enzyme to a high level in IHD patients even after treatment with Clopidogrel due to an impaired balance between NO bioavailability and oxidative stress. It has been supposed that the increase in Cp in IHD patients is a response to increased oxidative stress (antioxidant function) and to NO elevation after treatment with Clopidogrel (pro-oxidant function).

5. Conclusions

It has been concluded that IHD patients exhibited a significant increase in Cp oxidase activity and concentration. Treatment with Clopidogrel did not affect Cp levels in those patients. The pro-oxidant and antioxidant functions may drive cells to express the Cp enzyme at high levels in IHD patients, even after treatment with Clopidogrel, due to an impaired balance between NO bioavailability and oxidative stress.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

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