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PREDICTIVE ROLE OF CERULOPLASMIN AND HIGH SENSITIVITE C-REACTIVE PROTEIN AS INFLAMMATORY MARKERS IN ATHEROSCLEROTIC CORONARY ARTERY DISEASE PATIENTS

By

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ABSTRACT

Background: Coronary artery disease (CAD) remains the leading cause of morbidity and mortality in the industrialized world. The root cause of CAD is mainly atherosclerosis. It seems to be a chronic inflammatory condition, which later develops into an acute clinical event by the induction of plaque rupture, causing thrombosis. Ceruloplasmin is the major copper-carrying protein in the blood and in addition plays a role in iron metabolism.

Objective: To assess the predictive value of ceruloplasmin and high-sensitivite C-reactive protein in diagnosis of atherosclerotic coronary artery disease.

Patients and methods: The study was conducted on 60 patients presented with clinical features of coronary artery diseases, admitted to Cardiology Department at Sayed Galal University Hospital, throught the period from July 2017 and March 2020, and they were classified according to coronary angiography into 3 subgroups (one, two, multi-vessel disease). They were compared with 20 healthy individuals with identical demographic characteristics not suffering from any disease. Serum ceruloplasmin and serum hs-CRP levels were measured in both groups.

Results: A significant increase in serum ceruloplasmin and hs-CRP levels were observed in all patients of coronary artery disease as compared to control group (p=<0.001).

Conclusion: In patients of CAD, the serum ceruloplasmin and hs-CRP levels rise due to their property as acute phase proteins.

Keywords: Coronary artery disease, Ceruloplasmin, Hs-CRP, Acute phase protein.

INTRODUCTION

Coronary artery disease (CAD), also known as ischemic heart disease (IHD), atherosclerotic heart disease, atherosclerotic cardiovascular disease, and coronary heart disease. CAD is a group of diseases that includes: stable angina, unstable angina, myocardial infarction, and sudden coronary death. It is within the group of cardiovascular diseases of which it is the most common type *(Wong, 2014)*.

Many studies have shown that inflammation is an important factor in coronary artery disease (CAD).

Inflammation contributes to different stages in the pathogenesis of CAD. Due to inflammation acute phase response occurs. This response is induced by pro-inflammatory cytokines, which are released from the inflamed tissue by inflammatory and parenchymal cells and stimulates the liver to synthesize a number of acute phase proteins *(Badiger et al., 2014).*

These acute phase reactants have been identified as valuable risk markers for the prediction of cardiovascular events. Among these, ceruloplasmin and high sensitive C-reactive protein (hs- CRP) have been central to most studies in the field *(Gupta et al., 2016).*

Ceruloplasmin is an important extracellular antioxidant. Ceruloplasmin, known as ferroxidase, is an enzyme synthesized in the liver containing six atoms of copper in its structure *(Linder, 2016).*

Ceruloplasmin is @-2globulin protein. It carries 90% of the copper in our plasma. It is an acute phase protein which rises after any form of tissue injury *(Riwantoet al., 2013).*

Several prospective studies have indicated that the serum copper or ceruloplasmin level may be an independent risk factor for cardiovascular disease *(Venkataramana et al., 2012).*

The increased risk has been attributed to prooxidant function of ceruloplasmin, and recent experimental studies demonstrating the ability of ceruloplasmin to oxidatively modify low-density lipoprotein (LDL) seem to underline this concept *(Rochette et al., 2014).*

Hs-CRP predicts future coronary events merely because it is associated with all of the major risk factors for atherosclerosis, namely dyslipidemia, smoking, hypertension, diabetes, abdominal obesity, depression, other psychosocial factors and many others *(Mirzaii-Dizgah et al., 2012).*

Hs-CRP level was found to be a potent predictor of cardiovascular events than the LDL cholesterol level *(Mirzaii-Dizgah et al., 2012).*

This study was performed to evaluate the predictive value of ceruloplasmin and high-sensitivity C-reactive protein in diagnosis of atherosclerotic coronary artery disease.

SUBJECTS AND METHODS

This study was done at Al-Azhar university hospitals in the period between July 2017 and March 2020 on a total of 80 individuals (age and sex matched), those individuals were divided into 2 groups: patient's group and control group. The patients group was subdivided according to coronary artery occlusion diagnosed by coronary angiography into three subgroups:

Group 1: One vessel disease, 20 patients.

Group 2: Two vessel disease, 20 patients.

Group 3: Multi-vessel disease, 20 patients.

Control group includes 20 apparently healthy individuals, not suffering from any manifestations of either acute or chronic cardiac diseases.

Inclusion criteria:

Patients presented with typical angina pain (chest pain in the left side radiating to the arms, shoulder or neck). This pain is characteristically precipitated by exertion, eating, exposure to cold or emotional stress. It lasts for approximately 1-5 min. and is relieved by rest or sublingual nitroglycerine. The intensity of pain does not change with respiration, cough or change in position. Exclusion criteria: Patients with chronic liver diseases, chronic kidney diseases, chronic inflammatory diseases and malignancy.

All participating individuals gave a written informed consent for applying in the study.

Methods:

1. Full history taking and clinical examination, ECG, Echocardiography and Coronary angiography.
2. Sample Collection: Eight ml venous blood sample were withdrawn from all participants of the study (both patients and controls) and divided into two portions: the first portion (two ml) was put in EDTA tube for CBC which was done using Sysmex Kx-21 automated cell counter. The second portion (six ml) was put in plain tube and left to clot for 30 minutes then serum was separated and divided into two aliquots, one for routine biochemical tests performed on same day of collection by an enzymatic colorimetric reaction using a Modular P analyzer (Roche Diagnostics) while the other aliquot was stored deeply frozen at - 20° for estimation of serum ceruloplasmin and hs-CRP. Ceruloplasmin was done by ELISA using a commercially available ELISA kit supplied by Calbiotech's, Inc, USA.

Hs-CRP was estimated by immunoturbidimetric method using Respons 920 (DiaSys Diagnostics).

Statistical analysis:

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 2, IBM Corp. U.S.A. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Probability (P-value):

P-value <0.05 was considered significant.

For all analyses, Mann-Whitney U test and Student's t-test were used.

RESULTS

Our study included a total of 80 individuals. 60 patients recruited to Al-Azhar university hospitals and 20 apparently healthy individuals. The table shows the demographic characteristics of study population in control and CAD patients in whom family history of CAD was found significantly high as compared with control.

Systolic and diastolic blood pressures were also significantly high in the patients group as compared with controls.

The present study showed a significantly higher serum total cholesterol levels in CAD patients ($p<0.001$) than that in the controls.

Also a significantly higher serum total cholesterol, triglyceride, LDL-cholesterol

and VLDL-cholesterol levels (p<0.001) in those patients as compared to control with a significant reduction in HDL-cholesterol (p<0.001). Moreover a significantly higher serum random blood sugar level (p<0.001) in those patients as compared to control. In coronary artery disease patients the mean CK-MB value was also found to be significantly high as compared to the control subjects (p<0.001).

There was a significant increase in serum ceruloplasmin level in CAD patients as compared to control subjects (p<0.001). Serum hs-CRP level was also found significantly high in CAD patients as compared to control subjects (p<0.001).

Table: Demographic characteristics, Biochemical changes, cardiac marker level and Inflammatory markers level in CAD patients and control

Groups / Parameters	Patients (N = 60)	Control (N = 20)	P-value
Hypertension	65%	20%	<0.001
Family history of CAD	48.3%	5%	<0.001
CK-MB (IU/L)	59	13	<0.001
Total Cholesterol (mg/dl)	196	131	<0.001
Triglyceride (mg/dl)	221.8	59.1	<0.001
HDL-Cholesterol (mg/dl)	27	46.5	<0.001
LDL-Cholesterol (mg/dl)	147.5	64.5	<0.001
VLDL (mg/dl)	44.3	11.6	<0.001
Random blood sugar	157.6	78.3	<0.001
Ceruloplasmin (ng/ml)	52	28.5	<0.001
Hs-CRP (mg/L)	22.7	2.25	<0.001

DISCUSSION

Cp was shown to inhibit a variety of oxidative reactions, involving peroxide and superoxide, including the Fenton reaction that forms OH radicals from H2O2, and dismutation of superoxide *(Linder, 2016).* High levels of ceruloplasmin may be attributed to inflammation process *(Valkanova et al., 2013).* The prooxidant property of ceruloplasmin involves lipid peroxidation. Studies indicate that ceruloplasmin by itself can oxidize LDL in vitro and possibly in vivo. However, accessory factors derived from the vascular cells may be modulatory or requisite during lipoprotein oxidation within the vessel wall *(Procházková et al., 2011).*

Studies have reported about the cardio-protective nature of ceruloplasmin which could protect the myocardial tissue against the deleterious effects of oxygen free radicals. Studies observing the role of transition metal ion-mediated oxidation of LDL molecules, centered on the role of human ceruloplasmin in this oxidative process, as it is the principal copper containing protein in serum *(Venkataramana et al., 2012).*

In particular, some studies have shown the prognostic value of elevated Cp, even after adjustment for traditional risk factors, for determining the future risk of cardiac events in stable cardiac patients *(Wilson Tang et al., 2012).*

CRP has been shown to be a prognostic marker of adverse clinical cardiac events, such as death, acute myocardial infarction, and urgent revascularization *(Mirzaii-Dizgah et al., 2012).*

Inflammatory markers such as CRP reflect the extent of myocardial necrosis and correlate with cardiac outcomes following acute MI. Moreover, myocardial necrosis following acute MI induces free radical generation and triggers the inflammatory cascade. Reperfusion therapy may also lead to further intensification of the inflammatory reaction, with the recruitment of neutrophils into the reperfused myocardium. All of these interconnected processes may account for the massive increase of serum hs-CRP levels, a classical marker of inflammation, following an ischemic heart event *(Mirzaii-Dizgah et al., 2012).* In our study, there wasa highly statistical significant increase in serum ceruloplasmin and hs-CRP in pathological group (One vessel disease, two vessel disease, and multi-vessel disease) in comparison to Control group. This study showed that 85% abnormally high ceruloplasmin levels for patients suffering from coronary artery diseases (< 41.5 ng/ml) and 100% normal ceruloplasmin levels in control (> 41.5 ng/ml). Also, 95% abnormally high hs-CRP levels for patients suffering from coronary artery diseases (< 6 mg/L) and 100% normal hs-CRP levels in control (> 6 mg/L). Patients with diabetes mellitus, hypertension, family history of CAD, high cholesterol levels, high triglycerides, low HDL-C, high LDL-C and high VLDL-C are at high risk for cardiovascular disease.

The results of this study were matched with that of *Sezen and Sezen (2018)* who found that high levels of ceruloplasmin may be an etiologic or diagnostic agent or a prognostic marker in cardiovascular health disorders. Also, *Stakhneva et al. (2017)* observed elevated concentration of C-reactive protein, the biomarker of acute phase, in patients with myocardial infarction and unstable angina. *Gupta et al. (2016)* showed a significant increase in serum hs-CRP and ceruloplasmin level in all patients of acute myocardial infarction as compared to control group and these higher levels also correlate with higher CK-MB level.

Chowdhury et al. (2015) found positive correlation between serums Ischemia modified albumin (IMA) and positive acute phase reactants ceruloplasmin and hs-CRP in patients of CAD. Kennedy et al. (2014) reported that serum ceruloplasmin levels in coronary artery disease patients elevated versus controls and were associated with increased risk of future major adverse cardiac events. After adjusting for traditional risk factors, higher serum ceruloplasmin was still associated with higher risk of major adverse cardiac events at 3 years. Similarly, the elevated concentration of ceruloplasmin is independently associated with increased risk of mortality from all causes including cardiovascular events *(Grammer et al., 2014). Hammadah et al. (2014)* showed increased ceruloplasmin levels were associated with increased 5 year all-cause mortality. When controlled for coronary disease traditional risk factors, creatinine clearance, dialysis, body mass index, medications, history of myocardial infarction, BNP, left ventricular ejection fraction (LVEF), heart rate, QRS duration, left bundle branch blockage, and implantable cardioverter-defibrillator placement, higher Cp remained an independent predictor of increased mortality. Model quality was improved with addition of ceruloplasmin

to aforementioned co-variables. *Bhagwat (2013)* observed high concentration of serum ceruloplasmin and hs-CRP in patients with coronary arterydisease. Moreover, *Dadu et al. (2013)* found that ceruloplasmin was associated with incident HF, mortality, and CVD in the ARIC population.

Xu et al. (2013) found that ceruloplasmin levels in ischemic and nonischemic groups were higher than those in control group. Ceruloplasmin had a positive linear correlation with C-reactive protein and a negative linear correlation with LVEF. Ceruloplasmin showed an independent association with the extent of heart failure in nonischemic cardiomyopathy patients. Ceruloplasmin was significantly elevated in patients with ischemic or nonischemic cardiomyopathy and had linear correlation with C-reactive protein and LVEF. In nonischemic cardiomyopathy patients, the ceruloplasmin value was an independent biomarker associated with the extent of heart failure.

CONCLUSION

Serum ceruloplasmin and serum hs-CRP levels in patients of CAD were found to be abnormally high. The raised ceruloplasmin and hs-CRP levels in patients of CAD suggested involvement of inflammation in the etiopathogenesis of CAD and have prognostic utility in CAD. Serum ceruloplasmin and hs-CRP levels are potent predictors of prognosis in patients with CAD and elevated levels of these inflammatory markers at the time of admission indicate a poor prognosis in patients with CAD. Higher serum ceruloplasmin and hs-CRP levels on admission in patients of CAD were due to their property as acute phase proteins.

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الدور التنبؤى للسيرولوبلازمين والبروتين التفاعلى سى عالى الحساسية كدلالة للإلتهاب فى مرضى تصلب الشريان التاجى

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خلفيـــة البحـــث: تعتبـــر أمـــراض القلـــب والأوعيـــة الدمويـــة الســـبب الأول للوفـــاة فـــي العـــالم، والســـيرولوبلازمين عامـــل خطـــر مهـــم يمكنـــه التنبـــؤ باحتشـــاء عضـــلة القلـــب الحـــاد وأمـــراض القلـــب والأوعيـــة الدمويـــة، كمـــا يعتبـــر مســـتوى البـــروتين التفـــاعلي ســـى عالى الحساسية واحدا من أقوى تنبؤات أمراض القلب والأوعية الدموية.

الهـــدف مـــن البحـــث: تقيـــيم مســـتوى الســـيرولوبلازمين والبـــروتين التفـــاعلى ســـى عـــالى الحساسية فى مرضى شرايين القلب التاجية.

المرضـــى وطـــرق البحـــث: تـــم أخـــذ عينـــات الـــدم مـــن ســـتين مـــريض بـــأمراض القلـــب المحجـــوزين بقســـم أمـــراض القلـــب و الأوعيـــة الدمويـــة، مستشـــفى ســـيد جـــلال الجـــامعى، فـــى الفتـــرة مـــن يوليـــه 2017 إلـــى مـــارس 2020، وتـــم فصـــل المصـــل وإســـتخدامه لتحليـــل مســـتوى الســـيرولوبلازمين والبـــروتين التفـــاعلى ســـى عـــالى الحساســـية فـــي مصل الدم.

النتـــائج: لـــوحظ وجـــود إخـــتلاف ذو قيمـــة إحصـــائية بـــين المجموعـــات فيمـــا يخـــص دلالات الالتهابـــات، وكانـــت النتـــائج طبيعيـــة للمجموعـــة الضـــابطة أمـــا المرضـــى فكـــان خمـــس وثمـــانون بالمائـــه مـــنهم لـــديهم إرتفـــاع فـــى مســـتوى الســـيرولوبلازمين فـــى مصـــل الـــدم وخمـــس وتســـعون بالمائـــه مـــنهم لـــديهم إرتفـــاع فـــى مســـتوى البـــروتين التفـــاعلى ســـى عالى الحساسية فى مصل الدم.

الاســـتنتاج: هنـــاك علاقـــة بـــين إرتفـــاع نســـب الســـيرولوبلازمين والبـــروتين التفـــاعلى سى عالى الحساسية وأمراض الشريان التاجى.