The Effect of Biomarkers (Leptin, Hs-CRP, Ceruloplasmin) and their Relationship to Cardiovascular Disease

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Abstract

Background: Cardiovascular events can be seen in patients without classical cardiovascular risk factors, spurring the search for novel risk markers. Biomarkers are quantifiable bodily substances that indicate disease. Biomarkers have been studied concerning cardiovascular disease (CVD) to better understand their role in CVD development and progression. Biomarkers include Leptin, hs-CRP, and ceruloplasmin. These biomarkers cannot diagnose cardiovascular disease alone, but they can supplement risk factors and risk stratification.

Method: This is a case-control study that included 90 coronary artery disease patients proved by coronary angiography. Included 53 males and 37 females, ages ranging from 25–45 years, who attended Al-Sadr Teaching Hospital and Basra Oil Specialized Cardiac Center, from December 2022 to March 2023. The patient's group were divided into a high-risk group and a low-risk group according to the clinical presentation & numbers of coronary vessel disease. On the other hand, 90 healthy participants matched in their age and sex to the patient's group were included in the study. Leptin was measured by an enzyme-linked immunosorbent assay (ELISA) whereas ceruloplasmin and hs-CRP were assayed by electrochemiluminescence immunoassay (ECLIA) using Cobas e411.

Results: Leptin and hs-CRP levels were significantly higher ($p<0.05$) while ceruloplasmin was significantly lower ($P<0.05$) in patients with coronary artery disease compared to age-matched healthy control subjects. These novel risk markers showed no significant statistical differences between high & low-risk groups of patients with coronary artery disease

Conclusion: There is a positive relationship between the levels of leptin, hs-CRP & risk of CVD, which might be considered as a novel risk marker for the presence rather than severity of coronary artery disease.

Keywords: hs-CRP, cardiovascular disease, novel biomarkers.

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Introduction

Cardiovascular disease (CVD) is the term used to describe the medical conditions characterized by the presence of heart and blood vessel disorders. A wide range of cardiovascular diseases exists, encompassing angina, coronary events, stroke, heart failure, hypertension, rheumatic fever, cardiomyopathy, arrhythmias, valvular heart disease, and congenital heart disease.(1) The presence of fatty streak, which is considered a characteristic feature of atherosclerotic plaque, can arise from various circumstances. The development of atherosclerotic plaque is influenced by various factors, including intimal proliferation, smooth muscle cell aggregation, and the presence of extracellular matrix and lipid-laden macrophages (also known as foam cells). (2,3) Angina occurs when there is stenosis or occlusion of one or more coronary arteries, leading to insufficient blood supply to the myocardium. Atherosclerosis, a kind of coronary artery disease, represents the predominant etiology for the occurrence of coronary artery obstruction. Less frequently seen yet plausible etiologies of angina encompass conditions such as anemia, heart

failure, and arrhythmias. (4,5) The precise role of leptin in the cardiovascular system remains uncertain. Numerous investigations have established a correlation between leptin and chronic inflammation. Elevated concentrations of leptin have been associated with a heightened susceptibility to cardiovascular disease among those who are obese. Furthermore, individuals diagnosed with dilated cardiomyopathy exhibit elevated levels of leptin, a biomarker associated with the progression of heart failure that is not influenced by immunological responses. (6,7) Ceruloplasmin (Cp) is an enzymatic protein synthesized in the hepatic tissue, primarily responsible for the oxidation of nitric oxide (NO) and facilitation of low-density lipoprotein oxidation, concomitantly reducing the bioavailability of NO. The release of endogenous nitric oxide (NO) has an impact on cardiac function and the pathogenesis of heart failure (HF). Endothelial dysfunction and atherosclerosis are consequences of reduced nitric oxide (NO) bioavailability, mostly attributed to the presence of oxidized low-density lipoprotein (LDL).

High-sensitivity C-reactive protein (hs-CRP), leukocyte counts, and myeloperoxidase are examples of nonspecific inflammatory markers that have been linked to CVD. Additionally, Cp, which is an acute-phase reactant, is correlated with these aforementioned variables. These data offer additional support for the association between Cp and CVD. (8,9) The present study aimed to determine the values of serum leptin, ceruloplasmin, and hs-CRP in CVD patients and to see the differences between those parameters with the severity of the disease angiographically.

Methodology

This case-control study included 90 patients diagnosed with obstructive CAD by coronary angiography performed by an interventional cardiologist, 53 males and 37 females with CAD, their ages ranged from 25-45 years. The patients were classified according to their signs and symptoms and the number of vessels into two groups: Acute coronary syndrome, 2 vessels, 3

vessel disease, and left main stem disease all constitute a high-risk category. Chronic, stable angina or single-vessel illness with a non-critical coronary lesion are examples of low-risk categories. A control group of 90 healthy volunteers (53 males and 37 females). We excluded the children, and patients aged above 55 and under 25 years. Blood samples were collected for measurement of serum Leptin, hs-CRP and ceruloplasmin. Leptin was assayed by an Enzyme-linked immunosorbent assay (ELISA) according to the operational automated of Sunlog, China. hs-CRP and ceruloplasmin were assayed by an electrochemiluminescence immunoassay (ECLIA) by Cobas e411 ROCHE, Germany. The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before conducting the study. The study protocol the subject information and the consent form were reviewed and approved by a local committee on publication ethics at Basra Health Directorate under reference No. 1234 on 30/11/2022.

Statistical Analysis

Data are presented as means ± standard deviation (SD). Differences between groups' means were tested by t-test and chi-square test. Correlations between variables were also determined. P-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows (version 26, USA).

Results:

Regarding the baseline sociodemographic characteristics, there are no significant differences in sex, age or smoking habit between healthy controls and patients (P>0.05). Blood pressure was significantly high (P<0.01) in patients compared to the controls.

Table (1): Socio-demographic characteristics of the study population

Characteristic		Control group (n=90)	Case group (n=90)	P. value
Male	No.	52	53	
	%	57.8%	58.9%	0.88
Female	No.	38	37	
	%	42.2%	41.1%	
Age (year) Mean ± SD		39.41 ± 5.31	40.42 ± 4.28	0.92
Smoker	Yes	46	51	0.52
	No	44	39	
Hypertension	Yes	39	59	<0.01
	No	53	31	
Weight(kg)	mean± SD	75.72±9.35	77.09±10.66	0.335
Height(m)	mean± SD	1.62±0.08	1.60±0.06	0.239
BMI (kg/m2)	mean± SD	28.94±4.09	29.84±4.10	0.125

(Table 2) showed significantly lower levels of ceruloplasmin in the patient's group when compared to the control group (P<0.05). The table also showed highly significant levels (P<0.001) in each of leptin and hs-CRP among the patients group compared to the control.

Table (2): The difference between Leptin, Ceruloplasmin and hs-CRP of the study population

Parameters	Control group (n=90)		Case group (n=90)		P. value
	Mean	SD	Mean	SD	
Leptin	3.7±	0.9	4.2±	0.8	<0.001
Ceruloplasmin	0.24±	0.08	0.22±	0.06	<0.05
hs-CRP	1.3±	1.2	6.9±	12.8	<0.001

The comparison of Leptin, CP and hs-CRP in patients and control group according to sex is shown in (Table 3) which revealed a significantly high level of leptin and hs-CRP in the patients'

group (P<0.01), while there is a non-significant change in CP (P>0.05).

Table (3): Comparison of Leptin, Ceruloplasmin and hsCRP in male patients and control groups.

Parameters	Control group (n=52)	Case group (n=53)	P. value
	Mean ± SD	Mean ± SD	
Leptin	3.6 ± 0.95	4.1 ± 0.8	<0.01
Ceruloplasmin	0.2 ± 0.12	0.2 ± 0.06	0.36
hs-CRP	1.4 ± 1.09	5.5 ± 3.5	<0.001

The comparison of Leptin, CP and hs-CRP in patients and control group according to sex **is** shown in Table (4) which revealed significantly high levels of leptin and hs-CRP in the patients group (P<0.01), while there is a non-significant change in CP (P>0.05).

Table (4): Comparison of Leptin, Ceruloplasmin and hsCRP between female patients and control group.

Parameters	Control group. (n=38)	Case group (n=37)	P. value
	Mean ± SD	Mean ± SD	
Lepton	3.9 ± 0.8	4.3 ± 0.7	<0.05
Ceruloplasmin	0.2 ± 0.1	0.2 ± 0.09	0.751
hs-CRP	1.2 ± 1.2	4.8 ± 4.3	<0.05

The comparison of these parameters in both sexes in the patient group showed non-statistically significant differences (P > 0.05), Table 5.

Table (5): Comparison of the biomarkers studied in male and female patients' groups.

Parameters	Male group (n=53)	Female group (n=37)	P. value
	Mean ± SD	Mean ± SD	
Leptin	4.1 ± 0.8	4.3 ± 0.7	0.18
Ceruloplasmin	0.2 ± 0.06	0.2 ± 0.09	0.32
hs-CRP	4.4 ±3.81	3.6 ±3.1	0.30

Non-significant changes (P>0.05) in the levels of leptin, ceruloplasmin, and hs-CRP

Table 6 In comparison to patients' groups of high risk and low risk.

Table (6): The difference between Leptin, Ceruloplasmin and hs-CRP in high-risk and

Low-risk groups

Parameters	Control group. (n=38)	Case group (n=37)	P. value
	Mean ± SD	Mean ± SD	
Lepton	3.9 ± 0.8	4.3 ± 0.7	<0.05
Ceruloplasmin	0.2 ± 0.1	0.2 ± 0.09	0.751
hs-CRP	1.2 ± 1.2	4.8 ± 4.3	<0.05

Discussion

Cardiovascular disease (CVD) is the leading cause of death in the developing world. To determine the risk of cardiovascular disease, we studied the levels of leptin, ceruloplasmin, and hsCRP, in addition to the information from the patient, which included age, gender, weight, height, blood pressure, and smoking. Leptin and hs-CRP are significantly high (P <0.001), while serum ceruloplasmin is significantly low (P<0.05). These results were agreed with the study conducted by Syed et al. in 2020 who revealed that Leptin was significantly

higher in patients with CVD (10). Leptin's mechanism is unclear. The function of inflammation in AMI has been extensively studied. TNF-, a pro-inflammatory mediator, is linked to mortality and recurrent myocardial infarction. Leptin normally reduces TNF- expression, but hyperleptinemia, especially in obese people, can activate TNF- through the p38 and JNK MAPK pathway in adipose tissues, triggering an inflammatory response. In obese people, TNF- is associated with insulin resistance, suggesting a role in type 2 diabetes. The role of leptin in atherogenesis suggests a second route. By binding to Ob-R, leptin signals the hypothalamus, macrophages, endothelial cells, and smooth muscle cells. Plaque buildup causes inflammation, defective endothelial cells, and smooth muscle migration to the injured intima, which causes cardiovascular disease. (11,12,13) This result is disagreed with.(14) Serum leptin levels did not significantly change in the study of 122 AMI patients admitted to the ICU, but they were higher in patients who were overweight or had a high body mass index or waist circumference. The author concludes that high body mass index (BMI) and obesity, which are known to be connected with cardiovascular illnesses, may be independently associated with leptin levels.(15) Ceruloplasmin is an important antioxidant enzyme, and its decrease may result from increased oxidative stress, genetic factors certain genetic variations that can impact ceruloplasmin production or stability. These variations may affect the expression or function of ceruloplasmin, leading to a decrease in its levels in individuals with CVD. A study by Vermal et al. carried out on 250 CAD patients found that serum ceruloplasmin was significantly lower in the MI group compared to the non-MI group CAD patients. Most studies show a positive correlation between serum CP and CHD, but this correlation has been called into question by others. Chapman et al. demonstrated that CP has an additional antioxidant function by scavenging free radicals through the suppression of myeloperoxidase. These results do not agree with the results of our research. (16,17) hs-CRP is considered a marker of systemic inflammation, and elevated levels of hs-

CRP have been associated with an increased risk of developing CVD. Numerous studies have shown that individuals with high levels of hs-CRP are more likely to experience cardiovascular events, such as heart attacks or strokes.(1^) Serum C-reactive protein (CRP) levels are decreased with long-term use of statin in patients with acute myocardial infarction, which may be helpful in the treatment of AMI.(19). Comparing values of leptin, ceruloplasmin, and C-reactive protein (CRP) in high-risk and low-risk patients, it is possible to find non-significant differences ($P > 0.05$). Non-significance does not necessarily indicate the absence of an association between these biomarkers and CVD risk. It might reflect limitations in the study design, inadequate statistical power, or the complex nature of CVD pathogenesis. Further research, incorporating larger sample sizes, controlling for confounding variables, and considering additional biomarkers, is crucial to obtain a more comprehensive understanding of the relationship between leptin, ceruloplasmin, hsCRP, and CVD risk. The mean values for (leptin, and ceruloplasmin) are slightly higher in females than males, this difference may be due to the influence of sex hormones, as leptin is produced by adipose tissue and females generally have a higher percentage of body fat than males. Additionally, estrogen has been shown to increase leptin levels, which could contribute to the observed difference.(20) In conclusion, the results illustrated that the levels of leptin and C-reactive protein, might considered a risk factor in people with cardiovascular diseases, and an increase in these biomarkers (leptin and hsCRP) can be a marker of significant coronary lesions that need clinical attention.

Acknowledgements

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تأثير بعض المؤشرات الحيوية (اللبتين، سيروبلازمين، وبروتين سي التفاعلي) وعلاقتها بأمراض القلب الوعائية

الخلاصة: الأحداث القلبية والوعائية تستمربالحدوث في المرضى دون عوامل الخطر التقليدية، مما يحفز البحث عن علامات جديدة للخطر . المؤشرات الحيوية هي مواد قابلة للقياس في الجسم تشير إلى الإصابة بالمرض. تمت دراسة هذه المؤشرات فيما يتعلق بأمراض القلب والأوعية الدموية لفهم دورها في تطور وتقدم هذا المراض. تشمل هذه المؤشرات الحيوية (اللبتين، السير وبلازمين وبروتين سي التفاعلي)

لا يمكن لهذه المؤشرات الحيوية تشخيص أمراض القلب بمفردها، ولكن يمكن أن تكمل عوامل الخطر والفحوصات السريرية. ويمكن اعتبار الرصد الطبي الدوري والتفسير السليم ضروريان لقياس الصحة القلبية.

المنهجية: شملت هذه الدراسة ٩٠ مريضًا خضعوا لتشخيص الشرايين التاجية قسطريا. تضمنت٥٣ ذكرًا و٣٧ أنثى من مرض الشرايين التاجية، تراوحت اعمارهم من ٢٥ إلى ٤٥ سنه، ا تم فحصهم في مستشفى الصدر التعليمي ومركز القلب التخصصي في البصرة، واستمرت من ديسمبر ٢٠٢٢ إلى أذار 2023   على الجانب الآخر، شملت ٩٠ مشاركًا صحيًا مطابقين في العمر والجنس لمجموعة المرضى واعتبارهم كمجموعه ضابطه.   تم قياس اللبتين باستخدام تقنية الاختبار الإنزيمي المرتبط بالمناعة (ELISA). بالإضافة إلى ذلك، تم إجراء اختبارات للسيروبلاسمين وبروتين سي التفاعلي باستخدام تقنية الاختبار الإلكتروكيميلومينسي المرتبط بالمناعة (ECLIA) بواسطة Cobas e411.

النتائج: كانت مستويات اللبتين والسيروبلاسمين وبروتين سي التفاعلي أعلى بشكل ملحوظ$(P<0.05)$ في المرضى مقارنة بالأشخاص الأصحاء المطابقين في العمر ، وكانت مستويات السيروبلاسمين منخفضة بشكل ملحوظ $(P<0.05)$ بين المرضى مقارنة بالأشخاص الأصحاء في المجموعة الضابطة.

الاستنتاجات: اضهرت هذه الدراسة وجود علاقة بين مستويات اللبتين والبروتين سي التفاعل في الافراد المصابين بأمراض القلب والاوعية الدموية والتي يمكن اعتبارها علامات خطر جديده لوجود مرض الشريان التاجي بالإضافة الى شدته.

الكلمات المفتاحية : بروتين سي المتفاعل العالي حساسية , امراض القلب والاوعية الدموية , مؤشرات حيوية جديدة.