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Original Research Article

Association of Serum Ceruloplasmin and Uric Acid as Biomarkers for Coronary Artery Disease Diagnosis

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Abstract

Background: Coronary artery disease (CAD) is the leading cause of death worldwide, ranking among the top eight fatal diseases. Atherosclerosis, characterized by the buildup of plaque in the arteries, is the hallmark of CAD. Oxidative stress, caused by reactive oxygen species (ROS), plays a crucial role in the development of atherosclerosis. LDL oxidation is a potent initiator of the pathological processes leading to CAD. The current study aimed to assess the risk of CAD in patients by utilizing serum ceruloplasmin and uric acid as potential biomarkers.

Methods: This study was conducted in the Department of General Medicine, Prathima Institute of Medical Sciences, Naganoor, Karimnagar. A total of 50 subjects were included in the study. Group (T) labeled as "Cases," comprised 25 patients diagnosed with coronary artery disease (CAD). Group (C) referred to as "Controls," consisted of 25 individuals of various ages and both genders, who had come for routine health check-ups or volunteered for the study and were without any known medical conditions.

Results: The levels of all eight are significantly higher in the CAD group than in the control group. The level of ceruloplasmin is significantly higher in the CAD group than in the control group ($p=0.012$). The level of uric acid is significantly higher in the CAD group than in the control group ($p=0.044$). Overall, the findings of this table suggest that there is a strong positive correlation between serum ceruloplasmin and total cholesterol, LDL cholesterol, and uric acid in both the CVD group and the control group. There is a strong positive correlation between serum uric acid and both total cholesterol ($r = 0.664$) and LDL cholesterol ($r = 0.662$) in CAD cases.

Conclusion: Serum levels of Ceruloplasmin and Uric Acid were significantly elevated in CAD cases compared to the control group. Furthermore, a positive correlation was established between these parameters. Consequently, this study suggests that Serum Ceruloplasmin and Uric Acid may serve as biomarkers for coronary artery disease.

Keywords: Ceruloplasmin, Uric Acid, Coronary Artery Disease (CAD), Biomarkers.

Introduction

Coronary Artery Disease (CAD) stands as the primary global cause of mortality, ranking first among the eight major fatal diseases. [1] It is responsible for one-third of all deaths worldwide, with two-thirds occurring in developing nations. Notably, the prevalence of CAD in India is four times higher than in the United States. Data from the Framingham trial suggests that nearly 50% of men and 30% of women aged over 40 will develop CAD, leading the World Health Organization to declare it a modern epidemic. [2] CAD is a chronic inflammatory condition characterized by an altered cardiac function resulting from an imbalance between oxygen supply and demand. [3] Atherosclerosis is a defining feature of CAD, and oxidative stress induced by reactive oxygen species (ROS) plays a crucial role in its pathogenesis. Oxidative modification of low-density lipoprotein (LDL) leads to increased uptake by macrophages. [4] The accumulation of cholesterol and oxidized LDL in arterial walls leads to atherosclerosis. Therefore, LDL oxidation serves as a potent trigger for the pathological processes leading to CAD. [5]

Ceruloplasmin (CP), a copper-containing α-2 glycoprotein with a molecular weight of approximately 132 kDa, is an acute-phase protein. There is evidence suggesting that Cp can independently serve as a marker for the progression of coronary atherosclerosis. [6] Biochemical studies have shown that it acts as a potent catalyst for in vitro LDL oxidation. [7] Uric acid, the final product of purine degradation in humans, is produced from

xanthine by the enzyme xanthine oxidase. High serum uric acid has been identified as a risk factor and an independent prognostic factor in CAD patients. [4, 8] Uric acid has been found to promote in vitro LDL oxidation, a critical step in atherosclerosis progression. [9] Additionally, it can stimulate the adherence of granulocytes to the endothelium and the release of peroxide and superoxide free radicals. [10] Elevated levels of both ceruloplasmin and uric acid have been associated with adverse effects on endothelial function, increasing the risk of atherosclerotic plaque formation. [11]

When these plaques rupture and result in thrombus formation, they can obstruct coronary blood vessels, causing an acute reduction in blood supply to a portion of the myocardium, ultimately leading to myocardial infarction (MI), a severe clinical manifestation of CAD. [12] Therefore, it is essential to assess CAD risk in patients using serum ceruloplasmin and uric acid as markers, in addition to other parameters such as fasting blood glucose and a fasting lipid profile. The current study aims to assess the levels of Ceruloplasmin and Uric acid in individuals with coronary artery disease (CAD) as well as in a group of healthy controls.

Material and methods

This prospective study was conducted in the Department of General Medicine, at Prathima Institute of Medical Sciences, Naganoor, Karimnagar, Telangana. Institutional Ethical approval was obtained for the study. Written consent was obtained from all the participants of the study after explaining the nature of the study in the vernacular language.

Inclusion Criteria

1. Males and females
2. Cases that have been clinically diagnosed with coronary artery disease (CAD) based on ECG and angiographic evidence.
3. CAD patients, both with and without associated complications.
4. Healthy controls are matched in terms of age and sex and do not have any significant under-lying medical conditions.

Exclusion Criteria

1. Patients with acute or chronic liver conditions.
2. Individuals with renal disorders.
3. Patients with thyroid abnormalities.
4. Patients on anti-inflammatory drugs
5. Patients of Gout, Wilson's disease.

The study involved a comprehensive data collection process, which included obtaining detailed information through history-taking. This information encompassed demographic details, drug usage history, personal background, familial medical history, past and present medical conditions, and medication history. Additionally, the available case records were thoroughly reviewed to extract any pertinent data. The participants were divided into two groups based on the inclusion and exclusion criteria. A total of 50 subjects were included in the study. Group (T) labeled as "Cases," comprised 25 patients diagnosed with coronary artery disease (CAD). Group (C) referred to as "Controls," consisted of 25 individuals of various ages and both genders, who had come for routine health check-ups or volunteered for the study and were without any known medical conditions.

A volume of 5 milliliters of venous blood was collected from each subject through a sterile needle and syringe, extracted from the antecubital vein. Subsequently, the obtained blood samples were transferred into sterile centrifuge tubes, where they were allowed to form clots. After clot formation, each sample was subjected to centrifugation at 3000 revolutions per minute for a duration of 3 minutes to separate the serum. The resulting serum was carefully withdrawn using a micropipette and then transferred into Eppendorf tubes. The biochemical analysis was conducted within 24 hours of blood collection to ensure the integrity and accuracy of the results.

The measurement of serum ceruloplasmin was performed following the Houchin method [12], which involves the use of Para Phenylene Diamine (PPD), Acetate buffer, and sodium azide. Uric acid levels in serum were determined using the Uricase-Peroxidase kit method [13] and analyzed with a Semi autoanalyzer Erba Chem 7.

Statistical Analysis: The data collected sorted and uploaded on MS Excel spreadsheet. Analyzed by SPSS version 21 on Windows format. Continuous variables were denoted as mean, standard deviations, and percentages. The categorical variables were analyzed by chi-square test with p-values of < 0.05 considered as significant.

Results

Table 1 shows the levels of variables in two groups of people: one group with coronary artery disease (CAD) and the other group without CAD. The levels of all eight are significantly higher in the CAD group than in the control group. The level of ceruloplasmin is significantly higher in the CAD group than in the control group ($p=0.012$). The level of uric acid is significantly higher in the CAD group than in the control group ($p=0.044$). Glucose: The level of glucose is significantly higher in the CAD group than in the control group ($p=0.010$). Total cholesterol: The level of total cholesterol is significantly higher in the CAD group than in the control group ($p=0.001$). Triglycerides: The level of triglycerides is significantly higher in the CAD group than in the control group ($p=0.023$). HDL-C:

The level of HDL-C is significantly lower in the CAD group than in the control group (p=0.015). LDL-C: The level of LDL-C is significantly higher in the CAD group than in the control group (p=0.034). VLDL: The level of VLDL is significantly higher in the CAD group than in the control group (p=0.021). The findings suggest that diabetes, high cholesterol, high triglycerides, low HDL-C, and high LDL-C are risk factors for CAD.

Table 1: Showing biomarkers for coronary artery disease diagnosis

Variable (mg/dl)	Group T (Test)	Group C (Control)	P value
Ceruloplasmin	57.66 ± 8.14	29.67 ± 4.49	0.012
Uric acid	8.17 ± 1.01	4.98 ± 0.72	0.044
Glucose	182.35 ± 37.38	89.25 ± 7.18	0.010
Total cholesterol	251.73 ± 15.80	161.19 ± 11.27	0.001
Triglycerides	215.08 ± 18.91	137.84 ± 9.51	0.023
HDL-C	33.67 ± 4.22	38.64 ± 3.21	0.015
LDL-C	170.28 ± 12.57	101.29 ± 15.27	0.034
VLDL	43.20 ± 4.34	28.94 ± 1.22	0.021

Table 2 shows the correlation coefficients between serum ceruloplasmin and other biochemical parameters in two groups of people: a group (Test) with coronary artery disease and a control group without coronary artery disease. Overall, the findings of this table suggest that there is a strong positive correlation between serum ceruloplasmin and total cholesterol, LDL cholesterol, and uric acid in both the CVD group and the control group. There is also a moderate positive correlation between serum ceruloplasmin and triglycerides and VLDL cholesterol in the CVD group, but only a weak positive correlation between serum ceruloplasmin and triglycerides and VLDL cholesterol in the control group. There is a moderate negative correlation between serum ceruloplasmin and HDL cholesterol in both the CVD group and the control group. These findings suggest that serum ceruloplasmin may be a biomarker for CVD.

Table 2: Correlation of serum ceruloplasmin with other biochemical parameters in cases of coronary artery disease and controls

Ceruloplasmin Versus	Correlation coefficient 'r' values	
	CVD cases	Controls
Uric acid	0.712	0.321
Glucose	0.366	0.215
Total Cholesterol	0.610	0.501
LDL Cholesterol	0.521	0.499
HDL Cholesterol	-0.339	-0.343
Triglycerides	0.462	0.166
VLDL Cholesterol	0.427	0.118

Table 3: Correlation of serum uric acid with other biochemical parameters in cases of coronary artery disease and controls

Uric acid Versus	Correlation coefficient 'r' values	
	CVD cases	Controls
Glucose	0.515	0.231
Total Cholesterol	0.664	0.375
LDL Cholesterol	0.662	0.310
HDL Cholesterol	- 0.441	-0.112
Triglycerides	0.399	0.201
VLDL Cholesterol	0.625	0.237

Table 3 summarizes the correlation between serum uric acid and other biochemical parameters in individuals with coronary artery disease (CAD) and a control group without CAD.

Total Cholesterol and LDL Cholesterol: There is a strong positive correlation between serum uric acid and both total cholesterol (r = 0.664) and LDL cholesterol (r = 0.662) in CAD cases. This indicates that as uric acid levels increase, total cholesterol and LDL cholesterol levels also tend to increase.

VLDL Cholesterol: There is a strong positive correlation between serum uric acid and VLDL cholesterol in CAD cases (r = 0.625), indicating that as uric acid levels increase, VLDL cholesterol levels also tend to increase. Overall, these findings suggest that serum uric acid may be associated with the development of CAD. The stronger correlations observed in CAD cases compared to the control group further support this association.

Discussion

Coronary artery disease remains a prominent cause of illness and death among adults globally. There is mounting evidence suggesting that both chronic and acute excessive production of reactive oxygen species (ROS) in response to pathophysiological conditions plays a pivotal role in the development of cardiovascular diseases (CVD). [13] ROS serve as mediators in numerous signaling pathways, ultimately leading to vascular inflammation and the formation of atherogenic plaques. The peroxidation of lipoproteins, particularly low-density lipoprotein (LDL), significantly contributes to the onset and progression of atherosclerosis. [14] LDL within the intimal layer of blood vessels is in close proximity to endothelial cells and smooth muscle cells, both of which can induce LDL oxidation through the release of free radicals. Macrophages within the intimal region further enhance LDL oxidation by releasing free radicals [15, 16]. As a result, elevated concentrations of these oxidants in the bloodstream have been linked to an increased risk of cardiovascular diseases.

Biochemical investigations have demonstrated that Ceruloplasmin (Cp) exhibits considerable catalytic activity in LDL oxidation in vitro. Various mechanisms have been proposed to explain LDL oxidation, including copper ion-induced oxidation within the arterial wall by cells like macrophages, smooth muscle cells, and endothelial cells. [17] These cells require cell-derived superoxide and external transition metal ions for LDL oxidation [18]. Ceruloplasmin itself possesses pro-oxidant properties, contributing to DNA damage through the generation of hydroxyl radicals, possibly due to the release of Cu^{2+} from oxidatively damaged ceruloplasmin following conformational changes. Elevated levels of ceruloplasmin in coronary artery disease (CAD) have been associated with its role as an acute phase reactant protein. However, the exact cause of the remaining elevated ceruloplasmin levels may relate to its other properties, such as its pro-oxidant activity in LDL oxidation or its antioxidant functions. Some researchers have suggested that the increased ceruloplasmin levels in CAD are primarily attributed to inflammatory processes rather than its pro-oxidant activity [19].

Beyond its pro-oxidant and acute phase protein characteristics, a few studies, such as Osaki et al. [19] have demonstrated that ceruloplasmin acts as an essential extracellular antioxidant, safeguarding the intima against free radical damage. This antioxidant role is attributed to its ferroxidase activity, which catalyzes the conversion of Fe^{2+} to Fe^{3+}, thereby inhibiting ferrous ion-stimulated lipid peroxidation and participating in the breakdown of lipid peroxides. Ceruloplasmin's potential as an antioxidant in coronary artery disease may also be linked to its ability to scavenge free radicals, including superoxide anion radicals, thereby preventing the release of noradrenaline a potent vasoconstrictor induced by superoxide free radicals [20]. These findings align with prior research by other similar studies. [21-23] supporting the use of serum Ceruloplasmin as a biomarker in coronary artery disease.

Although the exact mechanism through which uric acid contributes to cardiovascular disease is not entirely clear, hyperuricemia has been associated with adverse effects on endothelial function, oxidative metabolism, platelet adhesion, and aggregation. [24] Serum uric acid levels may be a valuable tool for risk stratification in cardiovascular disease. It should be given careful consideration when evaluating overall cardiovascular risk. Serum uric acid levels reflect xanthine oxidase activity and oxidative stress production. Uric acid can act both as an antioxidant and a pro-oxidant and may contribute to endothelial dysfunction. It stimulates the production of monocyte chemoattractant protein 1 through the activation of p38 MAP kinase and nuclear transcription factors NF-κB and AP-1. These chemokines are significant contributors to vascular dysfunction and tissue injury, particularly following events like acute myocardial infarction (AMI). [25] This could explain why uric acid is considered a negative prognostic marker for AMI. The causes of hyperuricemia in hypertension, a potent risk factor for coronary artery disease, are not entirely clear, but several mechanisms have been proposed. Hypertension may initially elevate serum uric acid through increased serum lactate levels, which can result from renal microvascular disease and local tissue hypoxia associated with hypertension. [26] Elevated lactate levels are expected to decrease uric acid tubular secretion, thereby increasing serum levels. Intrarenal ischemia can also contribute to uric acid generation via xanthine oxidase. Metabolic changes, disturbances (such as hyperinsulinemia), or sympathetic activity may alter renal sodium handling, subsequently increasing arterial pressure, reducing renal blood flow, and decreasing uric acid secretion. This, in turn, leads to increased purine oxidation, elevated production of reactive oxygen species (ROS), vascular injury, and reduced nitric oxide [27, 28].

Conclusion

The results of this study provide valuable insights into the association between the measured biomarkers and coronary artery disease. Serum levels of Ceruloplasmin and Uric Acid were significantly elevated in CAD cases compared to the control group. Furthermore, a positive correlation was established between these parameters. Consequently, this study suggests that Serum Ceruloplasmin and Uric Acid may serve as biomarkers for coronary artery disease.

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