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Original Research Article

Serum ceruloplasmin and uric acid levels as biomarkers in coronary artery disease

Dr T. Anil Kumar[*1], Dr B. Lakshmi Keerthana[2]
[1,2]Senior Resident, Department of Biochemistry, Rangaraya Medical College, Kakinada, Andhra Pradesh, India

***Corresponding author**
Dr T. Anil Kumar
Email: anil.mbbs@yahoo.co.in

Abstract: Coronary Artery Disease (CAD) is one of the leading cause of death ranked "FIRST" worldwide among the eight killer diseases. Atherosclerosis is the hallmark of CAD. Oxidative stress induced by reactive oxygen species (ROS) is implicated in the pathogenesis of atherosclerosis. LDL oxidation is a potent trigger for the pathological events leading to CAD. Keeping this in view, present study is undertaken to assess the risk of CAD in patients using serum ceruloplasmin and uric acid as biomarkers. The study group consisted of 110 subjects of age group 20-50 years and of both sexes. Of these, 70 were CAD patients (Group 2), and 40 were healthy age and sex matched controls (Group 1). The serum levels of both ceruloplasmin and uric acid are elevated in cases (CAD patients) when compared to controls. The increase is statistically highly significant ($p < 0.001$). Very large positive correlation was obtained for between ceruloplasmin and uric acid values. Both the parameters also achieved positive correlation with blood glucose and lipid profile parameters with exception of HDL cholesterol which showed negative correlation. Thus Ceruloplasmin and Uric acid levels can be used as biomarkers in the assessment of CAD.
Keywords: CAD, Atherosclerosis, Oxidative stress, LDL oxidation, Ceruloplasmin, Uric acid.

INTRODUCTION:

Coronary Artery Disease (CAD) is one of the leading cause of death ranked "FIRST" worldwide among the eight killer diseases [1]. It accounts for one-third of all deaths worldwide, two thirds of which occur in the developing countries. CAD rates are now 4-fold higher in India compared to the U.S [2]. Based on data from the Framingham trial nearly 50% of males and 30% of females over the age of 40 will develop coronary artery disease. WHO has declared CAD as Modern Epidemic.

CAD is a chronic inflammatory disease. It is characterized by altered cardiac function due to imbalance between oxygen supply and demand [3]. Atherosclerosis is the hallmark of CAD. Oxidative stress induced by reactive oxygen species (ROS) is implicated in the pathogenesis of atherosclerosis. Oxidative modification of low density lipoprotein (LDL) leads to enhanced uptake by macrophages. Cellular accumulation of cholesterol and oxidized LDL in the arterial walls, leads to atherosclerosis. So LDL oxidation is a potent trigger for the pathological events leading to CAD [4].

Ceruloplasmin (Cp) is a copper containing α-2 glycoprotein with a molecular weight of approximately 132 kDa. It is an acute phase protein. It has been proposed that Cp can serve as an independent marker for the progression of coronary Atherosclerosis [5]. Biochemical studies have shown that it is a potent catalyst for invitro oxidation of low-density lipoprotein (LDL) [6].

Uric acid is the final breakdown product of purine degradation in humans. It is produced from xanthine catalyzed by the enzyme xanthine oxidase. High serum uric acid has been indicated as a risk factor [7] and as an independent prognostic factor in patients with CAD. Uric acid has been found to promote low-density lipoprotein (LDL) oxidation in vitro, a key step in the progression of atherosclerosis [8]. Uric acid can also stimulate granulocyte adherence to the endothelium and peroxide and superoxide free radical liberation [9].

Thus elevated levels of Ceruloplasmin and Uric Acid are found to be associated with deleterious effects on endothelial function which increases risk of atherosclerotic plaque formation [10]. Rupture of plaque with thrombus formation and occlusion of coronary blood vessels result in an acute reduction of blood supply to a portion of the myocardium leading to MI [11] which is a catastrophic clinical manifestation of CAD.

Keeping this in view, it is important to assess the risk of CAD in patients using serum ceruloplasmin and uric acid as markers. In addition, other parameters such as fasting blood glucose, fasting lipid profile were also estimated.

Aims & Objectives:

The aim of this study is to know whether inflammatory and oxidative stress marker – Ceruloplasmin and endogenous anti-oxidant – Uric acid levels can be used as biomarkers in CAD.

The objective of the present study is to -

- Estimate the levels of Ceruloplasmin and Uric acid in patients with CAD and healthy controls.
- Correlate the estimated parameters, and demonstrate their utility as a valuable tool in diagnosis of CAD.

MATERIALS & METHODS:

Study centre & Period:

This research was conducted at clinical laboratory, Department of Biochemistry, Andhra Medical College between October 2013 and June 2015.

Subjects Selection:

Patient selection was done by simple random sampling of individuals presenting to the outpatient clinic of Department of Cardiology, King George Hospital attached to Andhra Medical College, Visakhapatnam. An informed consent was taken from the CAD patients and controls before the collection of blood sample. The subjects were selected based on following inclusion and exclusion criteria.

Inclusion Criteria:

- Clinically, ECG and Angiographically proven CAD patients.
- CAD patients with and without complications.
- Controls were healthy individuals, age and sex matched without any major illness.

Exclusion Criteria:

- Patients with acute or chronic liver diseases.
- Renal diseases.
- Patients with thyroid disorders.
- Use of antioxidants, anti-inflammatory drugs, hypolipidemic drugs etc;
- Gout.
- Wilson's disease.

Study Pattern:

After obtaining consent, the study subjects were subjected to detailed history taking including demographic data, drug history, personal history, family history present and past medical history and drug intake. The available case records were scrutinized to collect any valid data.

• GROUP 1: CONTROLS - 40 ages matched controls of both sex, who came for routine health check-up / healthy volunteer.

• GROUP 2: CASES – 70 patients with CAD.

Specimen Collection:

Venous blood (5ml) was obtained from each of the subjects by vein puncture of the ante cubital vein using a sterile needle and syringe. The blood samples were then transferred into clean sterile centrifuge tubes and allowed to clot. Each clotted sample was centrifuged at 3000 rpm for 3 min to obtain the serum. The serum was removed using a micropipette and transferred to Eppendorf tubes. Biochemical assay was carried out within 24hrs of blood collection.

Assay of Markers:

Ceruloplasmin in serum was measured by Houchin method [12] using Para Phenylene Diamine (PPD), Acetate buffer and sodium azide. Uric acid in serum was measured by Uricase-Peroxidase kit method [13] on Semiautoanalyzer.

Statistical Analysis:

The data obtained were analyzed using Student's t-test where $p<0.001$ was considered as highly significant. All results were expressed as Mean ± S.D. Correlation between different estimated parameters was done by Pearson correlation coefficient.

RESULTS & OBSERVATIONS:

The results obtained for various parameters and their correlation are tabulated as follows -

Table-1: Observation of Biochemical parameters

Value (mg/dl)	Group 1 (N=40)	Group 2 (N=70)	p value
Ceruloplasmin	30.47 ± 5.58	58.44 ± 9.31	< 0.001
Uric Acid	5.88 ± 0.66	8.10 ± 1.05	< 0.001
Glucose	90.35 ± 8.09	194.32 ± 43.79	< 0.001
Total Cholesterol	168.82 ± 16.38	248 ± 18.0	< 0.001
Triglycerides	140.70 ± 10.74	210.4 ± 26.42	< 0.001
HDL Cholesterol	37.35 ± 4.53	31.83 ± 5.90	< 0.001
LDL Cholesterol	103.33 ± 18.15	173.85 ± 19.27	< 0.001
VLDL Cholesterol	28.14 ± 2.14	42.31 ± 5.56	< 0.001

From the above table-1 it was observed that, Mean ± SD of all the biomarkers are increased in cases when compared to the controls. Also it was observed that the increase in biomarkers were statistically highly significant ($p < 0.001$) in cases when compared to controls.

Table-2: Correlation of Ceruloplasmin with other Biochemical parameters

PARAMETER	CORRELATION CO-EFFICIENT (r value)	
	CASES	**CONTROLS**
Ceruloplasmin Vs Uric Acid	0.723	0.453
Ceruloplasmin Vs Glucose	0.379	0.255
Ceruloplasmin Vs Total Cholesterol	0.622	0.527
Ceruloplasmin Vs LDL Cholesterol	0.568	0.553
Ceruloplasmin Vs HDL Cholesterol	- 0.348	- 0.339
Ceruloplasmin Vs Triglycerides	0.453	0.163
Ceruloplasmin Vs VLDL Cholesterol	0.414	0.114

From the above table-2 it was observed that serum Ceruloplasmin showed positive correlation with serum Uric acid (very large correlation), Glucose and Lipid profile parameters with exception of HDL cholesterol which showed negative correlation.

Table-3: Correlation of Uric Acid with other Biochemical parameters

PARAMETER	CORRELATION CO-EFFICIENT (r value)	
	CASES	**CONTROLS**
Uric Acid Vs Glucose	0.562	0.227
Uric Acid Vs Total Cholesterol	0.678	0.377
Uric Acid Vs LDL Cholesterol	0.655	0.327
Uric Acid Vs HDL Cholesterol	- 0.429	- 0.104
Uric Acid Vs Triglycerides	0.406	0.202
Uric Acid Vs VLDL Cholesterol	0.655	0.246

From the above table -3it was observed that serum Uric acid showed positive correlation with Glucose and Lipid profile parameters with exception of HDL cholesterol which showed negative correlation.

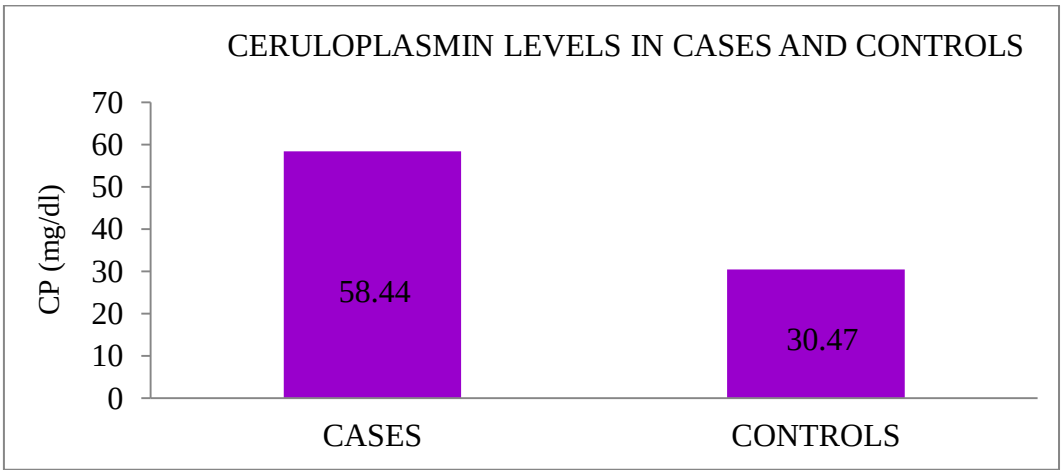

Fig-1: Bar diagram of Ceruloplasmin Levels in Cases and Controls

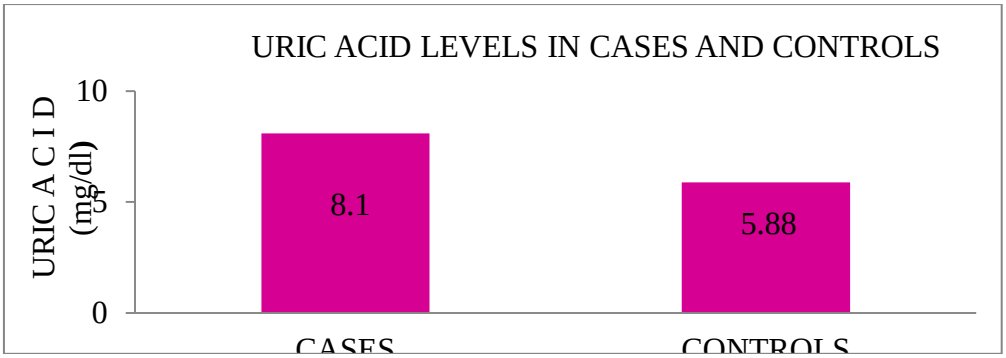

Fig-2: Bar diagram of uric acid Levels in Cases and Controls

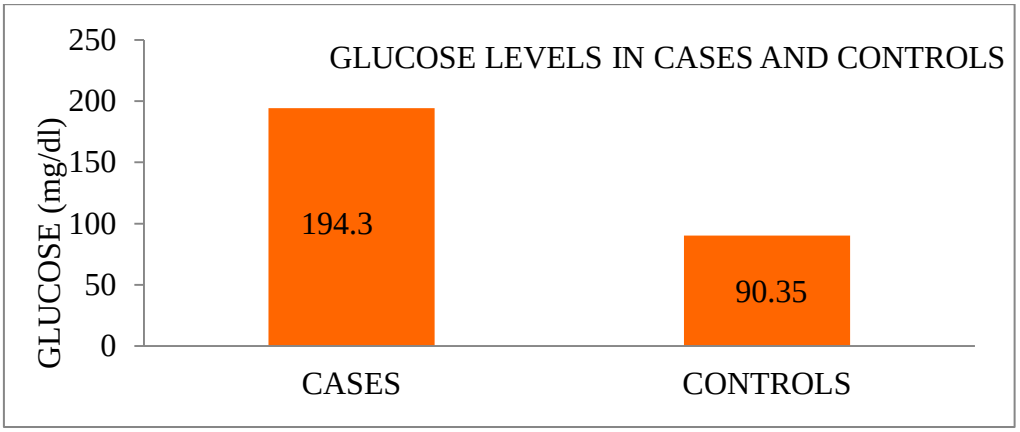

Fig-3: Bar diagram of glucose Levels in Cases and Controls

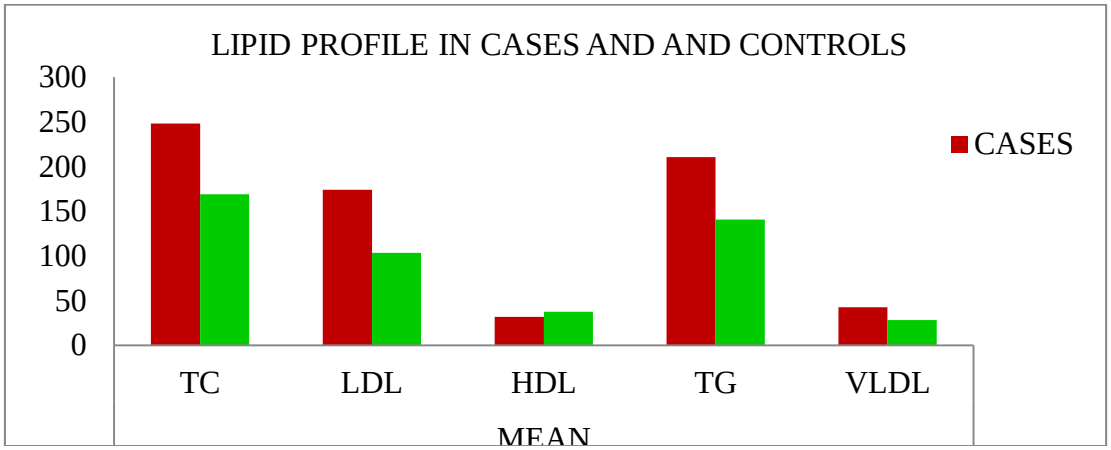

Fig-4: Bar diagram of lipid profiles in Cases and Controls

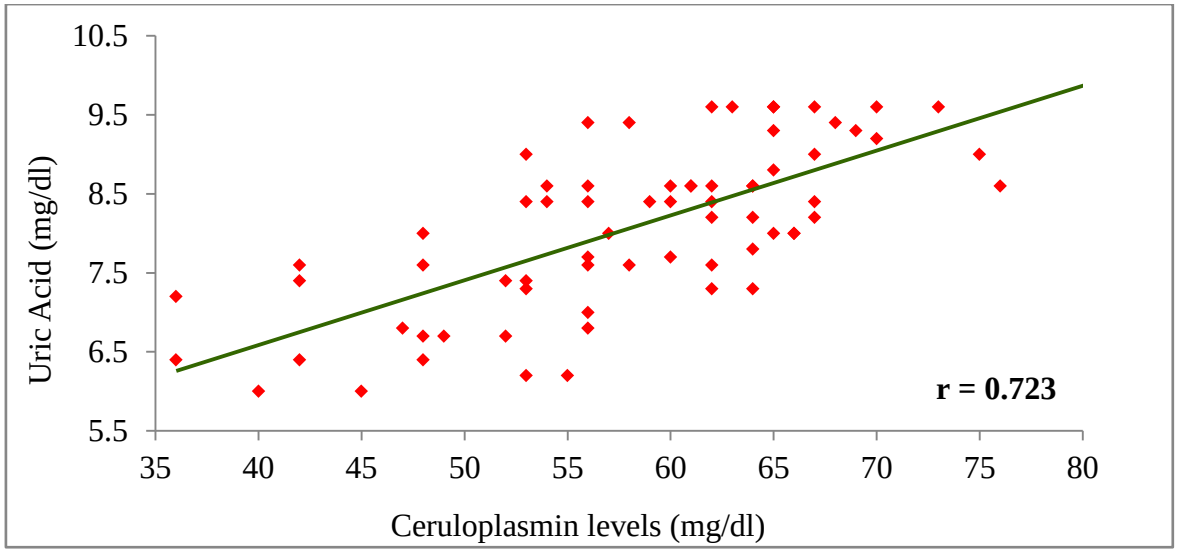

Fig-5:Correlation between Ceruloplasmin and Uric acid

DISCUSSION:

Coronary artery disease continues to be a leading cause of morbidity and mortality among the adult populations all over the world. Growing evidence indicates that chronic and acute overproduction of reactive oxygen species (ROS) under pathophysiological conditions is integral in the development of cardiovascular diseases (CVD). ROS mediate various signaling pathways that results into vascular inflammation and atherogenesis. Various animal models and human investigations also support the oxidative stress hypothesis of atherosclerosis. Oxidative stress is the unifying mechanism for many CVD risk factors, which additionally supports its central role [14].

The peroxidation of lipoproteins, especially LDL plays a significant role in the pathogenesis and progression of atherosclerosis. LDL in the intimal space is in close proximity to endothelial cells and smooth muscle cells, both of which can promote LDL oxidation by free radicals that are released from endothelial cells and smooth muscle cells. Macrophages of the intimal cells further contribute to LDL oxidation by releasing free radicals [15, 16].

Thus high blood concentrations of these oxidants have been linked with increased risk of cardiovascular diseases. A better understanding of the complexity of cellular redox reactions, development of a new class of antioxidants targeted to specific sub cellular centres, and the phenotype-genotype linkage analysis for oxidative stress will likely be avenues for future research in this area as we move towards the broader use of pharmacological and regenerative therapies in the treatment and prevention of CAD. Therefore measures of their activity in serum have been used to estimate the amount of oxidative stress [17].

INCREASED CERULOPLASMIN LEVELS:

Biochemical studies have shown that Cp is a potent catalyst of LDL oxidation in vitro. Different mechanisms have been proposed for oxidation of LDL. One of the mechanisms is copper ion induced oxidation of LDL. LDL can be oxidized to an atherogenic form (oxidized – LDL) within the arterial wall by macrophages, smooth muscle cells and endothelial cells. Cell derived superoxide and exogenous transition metal ions are required for oxidation of LDL by smooth muscle cells and endothelial cells [18].

Ceruloplasmin itself has pro oxidant activity. Along with H_2O_2, it mediates DNA damage through the production of hydroxyl radicals. This could be possible by released Cu2+ from oxidatively damaged ceruloplasmin through the conformational changes. The release of Cu^{2+} from ceruloplasmin during oxidative stress further increases the production of free radicals and could also aggravate cellular damage [19].

Ceruloplasmin also being an acute phase reactant protein, its levels rises immediately after cellular damage in CAD. According to Kerstin Klipstein Grobush *et al.;*[20] high levels of ceruloplasmin in coronary heart disease (CHD) can be partly attributed to its property as an acute phase protein. The remaining elevated ceruloplasmin levels may be due to other properties of ceruloplasmin, like its pro-oxidant activity by oxidation of LDL or its antioxidant activity. Further, they suggested that the elevated levels of ceruloplasmin in CHD are mainly attributed to inflammation processes rather than to the pro-oxidant activity of ceruloplasmin.

Besides being a pro-oxidant and an acute phase protein, few studies like Osaki *et al.;* [19] have shown

that ceruloplasmin is an important extracellular antioxidant and it protects intima against free radical injury. The antioxidant property of ceruloplasmin is through its ferroxidase activity catalyzing the oxidation of Fe^{2+} to Fe^{3+}. Thus it inhibits ferrous ion stimulated lipid peroxidation and is known to be involved in the decomposition of lipid peroxides.

The role of ceruloplasmin as an antioxidant in coronary artery disease may also be related to its free radical scavenging properties [21]. It scavenges superoxide anion radicals and thus prevents superoxide free radical induced release of noradrenaline which is a potent vasoconstrictor.

The findings of present study correlate well with findings of previous studies of Varma *et al.*[22], Awadallah *et al.*[23], Sirajwala *et al.* [24], Gocmen *et al.* [25], Abdullah *et al.* [26], and Venkatramana *et al.* [27]. Hence serum Ceruloplasmin can be used as biomarker in coronary artery disease.

INCREASED URIC ACID LEVELS:

Although the mechanism by which uric acid may play a pathogenic role in cardiovascular disease is unclear, hyperuricemia is associated with deleterious effects on endothelial dysfunction, oxidative metabolism, platelet adhesiveness and aggregation. Overall, serum uric acid may be a powerful tool to help stratify risk for cardiovascular disease. It should be carefully considered when evaluating overall cardiovascular risk.

Serum uric acid levels reflect the xanthine oxidase activity and oxidative stress production. Uric acid may function as an antioxidant and also a pro oxidant. It may also contribute to endothelial dysfunction. Uric acid synthesizes monocyte chemo attractant protein 1 by stimulating p38 MAP kinase and the nuclear transcription factor NF-κB and AP-1. These chemokines are important in causing vascular dysfunction and tissue injury especially after events like AMI [28]. This could explain that why uric acid can be considered a negative prognostic marker for AMI.

The causes of hyperuricemia in hypertension, a potent risk factor for coronary artery disease, are unclear, but several mechanisms have been proposed. First, hypertension may increase serum uric acid via elevated serum lactate levels. Hypertension initially produces renal microvascular diseases and local tissue hypoxia, as evidenced by increase in serum lactate. Lactate would be expected to decrease the tubular secretion of uric acid, leading to increased serum levels. Intrarenal ischemia can also contribute to generation of uric acid via xanthine oxidase.

It is also possible that metabolic alterations or disturbances (hyperinsulinemia) or sympathetic activity may produce changes in renal sodium handling, leading to increased arterial pressure, decreased renal blood flow and decreased uric acid secretion. This, in turn, increases purine oxidation resulting in increased production of reactive oxygen species (ROS), subsequent vascular injury, and reduced nitric oxide [29, 30 31].

The findings of present study correlate well with findings of previous studies of Bickel *et al.* [31], LIFE study [32], SHEP trial [33], Daniel *et al.* [34], Yildiz *et al.* [35]. Hence serum uric acid can be used as biomarker in coronary artery disease.

CONCLUSION:

The results of the present research provide valuable information and association between the measured biomarkers and coronary artery disease. Serum Ceruloplasmin and Uric acid levels were significantly elevated in CAD cases when compared to the control group. Positive correlation was also established between the parameters. Hence, the present study suggests that Serum Ceruloplasmin and Uric Acid may be used as biomarkers in coronary artery disease.

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