International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 4, 2024
https://theaspd.com/index.php

Copeptin & Troponin T: A Dual Biomarker Approach For Assessing The Severity In Coronary Artery Disease

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Date: 10-09-2024 | Revision Date: 21-10-2024 | Accepted Date: 15-11-2024 | Publishing Date: 31-12-2024

ABSTRACT

Background: *Cardiovascular diseases are leading causes of morbidity and mortality amounting to 25 % of the same in the Indian sub-continent. Coronary artery disease (CAD) is the most common heart disease, ranging from asymptomatic atherosclerosis and stable angina to acute coronary syndromes. Atherosclerotic plaques- the pathophysiological hallmark, need swift and deep ended comprehension for timely diagnosis subsequently leading to adequate redressal from unit patient to population levels. Copeptin, a 39-amino acid glycoprotein, being stable, is easily measurable in peripheral blood and has potential as a biomarker in cardiac diseases like heart failure and acute coronary syndromes. Troponin is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that are integral to muscle contraction. Measurements of blood level of cardiac-specific troponins I and T are extensively used as diagnostic and prognostic indicators in the management coronary artery disease. The objective of this study was to evaluate the differences in copeptin and troponins T levels between CAD patients and healthy controls as a dual biomarker approach to assess the potential of copeptin and troponins T as a dual biomarker for CAD.*

Methods: *This observational case-control study was conducted at S.C.B Medical College and Hospital, Cuttack, from 2020 to 2022 after approved by the Institutional Ethics Committee which involved 80 CAD patients along with an equal number of age- and sex-matched controls. Exclusion criteria included acute or chronic kidney disease, traumatic heart disease, head injury, severe morbidity, or refusal to participate. copeptin and troponins T were measured by Enzyme-linked immunoassay (ELISA).*

Results: *Severe CAD patients exhibited significantly higher copeptin and troponins T levels compared to controls as compared to moderate and mild CAD patients and the difference was statistically significant. ($p<0.001$)*

Conclusion: *CAD patients exhibited higher copeptin and troponins T levels compared to healthy controls, suggesting that copeptin and troponins T can serve as a dual biomarker for CAD.*

Key-words: *Cardiovascular Disease, atherosclerotic plaques, amino acid glycoprotein, cardiac-specific troponin*

INTRODUCTION

Coronary Artery Disease (CAD) can be defined as luminal narrowing of coronary arteries due to atherosclerosis, which causes myocardial oxygen deprivation and ischemia. CAD includes myocardial ischemia, angina pectoris and myocardial infarction etc [1]. Coronary Heart Diseases (CHD) are one of the major causes of disease-burden and death in India. Mortality data from the Registrar General of India shows that cardiovascular diseases are a major cause of death in India now i.e., 23% of total and 32% of adult deaths in recent year [2]

Copeptin, first described in 1972 by Holwerda is a 39-amino acid glycopeptide with leucine rich core segment. It is derived from the cleavage of precursor of arginine vasopressin, produced in an equimolar ratio in hypothalamus and processed during axonal transport [3]. Unlike Arginine Vasopressin (half-life: 5-20 min), Copeptin is a stable molecule and can be easily measured. The concentration of Copeptin has been shown to increase early after acute and severe cardiac events and can be evaluated as an early-rule-

out strategy for acute myocardial infarction in patients presenting with signs and symptoms of acute coronary syndrome.[4] Serum Copeptin is a marker of body's endocrine stress response mediated through hypothalamus-pituitary-adrenal system and activated by stress[5].As endogenous stress is increased at the onset of acute coronary syndrome, Copeptin could identify acute coronary syndrome patients when other biomarkers are still negative[6]

Troponin is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that are integral to muscle contraction. It was discovered by the German physician Hugo A. Katus at the University of Heidelberg, who also developed the troponin T assay [7]. Measurements of blood level of cardiac-specific troponins I and T are extensively used as diagnostic and prognostic indicators in the management coronary artery disease [8][9]. Serum level of troponin T increases within 6 hours of myocardial infarction peaks at 24 hours then remains elevated up to 10-14 days. The 99th percentile cut-off for cardiac troponin T (cTnT) is 0.01 ng/Ml[10] The reference range for the high sensitivity troponin T is a normal < 14 ng/L, borderline of 14-52 ng/L, and elevated of >52 ng/L[11].Serial estimation of cardiac troponins are done in any patient reporting with symptoms to the emergency clinic.

Hence, our study is to find out the role of Copeptin and troponin T as a dual biomarker in various degree of severity of coronary disease cases and to determine its potential to become an early biomarker before the unset of sign and symptoms in CAD patients.

MATERIALS AND METHOD

This observational case-control study was conducted in the Department of Biochemistry in collaboration with the Department of Cardiology, S.C.B Medical College and Hospital, Cuttack from 2020 to 2022 after approved by the Institutional Ethics Committee (IEC Application. N0- 683/04.06.2021) which involved 80 CAD patients aged between 18-85 years along with an equal number of age- and sex-matched controls from Department of Cardiology at S.C. B Medical College and Hospital, Cuttack.The Study subjects were selected by Simple random sampling method. The Body Mass Index (BMI) was calculated taking weight in Kg and height in meter square. Gensini Score (GS) was evaluated to know the severity of CAD according to angiographic findings and the case group was categorized into three groups i.e., mild, moderate and severe. The parameters taken into consideration were degree of stenosis, proximity of the lesions in the coronary tree and lesions in the left main coronary artery.

Inclusion Criteria:

All diagnosed coronary artery disease patients attending the Department of Cardiology of S.C.B Medical College and Hospital, Cuttack was included in the study after obtaining study specific informed consent

Exclusion Criteria:

Patients with acute or chronic kidney disease, traumatic heart disease, history of head injury, morbidly sick patients were excluded from the study.

Biochemical Analysis:

After obtaining the consent of cases and controls, 5 ml of fasting venous blood was collected, of which 4 ml was kept in clot activator vials for serum biochemical analysis and 1 ml in oxo-fluoride vials for plasma glucose estimation. Fasting plasma glucose (FBS), serum urea, creatinine, serum lipid profile were done by TBA 120 FR auto analyser using standard commercial kits. Serum Copeptin was estimated by ELISA methods and Troponin T was estimated by electrochemiluminescence method (eCLIA) in Cobas e 411 analyser. Human Copeptin ELISA Kit employs a two-site sandwich ELISA to quantitate Copeptin in samples where an antibody specific for Human Copeptin and biotinylated human antibody and streptavidin horse radish peroxidase (HRP) were used. A standard curve was generated by plotting the absorbance versus the respective human Copeptin concentration of each standard (fig) on a point-to-point curve. The concentration of human Copeptin in the patient sample and in controls were determined directly from standard curve generated. Troponin T test contains two monoclonal antibodies specific to cardiac troponin T (cTnT): one gold-labelled, the other biotinylated. The antibodies form a sandwich complex with the cTnT in the blood.

Statistical Analysis:

SPSS version 22 and MS excel were used for data analysis. Paired t-test (for normal data), Pearson Correlation, ANOVA and Regression were used to analyse the data. Mean and Standard deviation of scores are presented. P value <0.05 considered as statistically significant.

RESULTS:

Table 1: Mean Age and BMI distribution of cases & control

	Control (n=80)	Case (n=80)	p- value
Age(yrs)	58.8 ± 13.9	60.5 ± 15.3	> 0.05
BMI (kg/m^2)	24.1 ± 1.4	27.6 ± 3.7	0.002

The mean age of cases was 60.5 ± 15.3 and controls was 58.8 ± 13.9 and the difference was statistically not significant. The mean BMI of cases and controls was 27.6±3.7 and 24.1±1.4 respectively with a highly significant association having a p value of 0.002.

Table 2: The mean Gensini score of Mild, Moderate & Severe cases CAD

CAD categories	Mild (n=11)	Moderate (n=24)	Severe (n=45)	p-value*
Gensini score (GS)	11.1±3.2	26.6±4.3	58.9±16.1	0.001

The average GS measured were 11.1±3.2, 26.6±4.3 and 58.9±16.1 in the mild moderate and severe case of CAD categories, respectively with a statistically significant difference having a p value of 0.001.

Table 3: Comparison of Biochemical parameters among study groups

Parameters	Control (n=80)	Case(n=80)	p- value
FBS (mg/dl)	104.8± 21.09	156.95 ±70.25	<0.001*
Urea(mg/dl)	23.63± 7.58	23.96 ±7.3	0.775
Creatinine(mg/dl)	0.8 ±0.17	0.86± 0.27	0.087
Total Cholesterol(mg/dl)	177.31± 41.7	202.71± 64.8	0.004*
Triglyceride(mg/dl)	123.59± 47.77	131.6± 42.7	0.265
HDL (mg/dl)	46.55 ±6.74	41.53± 9.86	<0.001*
LDL (mg/dl)	110.81± 39.4	129.95 ±19.3	<0.001*

* p- value ≤ 0.05: Statistically significant

Table 3 highlights that CAD cases had significantly higher fasting blood sugar(p<0.001), total cholesterol(p=0.004) and LDL (p<0.001) levels as compared to controls. The HDL level was significantly lower in CAD groups as compared to controls with a p value of < 0. 001.Though the levels of Urea, creatinine and triglyceride were higher in CAD groups, but there was no Statistically significant difference observed in between controls and cases.

Table no 4: Mean value of Serum Copeptin & Serum Troponin T in cases and controls

	Control (n=80)	Case (n=80)	p- value
Serum Copeptin	0.62± 0.036	4.12 ± 2.23	0.001
Serum Troponin T	0.022 ± 0.01	3.36 ± 2.38	0.002

The mean value of serum Copeptin in cases and controls was 4.12 ± 2.23 & 0.62± 0.036 respectively with a statistically significant p value of 0. 001.Similar observation was noted in the serum level of Troponin T where the mean value in cases was 3.36 ± 2.38 & in control 0.022 ± 0.01 having a statistically significant p value of 0.002 as shown in table 4.

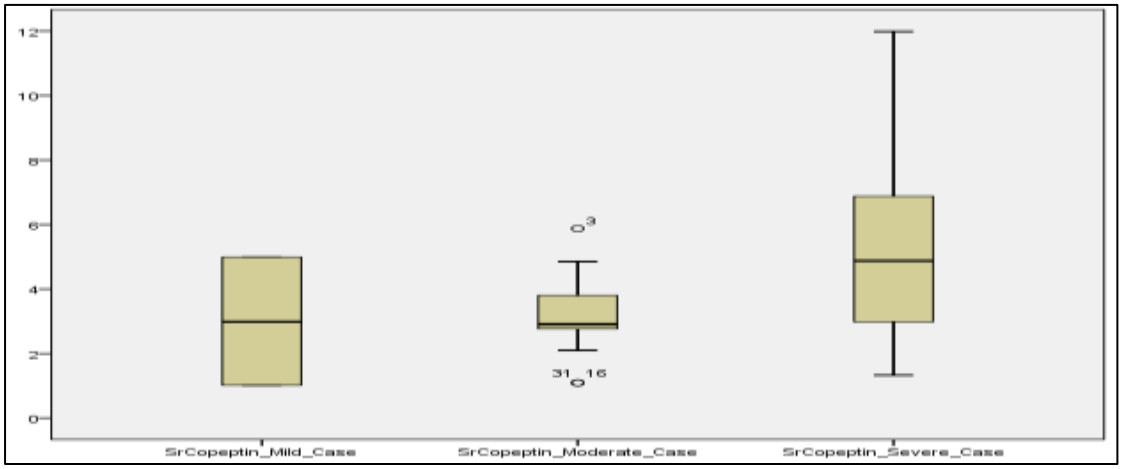

Fig -1:X axis -severity of CAD cases & Y axis-serum Copeptin level in pmol/L

From Box whisker plot (Fig-1), it is found that in first box Maximum value is higher in Mild cases than Moderate case, but in Severe cases it is the highest. Upper quartile in Moderate cases have most (more than 80%) distributions. Median in Mild and Severe box plot shows, distributions are normal. In moderate box plot distributions are positively skewed.

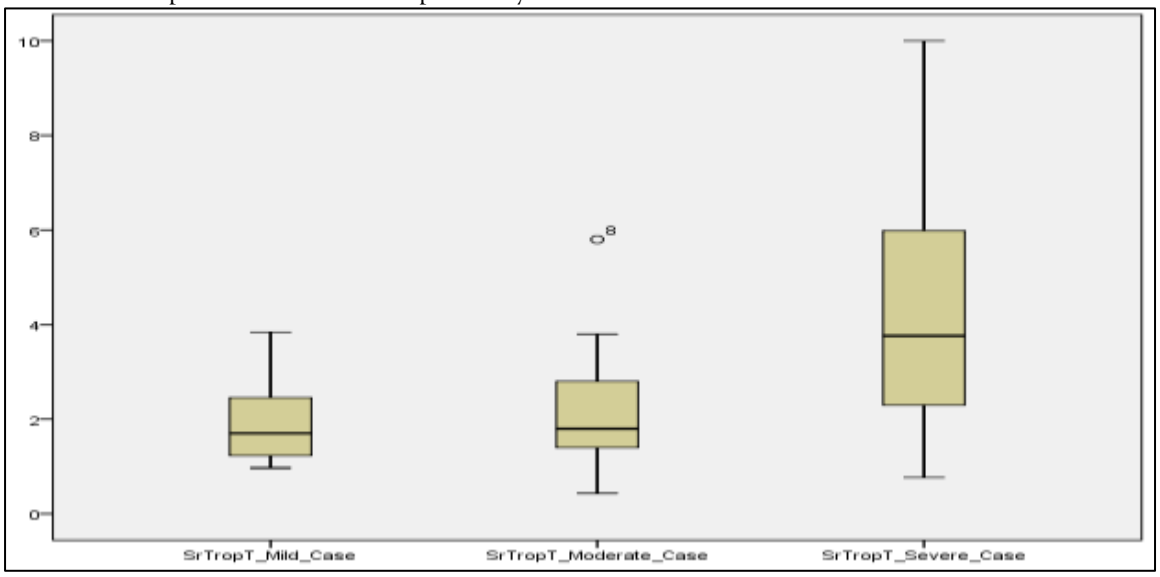

Fig -2:X axis -severity of CAD cases & Y axis-serum Troponin T level in ng/ml

From Box whisker plot (Fig 2) it is found that in first box maximum value is higher in Mild cases than Moderate cases, but in Severe cases it is the highest. Upper quartile in Severe cases have most (more than 70%) distributions. All three distributions are positively skewed.

Table 5: Correlation between Copeptin with Mild, Moderate and Severe degree of CAD

Comparison of serum Copeptin with various degree of CAD	r- value	p- value
Mild vs Moderate	0.612	0.048
Mild vs Severe	0.543	0.025
Moderate vs Severe	0.621	0.037

Table 5 shows a positive Pearson Correlation between serum Copeptin and Gensini score with r- value of 0.612, 0.543, 0.621 in Mild vs Moderate, Mild vs Severe & Moderate vs Severe degree of CAD (comparison based on Gensini Score) and this comparison of Mild vs Moderate, Mild vs Severe & Moderate vs Severe degree of CAD is highly significant; having p- value of 0.048,0.025 & 0.037 respectively.

Table 6: Correlation Serum Troponin T with Mild, Moderate and Severe degree of CAD.

Comparison Serum Troponin T with degree of CAD	r- value	p- value
Mild CAD vs Moderate CAD	0.698	0.035
Mild CAD vs Severe CAD	0.846	0.044
Moderate CAD vs Severe CAD	0.743	0.019

Table 6 shows a positive Pearson correlation between serum Troponin T and Gensini score with r- value of 0.698, 0.846 & 0.743 in Mild vs Moderate, Mild vs Severe& Moderate vs Severe degree of CAD (comparison based on Gensini Score). This comparison of Mild vs Moderate, Mild vs Severe & Moderate vs Severe degree of CAD is highly significant; having p- value of 0.035, 0.044 & 0.019 respectively.

DISCUSSION:

In the present study, serum copeptin and Troponin T level was found to be higher in cases as compared to the controls and the difference was found to be statistically significant. Also it was observed that the serum copeptin and Troponin T level was more elevated in the most severe category of CAD as compared to the mild and moderate category CAD with a significant positive correlation and significant p value while comparing Mild vs Moderate, Mild vs Severe & Moderate vs Severe degree.The finding of the present study is in accordance with the study conducted by Chai et al[12]where the copeptin levels were significantly higher than the controls. Copeptin being a non-specific but highly sensitive stress marker it is best evaluated in the patients who presented early with symptoms[13].Some study shows that the level of Copeptin rises significantly before the onset of cardiac symptoms.[13] Which could be potentially used as a very vital marker in patients without the classical symptoms of Coronary disease[14].Copeptin predicts development of CAD and cardiovascular mortality both in diabetic and non-diabetic[15] and in the present study the FBS level in CAD was significantly higher than the controls. Patients who developed cardiac failure or who died after an acute MI had significantly higher blood copeptin levels as shown in a study conducted by Khan et al [16]. Arginine vasopressin (AVP), also known as antidiuretic hormone, is one of the key hormones of the hypothalamic-pituitary-adrenal (HPA) axis. Copeptin, a peptide of 39 amino acid with leucine rich core segment. The C-terminal part of pro-AVP and is released together with AVP during processing of the precursor peptide [17]. The physiologic function of AVP is threefold, when released into the circulation, AVP mediates arteriolar vasoconstriction via the V1-receptor and exhibits an antidiuretic effect in the kidneys via the V2-receptor [18]. Copeptin and AVP are secreted from the neurohypophysis upon hemodynamic or osmotic stimuli AVP is also involved in the endocrine stress response. It is noteworthy that, in vivo, the kinetics of Copeptin are similar to those of AVP [19] but in contrast to AVP and cortisol, Copeptin is stable both in serum and plasma at room temperature and can be easily measured ex vivo as a 'shadow' fragment of AVP in the circulation [19,20], in ELISA and manual or fully automated chemiluminescence assays. This favourable discrepancy allows for the precise measurement of Copeptin as a surrogate marker for the unstable AVP. Copeptin results are available within one hour, which is crucial for any useful biomarker in the emergency department setting. Copeptin levels were already elevated at a time when Troponin T was still undetectable (0 hours to 4 hours) in 20 out of 81 patients with the final diagnosis of acute MI as shown in a study where a negative troponin and Copeptin at the time of ED presentation was enough to rule out acute MI [21].

Troponin is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that are integral to muscle contraction. Measurements of blood level of cardiac-specific troponins I and T are extensively used as diagnostic and prognostic indicators in the management coronary artery disease[22].The cardiac subtype of troponin T is especially useful in the laboratory diagnosis of CAD, because it is released into the blood-stream when damage to cardiac myocytes occurs[23].Serum level of Troponin T increases within 6 hours of myocardial infarction peaks at 24 hours then remains elevated up to 10-14 days. The 99th percentile cut-off for cardiac troponin T (cTnT) is 0.01 ng/ml [24]. Serial estimation of cardiac troponins are done in any patient reporting with symptoms to the emergency clinic. Cardiac troponins (cTn) exist as two specific isotypes; I and T (cTnI, cTnT). Elevated concentrations of

cTn are seen in a range of acute and chronic cardiac disease states, such as acute myocardial infarction (AMI), cardiac arrhythmias and heart failure [25]as well as in non-cardiac disease. In addition, cTn has been shown to be a robust marker of cardiovascular-and all-cause mortality, both in the general population, in patients with known coronary artery disease [26], in patients with acute coronary syndrome (ACS) [27].

In a study by Reichlin et al [28], combined use of plasma copeptin and troponin T levels was found to have higher sensitivity and specificity as compared to troponin T alone in ruling out a diagnosis of acute MI. So, a dual marker approach i.e combining copeptin and Troponin T may provide a more comprehensive assessment of CAD severity as conducted in the present study. Copeptin's ability to reflect cardiovascular stress and instability complements Troponin T's sensitivity to myocardial damage and this dual marker approach may enhance risk stratification, improve diagnosis and guide treatment decisions.

The strengths of the study:

Estimation of Serum Copeptin along with troponin T as a dual biomarker for CAD.

The limitations of the study:

The limitation of the study is, Serum Copeptin is sensitive but not specific only for CAD and Serum Copeptin levels may prove it difficult to predict the prognosis in cases with head injury or TBI (traumatic brain injury) or any diseases involving the hypothalamus.

SUMMARY AND CONCLUSION:

To conclude the study, the findings estimated and endorsed a substantial relationship between the levels of Serum Copeptin and Serum Troponin T levels in CAD patients in comparison to healthy controls and both the markers proved to be a strong predictor of severity of coronary artery disease. Thus, the dual marker approach of combining Serum Copeptin and Serum Troponin T offers a promising strategy for assessing CAD severity. By integrating these biomarkers, clinicians may improve risk stratification, diagnosis and treatment decisions, ultimately enhancing patient outcomes. Further research is needed to validate this approach and establish its clinical utility.

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