IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية
ISSN:1813-1638
The Medical Journal of Tikrit University
Journal Homepage: http://mjtu.tu.edu.iq
MJTU
The Medical Journal of Tikrit University

Evaluation the Sensitivity of Copeptin and Some Biochemical Parameters for Atherosclerosis

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Received: 00/00/2024
Revising: 00/00/2024
Proofreading: 00/00/2024
Accepted: 00/00/2024
Available:online:31/12/2024

KEY WORDS:
Atherosclerosis, Copeptin, Spexin, Galectin-9, Netrin-1, Sensitivity.

ABSTRACT
Background: This study assesses diagnostic sensitivity and specificity of a variety of biochemical markers, including Copeptin, Spexin, Galectin-9, Netrin-1, and conventional lipid profiles in early atherosclerosis detection.
Materials and Methods:A case-control study was conducted on ٩0 subjects which included atherosclerosis patients and healthy controls. Blood samples were tested for lipids and biomarker levels.
Results: Results showed significant differences in lipids such as cholesterol, LDL, and HDL in comparison between two groups coinciding with traditional atherosclerotic risk factors. Among new biomarkers, Copeptin demonstrated high accuracy. The others showed variable sensitivity and specificity.
Conclusion: Develop an understanding about the significance of these biomarkers in regard to their potential roles in atherosclerosis early diagnosis and management, in order to help develop more effective protocols for screening and treatment.

DOI: http://doi.org/10.25130/mjotu.00.00.00

INTRODUCTION

Atherosclerosis is a large and medium progressive, multifactorial disease of large and medium arteries. It is characterized by the deposit of plaques of lipids, cholesterol, calcium, and cellular debris at the intimal walls of the large and medium arteries. The disease eventually results in narrowing and hardening of the arteries, which can seriously impede blood flow and result in serious cardiovascular complications, including coronary artery disease, stroke, and peripheral artery disease. Since it is an asymptomatic condition until the advanced

stages, recognition and monitoring in early stages are essential to avoid complications. This present study is an attempt to find out the diagnostic utility of some novel biomarkers such as Copeptin, Spexin, Galectin-9, Netrin-1 in conjunction with conventional lipid profile parameters to detect atherosclerosis, based on their role in cardiovascular physiology and pathology. Therefore, the aim is to determine the sensitivity, specificity, and overall accuracy for atherosclerosis of these methods, which could enable more detailed and earlier diagnosis than conventional ones alone .

Atherosclerosis: is the chronic disease of the arteries, caused by the accumulation of plaque on the walls; the lumen gets narrowed and hardened. The plaque usually consists of fats, cholesterol, calcium, and other substances found in the blood. It is actually the major underlying cause of different forms of cardiovascular diseases: myocardial infarction, stroke, and peripheral vascular disease. Definition: The knowledge and identification of early biomarkers of atherosclerosis may bring about better prevention and treatment strategies [1].

Copeptin: It is the C-terminal part of the precursor peptide of vasopressin, an antidiuretic hormone causing vasoconstriction. It is a much more stable and readily measurable surrogate for vasopressin. High levels of Copeptin are associated with stress, cardiovascular diseases, and adverse outcomes in acute myocardial infarction and heart failure. It is also a very stable biomarker for atherosclerosis and increases with the severity of the disease [2] .

Lipid Profile: It helps the doctor to know if the patient needs medical intervention, lifestyle changes, or a healthy diet. Lipid Profile: This panel of blood measures the level of various kinds of lipids within the blood and includes both total cholesterol and low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL). It is very important in assessing the cardiovascular risk. High levels of LDL and VLDL, as well as reduced levels of HDL, are strong predictors of atherosclerosis and related cardiovascular events [3].

Spexin: Among the huge amount of peptide hormones, it is believed that Spexin has been identified to be associated with many physiological functions in mammals. It includes its role in energy homeostasis, weight regulation, and cardiovascular function. Deviations from these levels are associated with metabolic disorders and cardiovascular diseases, thus making it a possibility for a biomarker for atherosclerosis. Its role in inflammation and energy balance underscores its relevance in atherosclerosis progression [4].

Galectin-9: is an immune response modulation and inflammation protein, having interactions with glycan structures at the surface of cells. It has an immunoregulatory function, among other functions. Increased Galectin-9 levels are associated with inflammatory diseases, such as atherosclerosis. Its involvement in immune regulation and inflammation makes it a probable candidate for markers linked with the inflammatory processes of atherosclerosis [5] .

Netrin-1: is a guidance cue protein for cells and axon migration in development and plays some roles in inflammation and tissue repair. Recent studies have revealed the role of Netrin-1 in atherosclerosis pathogenesis through influencing immune cell trafficking and maintaining plaque stability. Netrin-1 levels correlated with atheroma burden and instability, thus offering itself as a possible biomarker for atherosclerosis [6] .

MATERIALS

[Study Design and Population This study was designed as a case-control investigation to evaluate the sensitivity and specificity of different biomarkers in diagnosing

atherosclerosis. The populations were divided into two groups:

• Patients Group: Individuals who are diagnosed with atherosclerosis based on the clinical symptoms and imaging studies.

• Control Group: Those who are healthy and free from cardiovascular and other chronic diseases.

Sample size A total of 90 participants (30 control group and 60 patients group) were included in the study.

Biochemical Parameters and Blood Sampling All participants submitted the blood samples after a minimum overnight fast of 12 h in order to ensure the fasting state during the measurement of lipid levels and other biochemical parameters. Blood samples were collected in plain tubes and serum tubes for serum separation. The samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which were then stored at -20°C until further analysis.

The ethical approval for the study was acquired from the institutional review board of the participating hospital. Informed consent in writing was taken from all the participants before including them in the study after providing adequate information regarding the aim of the study, procedures, and possible hazards during the period of the study].

Data Analysis

1. **Lipid Profile:**
    - Total Cholesterol (TC): The determination of TC was made by enzymatic colorimetric method using a commercially available kit (Roche Diagnostics, Indianapolis, IN).
    - Triglycerides (TG): Determined using the method of enzymatic colorimetric test Glycerol phosphate oxidase methods (Roche Diagnostics).
    - Low-Density Lipoprotein (LDL): Calculated using the Friedewald formula; LDL = TC - HDL - (TG/5).
    - High-Density Lipoprotein (HDL): Measured using direct enzymatic methods (Roche Diagnostics).
    - Very Low-Density Lipoprotein (VLDL): Triglyceride values were divided by a factor of 5 for the calculation.
2. **Novel Biomarkers:**
    - Copeptin: An enzyme-linked immunosorbent assay (ELISA) kit was used commercially to measure human Copeptin (Thermo Fisher Scientific, Waltham, MA).
    - Spexin: An ELISA kit was commercially utilized designed for human Spexin (Phoenix Pharmaceuticals, Inc., Burlingame, CA).
    - Galectin-9: A high-sensitivity ELISA kit was commercially used for measuring human Galectin-9.
    - Netrin-1: Measured using the ELISA with the Human Netrin-1 specific kit (Bio-Techne, Minneapolis).

Descriptive statistics (mean and standard error, SE) were calculated for all the biochemical parameters, giving an overall impression of the central tendency and variability within each group.

The comparative analysis was conducted using Student's t-test for the mean levels of the biochemical parameters in the patient and control groups. The level of significance was maintained at $P \leq 0.05$ for average importance and $P \leq 0.01$ for high importance.

Sensitivity and Specificity of the Analyzed Parameters

The sensitivity and specificity of every analyzed parameter have been calculated based on the ROC curve. AUC described the diagnostic accuracy of each analyzed biomarker.

The cut-off value of every parameter was determined from the cut-off value of ROC analysis to achieve maximal sensitivity and specificity.

Data analysis for the present study was done using SPSS software (Version 25.0, IBM Corp., Armonk, NY). The ROC curves were constructed in MedCalc software (Version 19.0.7, MedCalc Software Ltd., Ostend, Belgium).

RESULTS AND DISCUSSION

(Table 1): Comparison between patients and control groups in Lipid profile

Group	Mean ± SE (mg/dl)				
	Cholesterol	**Triglyceride**	**LDL**	**HDL**	**VLDL**
Patients	146.00 ±5.31	89.79 ±5.79	79.83 ±3.80	32.97 ±0.96	20.14 ±2.54
Control	109.72 ±5.41	77.33 ±6.32	54.18 ±3.76	45.10 ±3.63	16.96 ±1.39
T-test	16.337 **	19.22 NS	10.722 **	6.223 **	8.513 NS
P-value	0.0001	0.168	0.0001	0.0005	0.491

** (P≤0.01).

(Table 2): Comparison between patients and control groups in Spexin(spx), GAL9, Copeptin and Ntn-1

Group	Mean ± SE (pg/ml)			
	Spexin(spx)	**GAL9**	**Copeptin**	**Ntn-1**
Patients	103.05 ±6.39	21.10 ±2.11	64.04 ±8.44	74.28 ±7.56
Control	174.34 ±41.10	13.76 ±0.59	32.82 ±7.74	52.43 ±2.06
T-test	62.85 *	5.908 **	26.929 **	23.426 *
P-value	0.037	0.101	0.0074	0.050

* (P≤0.05), ** (P≤0.01).

(Table3):The values of cut off, Sensitivity , Specificity,accuracy and AUC of parameters for (patients and control groups

Variables	Cut-off value	Sensitivity %	Specificity %	Accuracy	AUC	P – value
TC	>117	76.92	77.27	0.5420	0.807	<0.0001
TG	>45	97.73	28.57	0.2630	0.561	0.4452
HDL	≤42.8	100.00	43.33	0.4333	0.723	0.0004
LDL	>69	67.74	86.21	0.5395	0.803	<0.0001
VLDL	≤16.8	63.08	64.00	0.2708	0.557	0.3997
TNF-α	>38.1693	36.84	85.71	0.2256	0.614	0.0750
Copeptin	>30.3125	85.25	79.31	0.6456	0.835	<0.0001
Netrin-1	>64.7067	40.35	96.15	0.3650	0.651	0.0122
Galectin-9	>17.2932	47.73	95.65	0.4338	0.737	0.0001
Spexin	≤73.9411	28.57	100.00	0.2857	0.649	0.0188

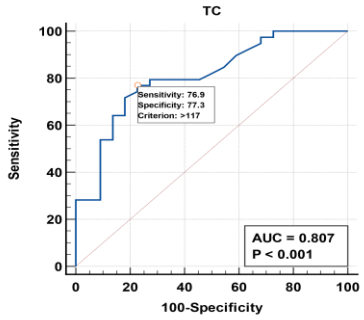

Figure 1: ROC curve of TC levels for patiens and control group

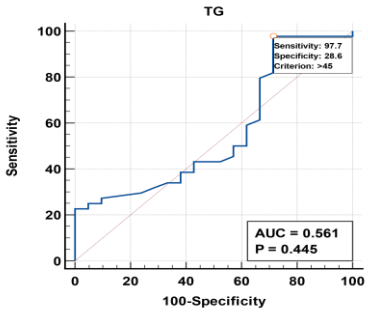

Figure 2: ROC curve of TG levels for patiens and control group

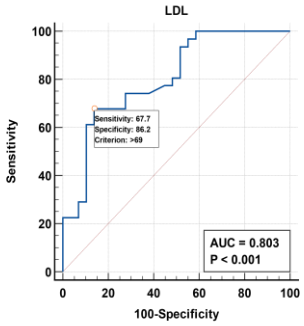

Figure 3: ROC curve of LDL levels for patiens and control group

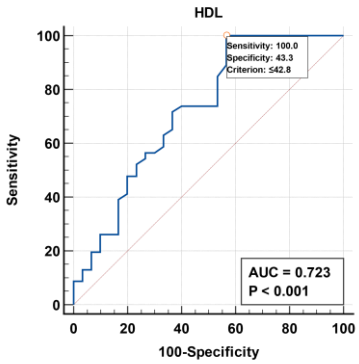

Figure 4: ROC curve of HDL levels for patiens and control group

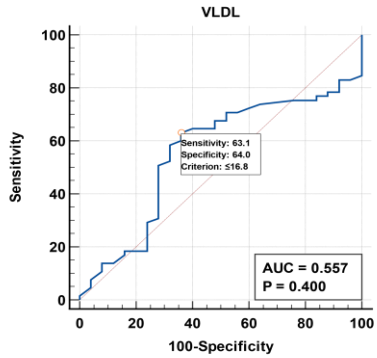

Figure 5: ROC curve of VLDL levels for patiens and control group

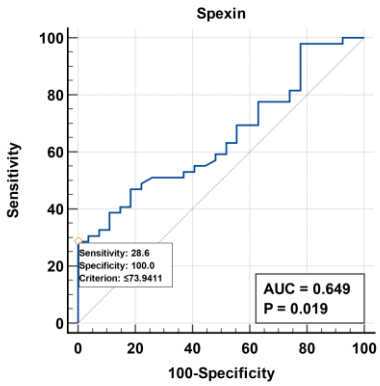

Figure 6: ROC curve of spexin levels for patiens and control group

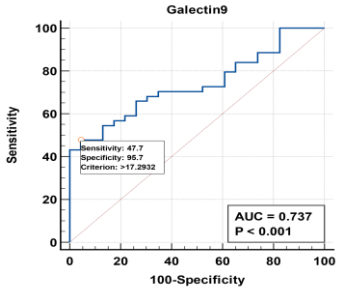

Figure 7: ROC curve of galectin-9 levels for patiens and control group

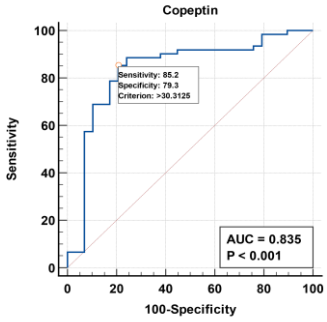

Figure 8: ROC curve of copeptin levels for patiens and control group

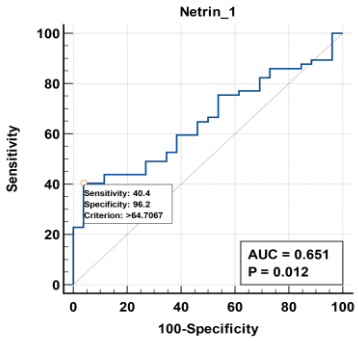

Figure 9: ROC curve of netrin-1 levels for patiens and control group

The results of this study therefore provide the full test evaluation of the sensitivity and specificity of various biochemical markers Copeptin, Spexin, Galectin-9, and Netrin-1 in the detection of atherosclerosis. This discussion appraises the significance of these findings, evaluates them against the current literature, and discusses their implications for clinical application. Significantly higher mean TC levels were recorded in atherosclerotic patients (146.00 ± 5.31 mg/dl) compared with the control group (109.72 ± 5.41 mg/dl), with a p-value of 0.0001. This relates well to the fact that raised cholesterol is a prime risk factor for atherosclerosis. The ROC analysis on this indicated the cut-off is >117 mg/dl, with a sensitivity of 76.92% and specificity of 77.27% (AUC = 0.807; P < 0.0001), indicating TC as a reliable marker for the diagnosis of atherosclerosis.

The level of TG was elevated in the patient group to 89.79 ± 5.79 mg/dl as against 77.33 ± 6.32 mg/dl in controls, but it did not achieve a statistical significance level (P = 0.168). It sensitively marked 97.73% specifically and marked 28.57% from cutoff more than 45 mg/dl (AUC= 0.561, P = 0.4452). These results suggest that TG may not be a strong diagnostic marker for atherosclerosis, probably due to their variability and influence in other metabolic conditions. The level of LDL was remarkably higher among patients with atherosclerosis (79.83 ± 3.80 mg/dl) compared to the controls (54.18 ± 3.76 mg/dl), with a p-value of 0.0001. The cut-off value calculated was >69 mg/dl, which gave out a sensitivity of 67.74% and specificity of 86.21% (AUC = 0.803, P < 0.0001), therefore re-emphasizing the importance of LDL in the genesis of atherosclerosis and in clinical diagnostics.

HDL was significantly lower in patients [32.97 ± 0.96 mg/dl] compared with controls [45.10 ± 3.63 mg/dl] at p-value 0.0005. At a cut-off value of ≤42.8 mg/dl, the test gave 100% sensitivity and 43.33% specificity [AUC = 0.723, P = 0.0004]. This is a well-defined association in regards to one of the relations of HDL and risk for atherosclerosis, which ultimately performs reverse cholesterol transport and elicits its anti. There was no significant difference in the VLDL level among cases (20.14 ± 2.54 mg/dl) and controls (16.96 ± 1.39 mg/dl) (P = 0.491). Using a cut-off value of ≤16.8 mg/dl shows moderate sensitivity, i.e., 63.08%, and specificity, i.e., 64.00% (AUC = 0.557, P = 0.3997). VLDL is less contributory to atherosclerosis than LDL; thus, the result might be not very.

Level of Copeptin was significantly higher in the patients group (64.04 ± 8.44 pg/ml) compared with controls (32.82 ± 7.74 pg/ml); p=0.0074. Through ROC analysis, >30.3125 pg/ml was detected with a sensitivity of 85.25% and specificity of 79.31% (AUC = 0.835, P <. Due to the previously discussed properties of Copeptin as a surrogate marker for vasopressin, it may represent stress and strain on the cardiovascular system, further solidifying its potential as a strong biomarker for atherosclerosis. Spexin levels in the patients were 103.05 ± 6.39 pg/ml, while they were 174.34 ± 41.10 pg/ml in controls, and the p-value was 0.037. With the cut-off value of ≤73.9411 pg/ml, the sensitivity and specificity were 28.57% and 100.00%, respectively (AUC = 0.649, P =. Although Spexin exhibits high specificity, the sensitivity is low enough to make it an inadequate single diagnostic marker. It would be valuable as part of a panel of biomarkers.

Patients had higher levels of Galectin-9 at 21.10 ± 2.11 pg/mL as compared to 13.76 ± 0.59 pg/mL in controls, with no statistical difference (p = 0.101). A cut-off >17.2932 pg/mL gave sensitivity of 47.73% and specificity of 95.65% (AUC = 0.737; P = 0.0001). Its high specificity suggests that Galectin-9 may serve to confirm atherosclerosis in suspected cases, especially in conjunction with other markers.

Cases showed higher serum levels of netrin-1 compared to the controls at 74.28 ± 7.56 pg/mL and 52.43 ± 2.06 pg/mL, respectively, with a P-value of 0.050. For the same, the cut-off value of > 64.7067 pg/ml gave a sensitivity of 40.35% and specificity of 96.15%, AUC = 0.651, P = 0.0122. Netrin-1 could be the reason for increased inflammation and migration of immune cells in atherosclerosis; hence, diagnostic performance was moderate. These findings are consistent with earlier literature reports that pointed out the importance of lipid profile parameters, in particular LDL and HDL, in the diagnosis and management of atherosclerosis. Statistically significant differences in the levels of TC, LDL, and HDL between patients and controls confirm

them as well-known risk factors for cardiovascular diseases [7,8] .

Copeptin exhibits high sensitivity and specificity given the several studies that have demonstrated its participation in stress-related cardiovascular conditions, agreeing with studies by [9,10] that demonstrated Copeptin as an independent prognostic marker in heart failure and myocardial infarction.

Thus, the lowering levels of Spexin in these patients and its regulation during metabolic and cardiovascular disturbances show great promise for clinical studies. Nevertheless, its low sensitivity dictates the need for verification by further studies. Galectin-9 and Netrin-1 are novel markers showing promise based on recent studies that identified their roles in inflammation and immune regulation. Increased levels of these markers, as shown in atherosclerotic patients, further suggest their potential roles in disease pathogenesis, which is supported by the studies on their immunomodulatory effects [11,12] .

Identification of reliable biomarkers for atherosclerosis is very important for early diagnosis and proper management. High sensitivity and specificity of Copeptin give some impetus to consider this test as a primary diagnostic measure, in particular, in laboratories where results of the classic lipid profile may be debatable. It might also increase diagnosis accuracy for personalized treatment strategies when used together with lipid profile parameters. The high specificity of Galectin-9 and Netrin-1 suggests that they may be useful in confirming a diagnosis of atherosclerosis in patients with an ambiguous lipid profile. Sensitivity was, on the other hand, low for spexin, albeit it might be useful in specific clinical scenarios when combined with other markers to improve overall diagnostic performance.

Future studies in larger multi-center study populations are required to validate these results and define the longitudinal changes in the levels of these biomarkers to atherosclerosis development and progression. Similarly, research on the combined diagnostic value of these biomarkers could offer a perspective on developing a full-panel test in atherosclerosis screening and risk assessment. This research is likely to be important since it unfolds the diagnostic utility of Copeptin, Spexin, galectin-9, and netrin-1 in comparison with conventional lipid profile parameters for atherosclerosis. Considering its good sensitivity and specificity values, in addition to the well-established importance of LDL and HDL, really holds out great promise of improving the early detection and management of atherosclerosis. Further studies are needed to validate these findings in clinical usage to enhance the outcomes of cardiovascular health] .

CONCLUSION

The study postulates that these biomarkers in the combination form may prove beneficial for early disease detection and disease management of atherosclerosis; however, this warrants further research to fully support the current findings.

CONFLICT OF INTEREST

The authors declare no competing interest.

ACKNOWLEDGEMENTS

I extend my thanks and gratitude to all the people who contributed to the completion of this study, especially the laboratory workers, patients, and healthy people from whom the study samples were taken.

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