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RESEARCH ARTICLE

Uyaner Kan et al: Circulating organokines in CAD and MetS

Circulating organokines in coronary artery disease and metabolic syndrome: FABP4, adiponectin, irisin, FSTL1

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ABSTRACT

Cardiovascular disorders are closely linked to metabolic syndrome and remain a leading cause of mortality worldwide, despite advances in early detection and treatment. Adipokines, cardiokines, and myokines play critical roles in maintaining systemic metabolic homeostasis. In this study, we measured serum levels of fatty acid binding protein 4 (FABP4), follistatin-like 1 (FSTL1), irisin, and adiponectin in 243 male patients undergoing elective coronary angiography. We investigated the associations of these biomarkers with coronary artery disease (CAD) and their correlation with metabolic syndrome status. FSTL1 levels were predicted using a Particle Swarm Optimization-enhanced Adaptive Neuro-Fuzzy Inference System (PSO-ANFIS) based on artificial intelligence. Patients with CAD exhibited significantly lower FABP4 levels ($p<0.0001$), and low FABP4 levels emerged as an independent predictor of CAD in logistic regression analysis (odds ratio 0.903, 95% CI 0.825-0.987, $p=0.025$). The combination of adiponectin, FSTL1, and irisin as a biomarker strategy demonstrated high sensitivity and specificity for diagnosing metabolic syndrome (AUC = 0.92, 95% CI 0.88-0.96). Both FSTL1 and adiponectin independently correlated with metabolic syndrome ($p<0.001$, odds ratio 1.039, 95% CI 1.025-1.054; $p<0.001$, odds ratio 0.979, 95% CI 0.971-0.988, respectively). The prediction of FSTL1 levels using PSO-ANFIS supports the concept of harmonization among metabolic messengers. These findings underscore the potential of FABP4 and FSTL1 as valuable biomarkers for diagnosing metabolic and cardiovascular diseases, thereby facilitating personalized interventions targeting organokine pathways.

Keywords: Metabolic syndrome, coronary artery disease, fatty acid-binding proteins, adiponectin, follistatin-related proteins, irisin.

INTRODUCTION

Adipose tissue, skeletal muscle, and the heart are active endocrine organs that synthesize and secrete bioactive molecules known as adipokines, myokines, and cardiokines under physiological and pathological conditions. Since atherosclerosis has been considered a chronic inflammatory disease, there has been intense interest in inflammation markers and related studies. These molecules and their interactions have been linked to inflammatory and oxidative damage, obesity, and obesity-related cardiovascular diseases [1].

Transcriptomic analyses of human adipose tissue have been pivotal in defining the adipokine concept. Adiponectin, named for its adipocyte-specific and abundant secretion as well as its collagen-like and nectin-like structural features [2], is a key adipokine with metabolic functions, including enhancement of glucose and lipid homeostasis and attenuation of oxidative stress and inflammation [3].

Adipokines play a role in the interaction between adipose tissue and peripheral organs, including the heart. Fatty acid-binding proteins (FABPs) are a group of cytosolic proteins involved in lipid transport and the coordination of inflammatory and metabolic pathways. Adipocyte-FABP (A-FABP, aP2 or FABP4) is a cytosolic protein found in adipocytes and is also expressed in macrophages and endothelial cells. Increased serum FABP4 concentrations are associated with obesity, insulin resistance, hypertension, inflammation, atherosclerosis, and metabolic syndrome. Therefore, FABP4 is proposed as an independent biomarker in metabolic and cardiovascular diseases [4].

Skeletal muscle makes up about 40% of body weight and is the largest organ in non-obese individuals. Numerous myokines expressed and secreted by skeletal muscle have been identified. Irisin, a myokine, is produced as a Type I membrane precursor protein called fibronectin Type III domain-containing protein 5 in muscle and is released into circulation after proteolytic degradation. Irisin is a well-known exerkine and has been shown to reduce insulin resistance in muscle [5]. It is thought to contribute to the beneficial effects of exercise on metabolism by promoting the transformation of white adipose tissue into brown adipose tissue.

Proteins expressed and secreted by cardiac cells in studies focused on transcription, protein, and secretome expression in cardiac tissues are termed cardiokines [6].

Cardiokines (or cardiomyokines) are peptides and proteins secreted by cells of the heart- myocytes, fibroblasts, endothelial cells, and vascular cells- under physiological or pathological conditions, exerting autocrine, paracrine, and potentially endocrine effects. These proteins, including FSTL1, are involved in regulating processes such as inflammation, fibroblast activation, cardiomyocyte hypertrophy, and cardiac remodeling [7].

Cardiovascular diseases constitute a group of disorders affecting the heart and blood vessels, and they remain a leading cause of death worldwide [8]. Metabolic syndrome is a metabolic dysfunction characterized by the coexistence of multiple risk factors associated with the development of cardiovascular diseases. Among the broad spectrum of cardiovascular conditions, coronary artery disease represents a significant health burden and is defined as a pathological process characterized by the accumulation of atherosclerotic plaques, either obstructive or non-obstructive, within the epicardial coronary arteries [9]. Asymptomatic atherosclerotic coronary artery disease is prevalent in the general population [10]. In this context, identifying metabolic messengers that can predict, prevent, or reduce mortality before the onset of cardiac damage is critical. This study aimed to explore the association between circulating organokines and CAD and metabolic syndrome.

MATERIALS AND METHODS

Study population

The study was conducted prospectively between October 2023 and May 2024 on male patients aged 18 years and older who applied to the Coronary Angiography and Cardiac Catheterization Laboratory. Patients diagnosed with neurological, musculoskeletal, or gastrointestinal diseases; thyroid, adrenal, or gonadal hormone disorders; infections; oncological diseases; and acute coronary syndrome were excluded. Based on the presence of metabolic syndrome (as defined by the National Cholesterol Education Programme Adult Treatment Panel III [11]) and coronary artery disease, patients were divided into four groups (Figure 1). Coronary artery disease was classified according to the SYNTAX score, which evaluates lesions causing ≥50% stenosis in vessels ≥1.5 mm, with the coronary tree divided into 16 segments based on the AHA classification as modified in the ARTS I and II trials [12].

Biochemical measurements

All venous blood samples were obtained from fasting participants in the morning, allowed to clot for 30 minutes, and centrifuged at 1500 g for 15 minutes. Serum aliquots were stored at −80 °C until analysis to minimize pre-analytical variability. ELK Biotechnology brand human sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits were used to measure serum adiponectin, FABP4, FSTL1, and irisin concentrations (intra-assay CV < 8%, inter-assay CV < 10%). The detection limits were 0.16 ng/mL, 31.25 pg/mL, 31.25 pg/mL, and 15.63 pg/mL, respectively. Additional information on biochemical measurements and calibration curves (Figure S1-S12) is presented in the supplementary material.

The Sysmex XN-1000 analyzer was used to test complete blood count, and the Premier Hb9210 automatic analyzer was used to test HbA1c. HDL, LDL, CRP, glucose, urea, and creatinine levels were measured by spectrophotometry on the Roche Cobas 8000 c702 analyzer. Insulin, high-sensitivity cardiac troponin T, and IL-6 levels were also measured by electrochemiluminescence immunoassay on the Roche Cobas 8000 e801 device.

Ethics approval and reporting guidelines

On June 16, 2023, the Necmettin Erbakan University Ethics Committee endorsed the study protocol (Approval No: 2023/4389). All participants provided written informed consent. The Declaration of Helsinki was followed when conducting the study. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) criteria were also followed in the study design.

Statistical analysis

GraphPad Prism v10, Jamovi v2.3.28, SPSS v26.0 and RStudio v2025.09.1 were all utilized for statistical analyses. Using G*Power 3.1, the sample size was determined to be 180 participants for four equally proportioned groups, with a 5% error margin, 80% power, and a medium effect size (0.25). To compensate for potential participant losses, the sample size was increased by 10% to ensure at least 50 participants in each group. Data collection was completed after the required sample size was achieved.

Descriptive data were presented as medians with interquartile ranges (IQR), means ± standard deviation (SD), numbers (n), or percentages (%). The Shapiro-Wilk test was used to determine whether the distribution was normal. Using Pearson's chi-square test with Bonferroni correction, the categorical variables were compared. Continuous variables were assessed using non-parametric tests (Mann-Whitney U test and Kruskal-Wallis test) except for age, which was analyzed using one-way ANOVA. Post-hoc tests (Dunn’s test, Tukey test, and Bonferroni correction) were applied to determine the sources of significant differences among three or more groups.

Relationships between adiponectin, FABP4, FSTL-1, irisin, and other variables were analyzed using Spearman’s correlation test. Binary logistic regression was conducted to identify independent factors associated with coronary artery disease and to evaluate the effects of parameters on metabolic syndrome, adjusting for other variables. Events per variable were evaluated for all multivariable models to ensure adequate statistical power. Statistical significance was set at $p<0.05$ for all tests.

Result prediction with particle swarm optimization-based adaptive neuro-fuzzy inference system

Prediction models based on artificial intelligence methods are frequently utilized in the literature [13–15]. One such approach is the Adaptive Neuro-Fuzzy Inference System (ANFIS), a hybrid artificial intelligence technique that combines the parallel processing capabilities of artificial neural networks with the expert-knowledge-based framework of fuzzy logic [16]. The operational structure and mathematical background of ANFIS, which was first proposed by Jang in 1993, are presented in Figure 2 [17].

A key factor in the success of the ANFIS architecture is selecting the types and weights of the membership functions, as well as determining the model weights and rule sets. While these selections are traditionally made using conventional methods, the predictive performance of ANFIS models can be enhanced by applying metaheuristic search algorithms. Among these new-generation metaheuristic algorithms, particle swarm optimization (PSO) has emerged as a powerful tool for modelling nonlinear systems analogous to biological systems [18]. Modelling processes based on PSO-enhanced ANFIS can thus be conducted with significantly higher accuracy.

In our study, artificial intelligence analyses were performed using MATLAB R2024b and Orange Data Mining 3.38.1. The weights and rules for the ANFIS model were determined using the particle swarm optimization algorithm. The algorithm parameters employed in this study were as follows: maximum iterations of 100, population size of 40, inertia weight of 1, inertia weight damping rate of 0.99, c1 (personal learning coefficient) of 1, and c2 (global learning coefficient) of 2. Gaussian membership functions were used, and 9 membership functions were defined for each input variable. Input values were normalized to the range [0–1]. After analyzing the outliers using the Tukey robust statistic, artificial intelligence analysis was performed on patients with complete data without imputation, and the same 220 patients were used in all methods. A fixed random seed was set to ensure reproducibility. To demonstrate the applicability of the proposed approach, the entire dataset was divided into two subsets: training and testing. 80% of the data was allocated to training, and the remaining 20% to testing. This division enabled assessment of whether the prediction models exhibited overfitting, a common issue encountered in such models.

RESULTS

Demographic and anthropometric features, clinical characteristics and biochemical parameters of study participants are presented in Table 1. When all four groups were evaluated collectively, the presence of coronary artery disease was found to influence FABP4 and irisin levels. In the presence of metabolic syndrome, notable differences were observed in adiponectin, irisin, and FSTL1 levels (Table 1 and Figure 3). A significant moderate correlation was found between fasting serum triglyceride levels and both serum FSTL1 ($r = 0.43$) and serum irisin levels ($r = 0.423$) ($p < 0.001$) when all participants were analyzed. Conversely, an inverse significant correlation was observed between serum adiponectin and FSTL1 levels ($r = -0.672$, $p < 0.0001$, Table S1).

In the coronary artery disease risk factor model, generated through binary logistic regression analysis and adjusted for confounding variables (dyslipidemia, defined as total cholesterol ≥200 mg/dL, LDL-C ≥130 mg/dL, triglycerides ≥150 mg/dL, HDL-C <40 mg/dL or use of lipid-lowering therapy; hypertension, age, smoking, and fasting glucose levels), irisin did not retain statistical significance as an independent predictor. In contrast, FABP4 remained significantly associated with the presence of disease (Table 2). According to the ROC analysis, FABP4 demonstrated an area under the curve (AUC) of 0.702 in diagnosing coronary artery disease. To demonstrate the diagnostic performance of FABP4 at different clinical priorities (higher sensitivity for screening and higher specificity for confirmatory evaluation), two cut-off values determined using Youden's J index were included: for 6.422 ng/mL, the sensitivity was 80.6% and for 0.584 ng/mL, the specificity was 92.1% (Table S2).

For the diagnosis of metabolic syndrome, a combined biomarker score was calculated as the logistic regression linear predictor including adiponectin, irisin, and FSTL1. The predicted probability (combined biomarker score) was calculated by Equation 1.

$$\text{Combined Score}=\frac{1}{1+e^{4.544-\,(0.201\times\text{Irisin})-\,(0.025\times\text{FSTL1})-\,(140.190\times 1/\text{Adiponectin})}} \quad (1)$$

The calculated cut-off value for this combination was 0.389, and the area under the ROC curve (AUC) for the combined biomarker score was 0.92. The appropriate cut-off values and characteristics of adiponectin, irisin, FSTL1, and the combined score suitable for diagnosing metabolic syndrome in our study are presented in Figure 4.

In multivariable logistic regression analyses, circulating adiponectin and FSTL1 levels remained significantly associated with the presence of metabolic syndrome after sequential adjustment for age, waist circumference, hypertension, glucose, triglyceride, and HDL levels (Table 3). In contrast, irisin showed no significant association with metabolic syndrome in any of the adjusted models.-

The FSTL1 levels of the patients were predicted using the PSO-based ANFIS method, with input variables including triglycerides, HDL, glucose, HOMA-IR, BMI, adiponectin, irisin, and immature granulocyte counts. To ensure the approach's validity, the patient order was randomized prior to analysis. Eighty percent of the patients were assigned to the training set, while the remaining 20% were used to evaluate the method's accuracy. Following the training phase, visual representations of the mean square error (MSE) and root mean square error (RMSE), including their means and standard deviations, are presented in Figure 5. The performance metrics (RMSE, MAE, R, and R^2) of all predictive models are presented in Table 4.

DISCUSSION

Our study investigated the associations of circulating organokines (FABP4, FSTL1, adiponectin, and irisin) with CAD and metabolic syndrome in male patients undergoing elective coronary angiography. We observed significant variations in the levels of these biomarkers, with each demonstrating unique associations relevant to cardiovascular and metabolic disease.

While studies in the literature show that FABP4 levels increase in atherosclerotic diseases [19–24], a meta-analysis found that a 1-SD decrease in FABP4 in Type 1 DM patients increased the risk of CAD by 2.4-fold [25]. In our study, FABP4 levels were low in patients with chronic coronary syndrome. It is known that the inflammatory response is significantly increased in acute coronary syndrome. However, in patients with stable (chronic coronary syndrome) included in this study, the inflammatory burden is generally lower. In addition, chronic treatments such as ACE inhibitors, antiplatelet agents, and statins may also affect these levels by modulating the inflammatory response [26,27]. Our results may be explained by the higher statin use among our CAD patients (57.3%) compared to those without CAD (27.2%). In fact, in our study, the association between chronic coronary syndrome and FABP4 persisted after adjustment for dyslipidemia with statin use, which may indicate that a low FABP4 level may still be a useful predictor in patients receiving treatment.

Consistent with previous reports demonstrating reduced circulating irisin levels in patients with stable coronary artery disease and inverse associations with disease severity [28,29], our study also found significantly lower irisin concentrations in the CAD group compared with non-CAD participants (Figure S13). However, this relation not maintained meaningful in multivariable regression analysis, suggesting that the relationship between irisin and CAD may be confounded by other cardiometabolic factors.

According to metabolic syndrome results, low adiponectin and high FSTL1 levels were each independently associated with the condition. This result is consistent with previous studies and current biological knowledge. Adiponectin is a well-known adipokine with insulin-sensitizing and anti-inflammatory effects, and its levels are low in obesity and metabolic syndrome [30–32]. In our study, adiponectin levels were significantly lower in patients with metabolic syndrome, and this finding also supports the role of adiponectin as a protective metabolic messenger. FSTL1, on the other hand, was significantly higher in individuals with metabolic syndrome. This finding is consistent with a recent study that identified FSTL1 as a marker of unhealthy metabolic conditions [33]. The independent association of FSTL1 with metabolic syndrome has been interpreted as potentially reflecting inflammation and cardiovascular stress associated with metabolic problems. Even though FSTL1 is known to protect the heart in acute conditions and has been associated with CAD severity in other studies [34,35], we did not find a significant difference in FSTL1 levels between our CAD and non-CAD groups. This result may be due to our patient selection (elective, mostly stable CAD) or the proportion of patients with metabolic syndrome but without CAD (group 2) in our study. The relationship between FSTL1 and heart disease may be context-dependent. FSTL1 may increase primarily in response to metabolic problems and inflammation rather than reflecting the degree of atherosclerosis in stable patients. Further researches necessitate to understand the roles of FSTL1 as both a cardiac and metabolic protein and to determine whether altering FSTL1 levels affects metabolic or cardiac health.

One more remarkable molecule in our study was irisin, a myokine. Higher irisin levels were detected in patients with metabolic syndrome. How irisin behaves in metabolic diseases remains controversial. Many previous studies agree with our findings [36–38]. Irisin has been identified to be higher in people with Type 2 diabetes and has been coupled with endothelial activation [39]. These increments are generally judged as a compensatory act. In this mechanism, higher irisin levels may help avert weight gain and insulin resistance by boosting energy consumption and enhancing muscle glucose uptake [40]. Our findings go along with this opinion, suggesting that the coupling between muscle and fat tissue is active in metabolic syndrome. However, some reports disagree with us. They found that irisin levels were either lower or remained steady in obesity and metabolic disorders [41,42]. Dissimilarities across patient groups, such as exercise habits, muscle mass, disease status, and lack of analytical standardization, may be possible causes for this discrepancy [43]. While irisin has been outlined in other works as a predictor of coronary artery disease, MetS, and poor cardiovascular outcomes, it did not turn up as an independent predictor in our set. On the whole, our findings and the diverse results in the literature signify that the function of irisin in metabolic syndrome is intricate. Irisin may rise as an adaptable reply, but its convenience as a standalone biomarker is restricted due to variability. Standardizing irisin measurements and examining various subgroups, such as active and inactive persons, necessitates more effort to reveal when irisin best indicates metabolic risk. Increased irisin levels in the metabolic syndrome group still support the thought that signals from muscle, fat, and heart interact in this situation.

The multimarker approach using adiponectin, FSTL1, and irisin demonstrates high diagnostic performance for metabolic syndrome (AUC = 0.92). High sensitivity and specificity values were found by jointly evaluating interactive signals between different organ systems. A more comprehensive metabolic risk assessment has been achieved through the integration of different metabolic indicators. Despite its strong diagnostic performance, challenges such as assay cost, test accessibility, and lack of standardization that may be encountered during its integration into routine clinical practice cannot be overlooked.

According to the authors' knowledge, this is the first AI-based PSO-ANFIS model for estimation in a clinical dataset, and it has yielded the best result with an R^2 value of 0.84 compared to other methods. The success of our model primarily indicates that metabolic messengers are interconnected. This supports the view that FSTL1 reflects signals from multiple metabolic pathways and responds to overall metabolic stress. Secondly, our success with the AI model illustrates how computational intelligence can complement traditional biostatistics. Whereas classical methods mainly reveal linear relations, the ANFIS framework traps complex, non-linear interactions that might otherwise go unnoticed.

Nevertheless, the present model should be regarded as proof of concept. Even though it predicts FSTL1 accurately, its clinical application remains experimental. In circumstances where direct measurement of FSTL1 is unavailable or costly, the model may provide a useful estimate or assist in tracking responses to therapy or lifestyle interventions. Further validation in larger and independent cohorts will be necessary before any clinical relocation.

From a clinical viewpoint, assessing a broader panel of organokines could improve risk stratification for coronary artery disease (CAD) and metabolic syndrome, helping clinicians identify individuals who may benefit from more intensive management of modifiable risk factors. The strong links observed between FABP4 and FSTL1 and cardiometabolic disease also suggest that these molecules might represent future therapeutic targets. For instance, pharmacologic inhibition of FABP4 is already being explored in metabolic disorders [44,45], and interventions that enhance adiponectin signaling, whether through PPAR-γ agonists [46] or lifestyle changes, are known to produce metabolic improvements. AMP-activated protein kinase (AMPK), which exerts its effects through adiponectin and SGLT-2 inhibitors, is a key mediator linking these organokines to cardiometabolic protection and can be exploited as a therapeutic target [47,48].

Even if specific therapies for FSTL1 or irisin do not yet exist, a better understanding of their functions could open new therapeutic possibilities. Modulating FSTL1 activity may provide beneficial effects on inflammatory or fibrotic pathways in the heart and vasculature. The metabolic benefits of exercise can be mimicked by irisin signaling.

Consistent with studies linking adipokines from the C1q/TNF-related protein (CTRP) family to subclinical atherosclerosis, such as increased carotid intima-media thickness, our findings support the relevance of adipokine-related mechanisms to vascular dysfunction by demonstrating that FABP4 contributes to the association with coronary artery disease [49].

Generally speaking, our findings foster uninterrupted investigation into whether modifying organokine levels might eventually decrease cardiovascular events or improve metabolic effects. Since this study used a cross-sectional design, a causal relationship between biomarkers and clinical outcomes cannot be established. The cohort may differ from those at low- to moderate-risk of CAD because this study included only patients undergoing coronary angiography. Antidiabetic, antihypertensive and lipid-lowering therapies may affect the biomarker levels we measured and a limitation is that they could not be evaluated together with their doses in our study. Considering that the sample size is relatively small, the findings of the AI model should be appraised well and verified in external datasets.

A main restriction of this research is that the cohort was bound to middle-aged and elderly male patients. This scheme preference was made intentionally to reduce variability emerging from sexuality -specific biological and hormonal impacts. It is common knowledge that circulating concentrations of adiponectin, FABP4, irisin, and

FSTL1 alter in consonance with sex hormones, menopausal status, fat distribution, and skeletal muscle mass. Adiponectin levels are normally higher in women than in men [50]. FABP4 concentrations and their correlation with cardiometabolic risk factors may vary between genders. Correspondingly, irisin secretion and its metabolic effects are affected by muscle mass and physical activity, both of which exhibit gender-dependent types. Further research should take into account the limitations of our study, especially the male-only elective angiography-based population, which restricts generalizability. Longitudinal or interventional comprehensive works that cover patients across a broader risk spectrum are required to outcome our results.

CONCLUSION

This research underlines FABP4 and FSTL1 as remarkable biomarkers connected to CAD and metabolic syndrome, respectively, and verifies the value of adiponectin and irisin in metabolic regulation. Synchronous evaluation of these organokines greatly flourishes the diagnostic accuracy of metabolic syndrome and promotes multi-marker approaches in clinical implementation. Besides, AI-based predictive models emphasize the potential of innovative analytical methods in biomarker research and facilitate improved personalized medical strategies for cardiometabolic illnesses.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Clinical and demographic characteristics of the participants

	n	**GROUP 1 (*n*=62)**	**GROUP 2 (*n*=52)**	**GROUP 3 (*n*=65)**	**GROUP 4 (*n*=64)**	***p***
Age	243	56.2 ± 10.6	56.2± 12.2	62.7 ± 9.9	62.3 ± 9.2	0.0001*
BMI (kg/m^2)	243	26.1 (24.4-29.7)	30.2 (27.7 - 34.2)	26.1 (23.5- 30)	28.7 (26.4-30.5)	<0.0001#
WC (cm)	199	96.5 (90-100)	100 (100-110)	98 (90-105)	103.5 (100-110)	<0.0001#
WHtR	199	0.55 (0.52-0.59)	0.60 (0.58-0.64)	0.56 (0.52-0.63)	0.61 (0.58-0.65)	<0.0001#
Smoker: (%)	213	37	27.5	32.2	35	0.788†
DM: n (%)	243	5 (8.1)	29 (55.8)	9 (13.8)	42 (65.6)	<0.0001†

Hypertension: n (%)	243	20 (32.3)	35 (67.3)	25 (38.5)	45 (70.3)	<0.0001[†]
Statin usage: n (%)	243	13 (20.9)	18 (34.6)	28 (43)	46 (71.9)	<0.0001[†]
SYNTAX score	243	0	0	13 (8-21)	15 (9.3-21)	<0.0001[#]
Triglyceride (mg/dL)	242	107 (79.4- 135)	186 (158- 257)	122 (93.4- 145)	178 (128- 256)	<0.0001[#]
T. cholesterol (mg/dL)	243	166 (146- 192)	183 (145- 204)	155 (127- 193)	150 (128- 182)	0.0069[#]
HDL (mg/dL)	243	43.1 (39.8- 49)	37.65 (32.3- 42)	44.2 (40.4- 52.5)	35.9 (31.2- 41.8)	<0.0001[#]
LDL (mg/dL)	243	100 (77.4- 116)	102 (70.7- 124)	77.8 (54.2- 120)	74.6 (56.8- 96.1)	0.001[#]
Glucose	242	88.2 (80.3-	99 (90.6- 127)	93.8 (83.7-	105.7 (93- 144)	<0.0001[#]

(mg/dL)		94.4)		103)		
Insulin (mU/L)	242	11.3 (5.44- 21)	13.7 (7.89- 27.2)	10.9 (4.95- 22.9)	12.6 (6.71- 30.3)	0.2464 #
HOMA-IR	242	2.25 (1.28- 4.63)	3.9 (1.88- 7.48)	2.55 (1.1- 5.25)	4.65 (2.03- 9.75)	0.0083#
HbA1c (%)	224	5.7 (5.4- 6)	6.2 (5.8- 7.3)	5.8 (5.6- 6.1)	6.5 (5.9- 8.3)	<0.0001#
Urea (mg/dL)	242	28.8 (23.5- 32.1)	28.7 (23.9- 35.7)	33.9 (29.4- 40.1)	32.5 (25.9- 38.8)	0.0007#
Creatinine (mg/dL)	242	0.88 (0.82- 1.04)	0.94 (0.82- 0.99)	0.95 (0.83- 1.1)	0.93 (0.83- 1.03)	0.5131#
CRP (mg/L)	242	2.03 (1.07- 4.76)	2.27 (1.18- 6.24)	1.68 (0.8- 5.78)	2.76 (1.27- 5.36)	0.5146#
IL-6 (pg/mL)	243	2.15 (1.5- 7.9)	2.6 (1.5- 5)	2.7 (1.5- 7.6)	3.15 (1.5- 6.95)	0.8965#

hs-cTnT (ng/L)	240	6.09 (4.12-8.83)	6.24 (4.65-11.7)	6.21 (5.06-12.9)	8.06 (5.79-13.5)	0.1079#
Adiponectin (ng/mL)	243	160 (129-191)	51 (39- 77.3)	130 (89.5-163)	52.5 (32-117)	<0.0001#
FABP4 (ng/mL)	243	5.53 (2.19-10.2)	4.69 (1.64-7.62)	2.93 (0.51-7.62)	0.53 (0.1- 4.15)	<0.0001#
FSTL1 (ng/mL)	243	31.1 (18.4-45.7)	131 (97.8- 158)	33 (25.3- 43.8)	108 (64- 139)	<0.0001#
Irisin (ng/mL)	242	3.89 (2.60-5.78)	7.93 (4.59-10.8)	3.25 (1.66-5.51)	4.97 (2.96-8.06)	<0.0001#

SYNTAX score: calculated based on coronary lesions producing ≥50% diameter stenosis in vessels with a diameter ≥1.5 mm. The clinical and demographic characteristics of the participants, along with the biochemical analyses, and ELISA measurement results, grouped according to the study categories, are summarized as mean ± standard deviation, n (%) and median (25-75th percentile). * ANOVA; # Kruskal–Wallis test; † Pearson's chi-squared test. Group 1: CAD– / MetS–; Group 2: CAD– / MetS+; Group 3: CAD+ / MetS–; Group 4: CAD+ / MetS+. While significant differences in age, BMI, and waist circumference were observed among the four groups, no consistent linear trend was noted across all groups; differences were primarily between specific group pairs. Abbreviations: BMI: Body mass index; WC: Waist circumference; WHtR: Waist height ratio; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; HOMA-IR: Homeostasis model assessment index; HbA1c: Hemoglobin A1c; DM: Diabetes mellitus; T. cholesterol: Total cholesterol; CRP: C reactive protein; IL-6: Interleukin 6; hs-cTnT: High-sensitive cardiac troponin T; p: Significance.

Table 2. Multivariable-adjusted relationships of circulating FABP4 with coronary artery disease

Model adjust	**OR**	**%95 CI**	***p***
Age	0.857	0.795- 0.924	0.000064
Age, dyslipidemia	0.88	0.814-0.952	0.001
Age, dyslipidemia, HT	0.89	0.822-0.965	0.005
Age, dyslipidemia, HT, smoking	0.884	0.810-0.966	0.006
Age, dyslipidemia, HT, smoking, glucose	0.903	0.825-0.987	0.025

CAD classified by SYNTAX score (≥50% stenosis in ≥1.5 mm vessels). Dyslipidemia is defined as total cholesterol ≥200 mg/dL, LDL-C ≥130 mg/dL, triglycerides ≥150 mg/dL, HDL-C <40 mg/dL in men, and/or use of lipid-lowering therapy—Hosmer-Lemeshow test *p*=0.791; Nagelkerke R^2=0.361; correct classification rate for CAD status: 76.9%. FABP4 was modeled as a continuous predictor, and an OR less than one indicates that higher values of the predictor are associated with a lower probability of coronary artery disease. Multivariable-adjusted logistic regression analysis showing the independent association of FABP4 levels with the presence of coronary artery disease after sequential adjustment for conventional risk factors. Abbreviations: OR: Odds ratio; CI: Confidence interval; HT: Hypertension; p: significance.

Table 3. Multivariable-adjusted relationships of circulating FSTL-1 and adiponectin with metabolic syndrome

	Adiponectin			**FSTL1**		
Model adjust	OR	%95 CI	*p*	OR	%95 CI	*p*
Age	0.975	0.969-0.981	<0.001	1.046	1.035-1.057	<0.001
Age, WC	0.978	0.971-0.984	<0.001	1.045	1.032-1.057	<0.001
Age, WC, HT	0.976	0.968-0.983	<0.001	1.045	1.032-1.058	<0.001
Age, WC, HT, glucose	0.976	0.969-0.984	<0.001	1.044	1.030-1.057	<0.001
Age, WC, HT, glucose, triglyceride	0.978	0.970-0.987	<0.001	1.041	1.027-1.055	<0.001
Age, WC, HT, glucose, triglyceride, HDL	0.979	0.971-0.988	0.000003	1.039	1.025-1.054	<0.001

Odds ratios and 95% confidence intervals were calculated using logistic regression models with progressive adjustment for potential confounders including age, waist circumference, hypertension, glucose, triglyceride, and HDL levels. The model containing adiponectin achieved a Nagelkerke R^2 value of 0.722 and correctly classified 87.4% of patients. The model containing FSTL1 obtained a Nagelkerke R^2 value of 0.778 with a correct classification rate of 91.5%. Both adiponectin and FSTL1 remained significantly associated with metabolic syndrome after full adjustment. Abbreviations: HDL: High-density lipoprotein; WC: Waist circumference; HT: Hypertension; p: Significance; OR: Odds ratios; CI: Confidence interval.

Table 4. Comparison of machine learning model performance metrics in predicting FSTL1 results

	Train Set			**Test Set**			
Model	**RMSE**	**MAE**	**R**	**RMSE**	**MAE**	**R**	**R^2**
Linear regression	32.4	26.5	0.73	31.1±4.23	25.4±4.18	0.64±0.27	0.45±0.32
Random forest	32.5	25.1	0.73	31.4±7.79	25.0±6.04	0.67±0.14	0.47±0.20
k-Nearest neighbors	36.1	27.4	0.65	35.4±8.27	27.7±6.46	0.55±0.23	0.34±0.25
Decision tree	39.2	29.5	0.57	38.1±10	29.2±7.22	0.42±0.33	0.2±0.41
Support vector machine	46.4	37.4	0.22	44.2±9.73	36.2±7.8	0.25±0.2	0.02±0.21
PSO-ANFIS	26	20.2	0.82	16.5±6.34	12.8±4.88	0.91±0.06	0.84±0.1

The test set performance of the models used in FSTL1 level prediction was evaluated using 10-fold cross-validation, and the results are reported as the mean ± standard deviation. Abbreviations: PSO-ANFIS: Particle swarm optimization-enhanced adaptive neuro-fuzzy inference system; RMSE: Root mean square error; MAE: Mean absolute error; R: Correlation coefficient; R^2: Coefficient of determination.

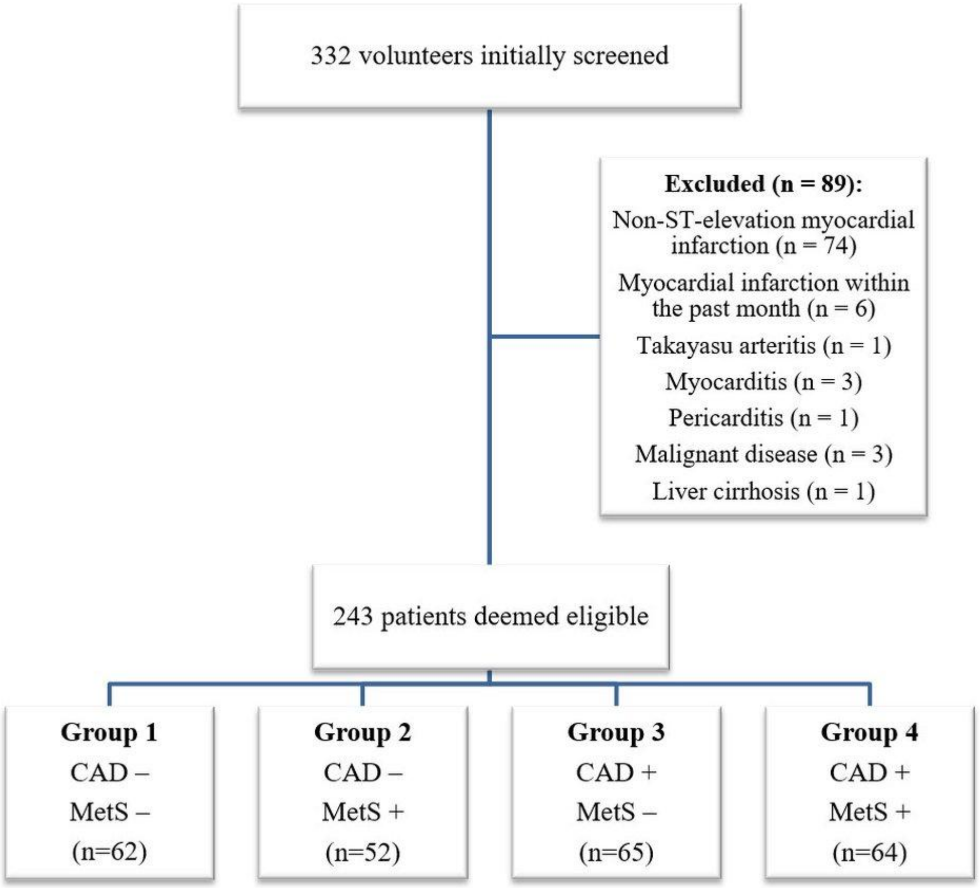


Figure 1. Classification of patients into study groups.

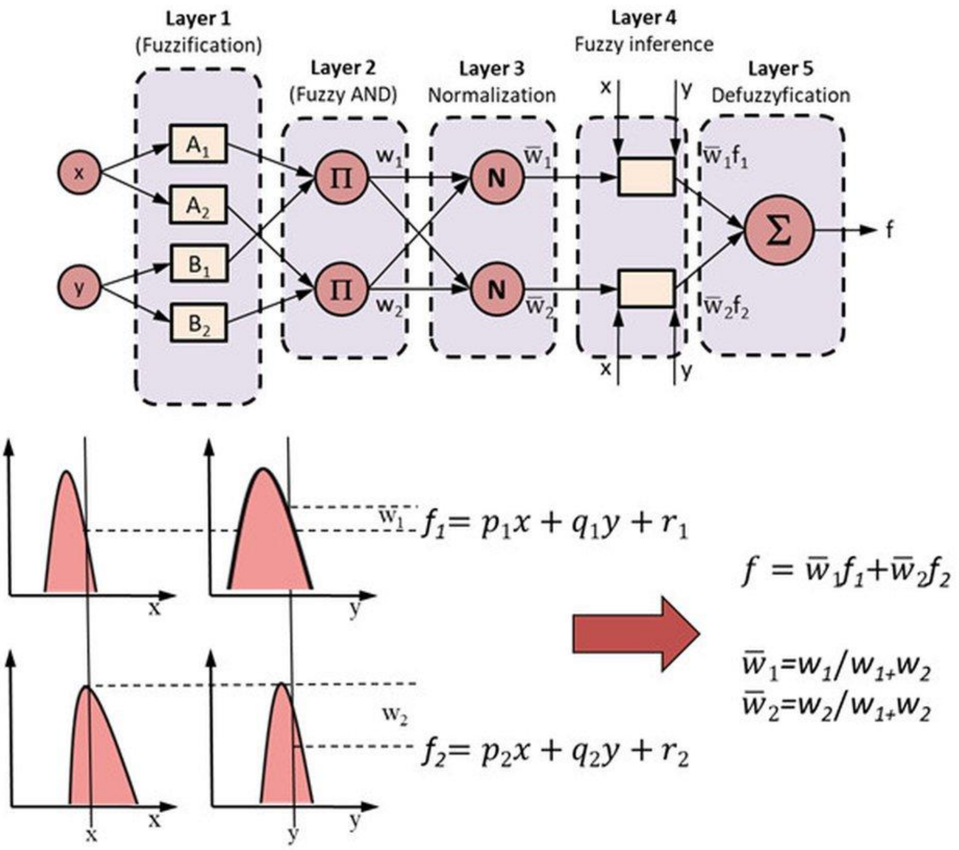


Figure 2. Background of Adaptive neuro-fuzzy inference system. The operational structure and mathematical background of the Adaptive neuro-fuzzy inference system are illustrated.

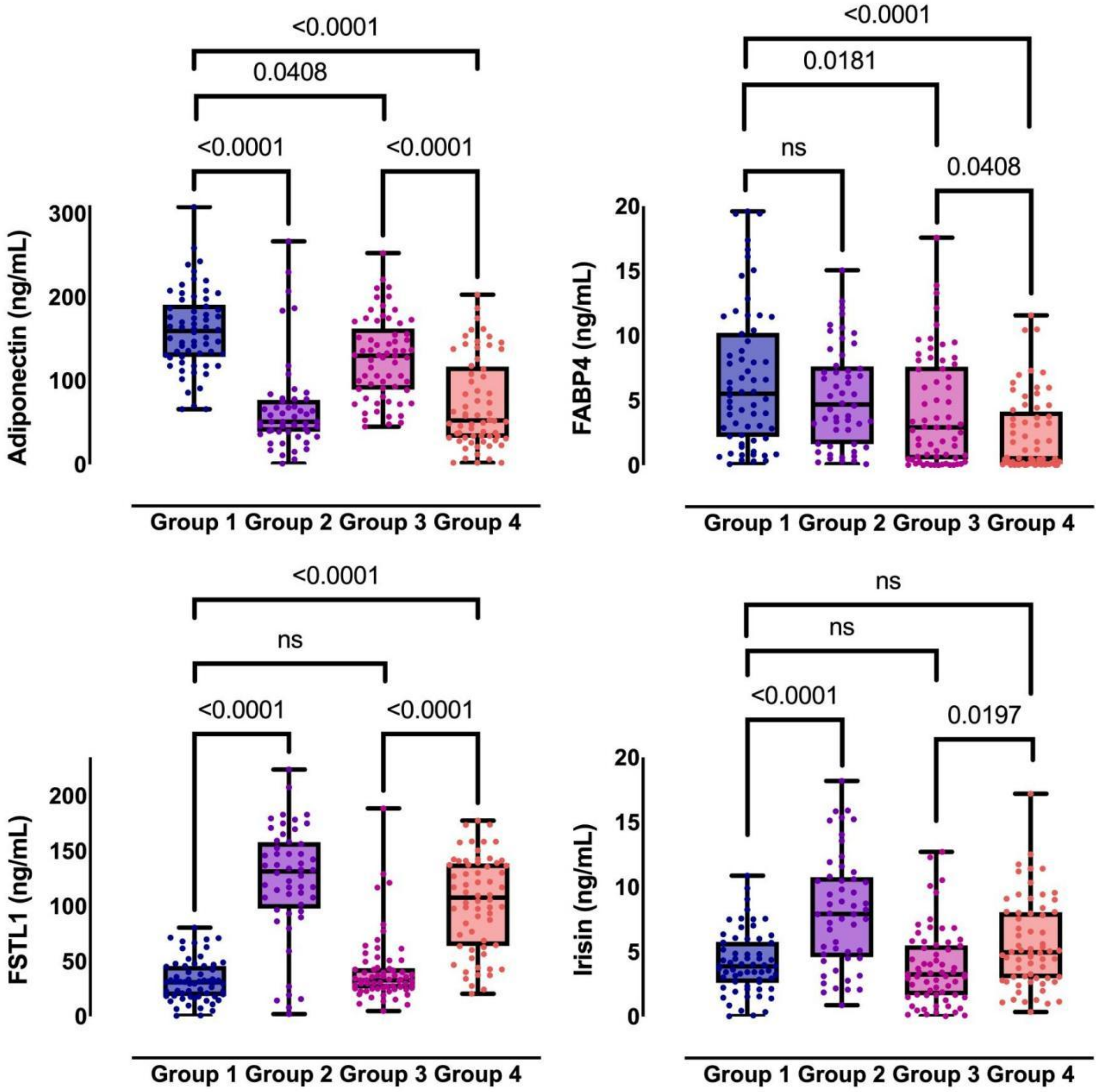
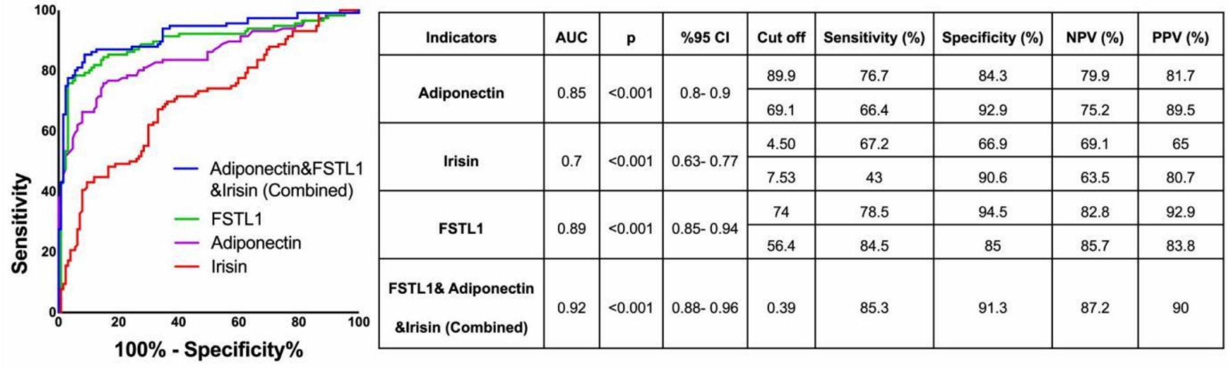


Figure 3. Adiponectin, FABP4, FSTL1, and irisin levels of patients across study groups according to the presence of MetS and CAD. Group 1 (*n*=62): MetS-negative / CAD-negative; Group 2 (*n*=52): MetS-positive only; Group 3 (*n*=65): CAD-positive only; Group 4 (*n*=64): MetS-positive / CAD-positive. Data are presented as median with IQR, as detailed in Table 1. Pairwise significance markers represent multiplicity-adjusted post-hoc tests (Dunn with Bonferroni). P-values were adjusted for multiple comparisons across the four biomarkers using the FDR method. Statistical significance is indicated as ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$. Abbreviations: FABP4: Fatty acid-binding protein 4; FSTL1: Follistatin-like 1; MetS: Metabolic syndrome; CAD: Coronary artery disease; n: Sample size; IQR: Interquartile range; FDR: False discovery rate; ns: Not significant; p: Significance.



Indicators	AUC	p	%95 CI	Cut off	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Adiponectin	0.85	<0.001	0.8- 0.9	89.9	76.7	84.3	79.9	81.7
				69.1	66.4	92.9	75.2	89.5
Irisin	0.7	<0.001	0.63- 0.77	4.50	67.2	66.9	69.1	65
				7.53	43	90.6	63.5	80.7
FSTL1	0.89	<0.001	0.85- 0.94	74	78.5	94.5	82.8	92.9
				56.4	84.5	85	85.7	83.8
FSTL1& Adiponectin &Irisin (Combined)	0.92	<0.001	0.88- 0.96	0.39	85.3	91.3	87.2	90

Figure 4. ROC curves comparing the discriminatory performance of adiponectin, FSTL1, irisin and combined biomarker score in identifying patients with MetS. Mean AUC values and their 95% confidence intervals estimated with 200 bootstrap samples were calculated. DeLong test comparing ROC curves of FSTL1 and the combined biomarker: Z = −2.019, *p* = 0.044. Additionally, optimal cut-off points determined using the Youden index and corresponding sensitivity and specificity values are presented. Abbreviations: AUC: Area under the curve; p: Significance; CI: Confidence interval; NPV: Negative predictive value; PPV: Positive predictive value.

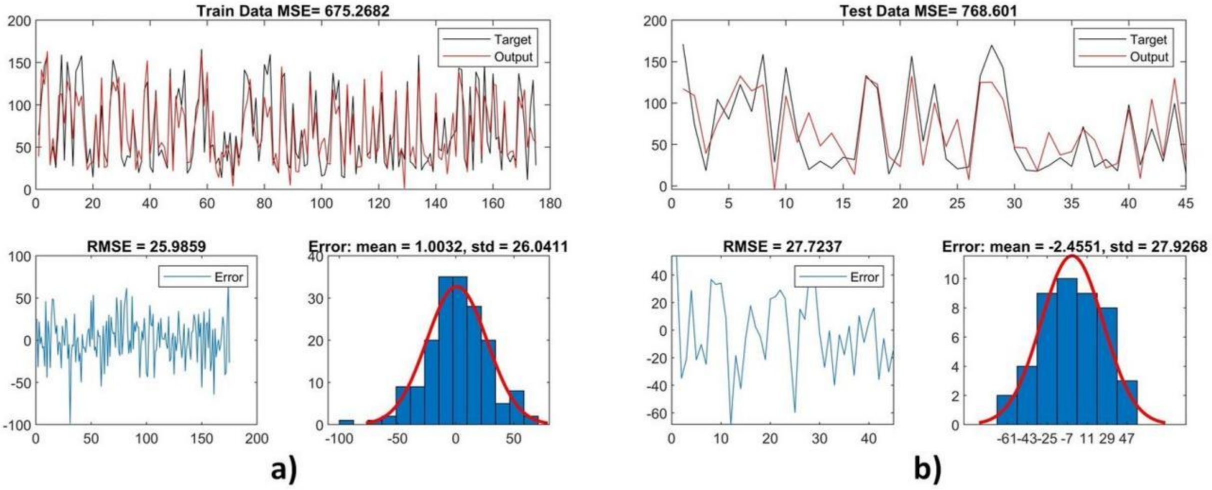


Figure 5. FSTL1 prediction using the particle swarm optimization-based adaptive neuro-fuzzy inference system method. (A) Train result; (B) Test result. The model performance for PSO-ANFIS using a single 80–20 train–test split is presented. Abbreviations: RMSE: Root mean squared error; MSE: Mean squared error.

SUPPLEMENTAL DATA

Supplemental data are available at the following link:

https://www.bjbms.org/ojs/index.php/bjbms/article/view/13188/4036