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Original Article

Implications of the Serum Concentrations of Neuregulin-4 (Nrg4) in Patients with Coronary Artery Disease: A Case-Control Study

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Abstract

Background: *Neuregulin-4 (Nrg4), a novel brown fat-enriched factor, has been reported to play a crucial role in developing metabolic disorders. The current case-control study aimed to investigate the association between serum Nrg4 and coronary artery disease (CAD).*

Methods: *This study enrolled 43 patients with CAD and 43 subjects with normal coronary arteries diagnosed by coronary angiography. Anthropometric and biochemical parameters were measured and recorded. The serum Nrg4 level was determined using the enzyme-linked immunosorbent assay. The relationships between circulating Nrg4 and CAD and other clinical parameters were analyzed. A receiver operating characteristic analysis was applied to assess the utility of Nrg4 in identifying CAD.*

Results: *The study population comprised 86 patients, including 64 men (74.4%), at a mean age of 57.83±6.01 years. Patients with CAD had significantly lower serum Nrg4 than the control group (P<0.001). The serum Nrg4 level was negatively correlated with anthropometric variables, including the body mass index, waist circumference, and the waist-to-hip ratio, fasting blood glucose, and the triglyceride-glucose index (P<0.05). In multivariable-adjusted regression analysis, the odds of CAD decreased by 46% per 1 SD elevation in the serum Nrg4 level (OR, 0.54; 95% CI, 0.40 to 0.73; P<0.001) after controlling for potential confounders. Nrg4 showed a significantly high area under the curve value (AUC, 0.85; 95% CI, 0.75 to 0.94) with 81.4% sensitivity and 95.3% specificity to identify CAD.*

Conclusion: *Generally, the serum level of Nrg4 declines in patients with CAD, which might be an independent risk factor for CAD.*

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Introduction

Despite the enormous improvements in diagnosing and treating coronary artery disease (CAD), it is the most prominent cause of mortality and loss of disability-adjusted life years worldwide.[1] Besides numerous well-established risk factors for CAD, including hypertension, hyperlipidemia, and diabetes, growing evidence has highlighted the role of excess adiposity and adipose tissue dysfunction as risk factors for CAD development.[2-4]

Adipose tissue, both white and brown, is a highly dynamic endocrine organ with a central function in regulating energy metabolism.[5,6] Adipokines, bioactive factors secreted by adipose tissue, are arising as predictors of the functional status of adipose tissue since they mediate the metabolic cross-talk between adipose tissue and the other organs.[7,8] Altered concentrations of circulating adipokines, such as leptin, adiponectin, resistin, and irisin, have been suggested to be associated with CAD.[3,9]

Among adipokines is neuregulin-4 (Nrg4), a member of the epidermal growth factor (EGF) family of extracellular ligands that plays a significant role in modulating energy metabolism and the pathogenesis of metabolic disorders.[10] Although Nrg4 was first identified in the pancreas,[11] evidence has shown that its highest level is expressed in brown adipose tissue (BAT).[12] Nrg4 expression is upregulated during brown adipocyte differentiation and browning of white adipose tissue (WAT).[13] Nrg4 functions in an autocrine, paracrine, or endocrine manner after the proteolytic cleavage of the EGF-like domain.[11]

Animal models and human studies have indicated decreased Nrg4 expression in obesity.[14] Human cross-sectional and case-control studies have reported lower serum concentrations of Nrg4 in patients with nonalcoholic fatty liver disease (NAFLD),[15,16] metabolic syndrome,[17] and type 2 diabetes mellitus,[18] indicating the potential protective role of Nrg4 in the development of obesity-associated metabolic disturbances. The cardioprotective actions of Nrg4 and its signaling pathway have been reported in experimental research, including the inhibited proliferation of vascular smooth muscle cells and endothelial cell apoptosis, reducing the progression of atherosclerosis.[19-21] A negative association between serum Nrg4 levels and carotid intima-media thickness and carotid plaques has also been observed in individuals with obesity.[22]

According to preliminary evidence, Nrg4 might be a potential risk factor for CAD. However, little clinical information is available regarding Nrg4 in patients with CAD. Here, we aimed to explore the association between serum Nrg4 levels and CAD by conducting a case-control study.

Methods

Patients aged 50 to 65 years with a body mass index (BMI) of 18.5 to 35 kg/m^2 admitted to Tehran Heart Center were screened. Forty-three patients with stable CAD (having 1 or 2 blocked arteries) and 43 with normal coronary arteries based on diagnosis by coronary angiography were enrolled in the study. Current smokers and patients with previous coronary artery bypass grafting, a history of myocardial infarction, stroke, or percutaneous coronary intervention during the preceding 3 months were excluded from the study. Furthermore, patients with diabetes, severe renal dysfunction, autoimmune diseases, familial hypercholesterolemia/hypertriglyceridemia, chronic inflammatory diseases, thyroid problems, or cancer were also excluded.

Before data collection, the purpose and design of the study were explained to all the participants, and written informed consent was obtained from them. The current study was in accordance with the declaration of Helsinki and received approval from the Research Ethics Committee of Tehran University of Medical Sciences and the National Institute for Medical Research Development before the study initiation.

Data regarding the age, sex, weight, height, waist, and hip circumferences of the subjects were collected and recorded. BMI was calculated as the weight (kg) divided by the square of the height (m^2). The waist-to-hip ratio was determined by dividing waist circumference (cm) by hip circumference (cm). Blood pressure was measured using an aneroid sphygmomanometer and stethoscope with the participant in a comfortable sitting position.

After an overnight fast, a blood sample was collected from each subject and stored at −80 °C until the experiments. According to the manufacturer's protocol, the serum Nrg4 concentration was measured using an enzyme-linked immunosorbent assay commercial kit (Shanghai Crystal Day Biotech Co, Ltd, China).

Blood biochemical analyses, including fasting blood glucose, triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein, were performed with a BT1500 autoanalyzer (Biotecnica Instruments, Italy) using relevant commercial kits (Pars Azmoon Co, Tehran, Iran).

The triglyceride glucose index was estimated with the following formulas: ln [fasting triglyceride (mg/dL) × fasting blood glucose (mg/dL)/2].

All the statistical analyses were conducted using IBM SPSS statistics version 24.0 (SPSS, Inc, Chicago, IL, USA). First, the normal distribution of quantitative variables was examined using the Shapiro-Wilk test. Variables with normal distributions were presented as the mean (standard deviation), and variables with skewed distributions were presented as the median (the interquartile range). Categorical variables are expressed as numbers (percentages). Differences between the 2 groups were

assessed with the independent samples *t* or Mann-Whitney *U* test. The χ^2 test was used to compare categorical variables. The subjects were classified into tertiles according to serum Nrg4 levels. One-way analysis of variance (ANOVA) or the Kruskal-Wallis test was utilized to test differences in normally and non-normally distributed variables between different tertiles of serum Nrg4 levels, respectively. Bivariate correlations between serum Nrg4 levels and other variables were performed with the Pearson correlation coefficient. Receiver operating characteristic (ROC) curve analyses and areas under the curve (AUC) were applied to evaluate the utility of the serum Nrg4 level and its cutoff values in determining the presence of CAD. Univariate and multivariable logistic regression analyses were conducted to assess the raw and adjusted association between Nrg4 and CAD. Two-tailed values of a *P* value of less than 0.05 were considered statistically significant.

Results

The demographic and clinical characteristics of the study population (n=86) are shown in Table 1. In total, 64 males and 22 females were included. The mean age of the subjects was 57.44±6.36 years in the CAD group and 58.21±5.68 years in the non-CAD group (P>0.05). The distribution of sex was significantly different between the 2 groups (P=0.012). BMI, waist circumference, the waist-to-hip ratio, fasting blood glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, and creatinine showed no statistical differences between the groups. The serum Nrg4 level was significantly lower in the CAD group than in the non-CAD group (1.63 [0.30, 2.97] vs 4.13 [3.57, 5.10] ng/mL (median [interquartile range]); P<0.001).

Table 2 presents the clinical characteristics of the study population across the tertiles of serum Nrg4 concentrations. No significant differences existed concerning age, sex, anthropometric indices (BMI, waist circumference, and the waist-to-hip ratio), fasting blood glucose, lipid profile, the triglyceride glucose index, systolic blood pressure, diastolic blood pressure, and creatinine among tertiles of serum Nrg4 levels. Nonetheless, there was a significant difference in the number of patients with CAD across the tertiles of serum Nrg4 concentrations (P<0.001).

Figure 1 illustrates the results of the AUC calculations for detecting the presence of CAD according to serum Nrg4 levels. Based on the ROC curve analysis, the serum Nrg4 level showed a significantly high AUC value (AUC, 0.85; 95% CI, 0.75 to 0.94) with 81.4% sensitivity and 95.3% specificity for predicting the presence of CAD. The cutoffs of serum Nrg4 levels were 3.13 ng/mL for detecting CAD, based on the Youden J index.

The crude and multivariable-adjusted odds ratios (ORs) for the association between the serum Nrg4 level and CAD are displayed in Table 3. In model 1, the crude model without any adjustments for confounding variables, the odds of CAD declined by 40% per 1 SD increase in the serum Nrg4 level (OR, 0.60; 95% CI, 0.46 to 0.80; P<0.001). After

Table 1. Clinical and biochemical characteristics of the study participants with and without CAD

Variables	With CAD (n=43)	Without CAD (n=43)	*P*
Age (y)*	57.44±6.36	58.21±5.68	0.557
Male/Female (%)	37 (86.0) / 6 (14.0)	27 (62.8) / 16 (37.2)	0.012
BMI (kg/m^2)	28.77±3.44	28.09±3.91	0.389
WC (cm)*	98.69±9.44	98.09±9.01	0.764
WHR*	0.96±0.06	0.94±0.05	0.178
FBG (mg/dL)#	97.00 (91.00, 106.00)	99.00 (89.00, 102.00)	0.819
TG (mg/dL)#	132.00 (98.00, 175.00)	119.00 (84.00, 161.00)	0.280
TC (mg/dL)*	156.95±39.44	155.29±39.44	0.846
LDL-c (mg/dL)*	93.81±32.70	92.71±29.34	0.870
HDL-c (mg/dL)*	40.74±9.07	39.22±8.10	0.413
TyG (index)*	8.82±0.49	8.65±0.48	0.120
Nrg4 (ng/mL)#	1.63 (0.30, 2.97)	4.13 (3.57, 5.10)	< 0.001
SBP (mmHg)#	120.00 (120.00, 130.00)	120.00 (110.00, 130.00)	0.164
DBP (mmHg)#	75.00 (70.00, 80.00)	70.00 (60.00, 80.00)	0.147
Cr (mg/dL)*	1.08±0.17	1.03±0.16	0.199

*Data are presented as the mean (the standard deviation). The independent samples t test was used for the comparisons.
#Data are presented as the median (the interquartile range). The Mann-Whitney U test was used for the comparisons.
CAD, Coronary artery disease; BMI, Body mass index; WC, Waist circumference; WHR, Waist-to-hip ratio; FBG, Fasting blood glucose; TC, Total cholesterol; TG, Triglyceride; LDL-c, Low-density lipoprotein cholesterol; HDL-c, High-density lipoprotein cholesterol; TyG, Triglyceride-glucose; Nrg4, Neuregulin-4; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; Cr, Creatinine

Table 2. Characteristics of the study participants across the tertile of serum Nrg4 concentrations

	Tertile 1	Tertile 2	Tertile 3	*P*
Serum Nrg4 level (ng/mL)	≤1.92	1.93-4.01	≥4.02	
Number of subjects (%)	29 (33.7)	29 (33.7)	28 (32.6)	
CAD/non-CAD (%)	28 (96.5) / 1 (3.5)	9 (31.0) / 20 (69.0)	6 (21.4) / 22 (78.6)	< 0.001
Male/female (%)	23 (79.3) / 6 (20.7)	20 (69.0) / 9 (31.0)	21 (75.0) / 7 (25.0)	0.677
Age (y)*	57.21±6.25	59.86±4.98	56.36±6.35	0.069
BMI (Kg/m^2)*	29.13±3.83	27.32±3.32	28.85±3.72	0.129
WC (cm)*	99.74±10.10	97.47±7.10	97.95±10.21	0.616
WHR*	0.96±0.06	0.95±0.05	0.94±0.06	0.159
FBG (mg/dL)#	100.00 (92.00, 109.00)	96.00 (89.00, 102.00)	98.50 (87.50, 101.00)	0.113
TG (mg/dL)#	141.00 (98.00, 175.00)	116.00 (104.00, 150.00)	127.00 (85.50, 190.50)	0.533
TC (mg/dL)*	158.10±41.11	154.14±36.66	156.15±41.13	0.930
LDL-c (mg/dL)*	93.59±34.11	90.38±24.64	95.92±33.91	0.797
HDL-c (mg/dL)*	41.62±8.46	40.62±7.71	37.62±9.32	0.190
TyG index*	8.86±0.53	8.67±0.39	8.67±0.53	0.231
Cr (mg/dL)*	1.06±0.18	1.03±0.18	1.09±0.15	0.463
SBP (mmHg)#	120.00 (120.00, 130.00)	120.00 (110.00, 130.00)	120.00 (110.00, 130.00)	0.296
DBP (mmHg)#	78.00 (74.00, 80.00)	70.00 (60.00, 82.00)	70.00 (65.00, 80.00)	0.139

*Data are presented as the mean (the standard deviation). One-way ANOVA was used for the comparisons.
#Data are presented as the median (the interquartile range). The Kruskal-Wallis test was used for the comparisons.
CAD, Coronary artery disease; BMI, Body mass index; WC, Waist circumference; WHR, Waist-to-hip ratio; FBG, Fasting blood glucose; TC, Total cholesterol; TG, Triglyceride; LDL-c, Low-density lipoprotein cholesterol; HDL-c, High-density lipoprotein cholesterol; TyG, Triglyceride-glucose; Nrg4, Neuregulin-4; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; Cr, Creatinine

adjustments for potential confounders, the ORs for CAD remained statistically significant (OR, 0.54; 95% CI, 0.40 to 0.73; P<0.001).

The correlations between the circulating Nrg4 concentration and anthropometric and metabolic variables are shown in Table 4. There were significant inverse correlations between the serum Nrg4 level and BMI (r= −0.21, P=0.048), waist circumference (r=−0.25, P=0.018), the waist-to-hip ratio (r= −0.24, P=0.027), fasting blood glucose (r= −0.24, P=0.025), and the triglyceride-glucose index (r= −0.21, P=0.048).

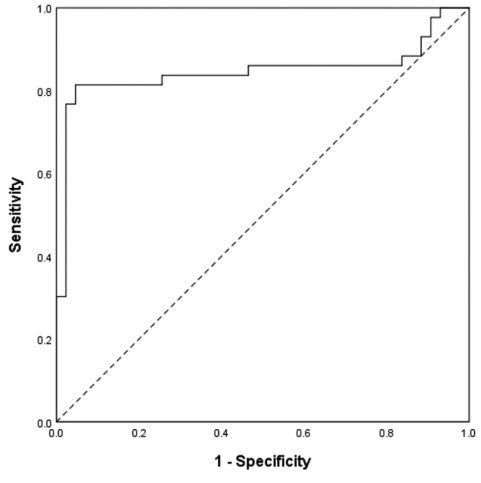

Figure 1. The image illustrates the receiver operating characteristic (ROC) curve for the presence of CAD according to serum neuregulin-4 levels.

Table 3. Odds ratios for CAD according to serum Nrg4 levels

Model	OR	95% CI		P
		Lower	Upper	
Model 1	0.60	0.46	0.80	< 0.001
Model 2	0.58	0.44	0.77	< 0.001
Model 3	0.54	0.40	0.73	< 0.001

OR, Odds ratio; CI, Confidence interval
Model 1, Crude model;
Model 2, Adjusted for age and sex;
Model 3, Adjusted for age, sex, body mass index, waist circumference, waist-to-hip ratio, total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and fasting blood glucose.

Table 4. Correlations between the serum Nrg4 level and anthropometric and metabolic variables

Variable	Correlation coefficient	*P*
Age (y)	-0.16	0.130
BMI (Kg/m^2)	-0.21	0.048
WC (cm)	-0.25	0.018
WHR	-0.24	0.027
FBG (mg/dL)	-0.24	0.025
TG (mg/dL)	-0.08	0.447
TC (mg/dL)	-0.002	0.984
LDL-c (mg/dL)	0.01	0.907
HDL-c (mg/dL)	-0.12	0.251
TyG index	-0.21	0.048

BMI, Body mass index; WC, Waist circumference; WHR, Waist-to-hip ratio; FBG, Fasting blood glucose; TC, Total cholesterol; TG, Triglyceride; LDL-c, Low-density lipoprotein cholesterol; HDL-c, High-density lipoprotein cholesterol; TyG, Triglyceride-glucose

Discussion

In the current study, we found that the serum level of Nrg4 declined significantly in patients with CAD compared with the control group. The number of CAD patients was significantly decreased across the tertiles of serum Nrg4 levels. The level of Nrg4 in the serum was inversely correlated with obesity indicators, including BMI, waist circumference, and the waist-to-hip ratio, and fasting glucose and the triglyceride-glucose index. Our findings demonstrated that a higher level of circulating Nrg4 could decrease the odds of CAD by 40% per 1 SD increase in the serum Nrg4 level. In addition, the circulating Nrg4 level had 81.4% sensitivity and 95.3% specificity for identifying CAD. These results indicate that circulating Nrg4 might play a protective role against CAD and its capability as a marker for predicting the presence of CAD.

In line with our results, a clinical study by Tian et al[23] on patients with CAD showed that those with a higher SYNTAX score (as a marker of CAD extension) had lower serum Nrg4. Our findings are also in agreement with an investigation by Rahimzadeh et al,[24] who found a negative association between the serum level of Nrg4 and the risk of acute coronary syndromes independent of traditional cardiovascular risk factors. Furthermore, the serum Nrg4 level was inversely associated with the number of vessels diagnosed with stenosis in that study.

BAT is a unique type of adipose tissue that contributes to systemic metabolic homeostasis by producing heat through the activation of uncoupled mitochondrial respiration in response to cold exposure.[25] BAT is metabolically active in adult humans, although its activity is disrupted in obesity.[26,27] Since activating BAT increases whole-body energy expenditure and results in reduced adiposity, targeting BAT activity and the browning of WAT might be a potential approach in the combat against obesity and the associated comorbidities such as diabetes and cardiovascular disease.[27,28] Recent evidence suggests that BAT might contribute to regulating systemic metabolism independent of thermogenic properties through the secretion of endocrine factors.[14,29] Nrg4 is among the first regulatory molecules identified to be secreted by BAT and white adipocytes upon cold exposure, and its expression has been associated with BAT activity and the browning of WAT.[13,30] It also has cardioprotective properties and beneficial effects on metabolic risk factors.[14,31]

The underlying mechanisms regarding the role of Nrg4 in the pathogenesis of CAD are not yet fully understood. Evidence suggests that the protective effects of Nrg4 might occur directly or indirectly in the cardiovascular system. The Nrg4-associated downstream signaling pathway, the ErbB4, is a key transcriptional regulator of cardiomyocyte proliferation and plays an important role in the repair of heart injury.[32-34] Clement et al[19] demonstrated that ErbB4 was upregulated in the rat's endothelium after balloon injury to the carotid artery. In the same study, activating ErbB4 attenuated neointima formation following vascular injury and inhibited vascular smooth muscle cell proliferation. This signaling pathway could also prevent atherosclerosis progression through the attenuation of endothelial cell apoptosis.[20,21]

The indirect mechanisms proposed for the Nrg4 functions affecting the cardiovascular system include its effects on adiposity, lipoprotein metabolism, insulin resistance, and inflammation.[14] An animal study by Wang et al[35] indicated that Nrg4 KO mice gained more weight and increased adiposity following high-fat diet feeding. A negative association between Nrg4 levels and obesity indicators, including BMI, waist circumference, and the waist-to-hip ratio, has also been reported in human cross-sectional studies,[17,36] similar to our results.

Nrg4 is a critical factor in regulating BAT activity. Increased BAT activity highly contributes to correcting metabolic disorders by utilizing glucose and fatty acids for thermogenesis.[14] It also improves insulin sensitivity by increasing peripheral glucose metabolism.[37] Recent experimental studies indicated that Nrg4 null mice experienced glucose intolerance and insulin resistance following diet-induced obesity, and the overexpression of Nrg4 by gene transfer protected the animals against these events.[35,38] Clinical studies have also revealed lower circulating Nrg4 levels in patients with impaired glucose tolerance.[18,39,40] Further, Cai et al[17] reported that fasting blood glucose was significantly decreased across quartiles of serum Nrg4 in obese adults. We also observed a negative correlation between the Nrg4 concentration and fasting blood glucose. A case-control study on patients with NAFLD reported that the serum Nrg4 level was inversely correlated with the triglyceride-glucose index,[16] a product of blood glucose and triglyceride associated with cardiovascular disease, which is entirely consistent with our findings.

Inflammation is another indirect mechanism influenced by Nrg4, which is not assessed here. Some studies have suggested a protective role for Nrg4 in various inflammatory diseases, such as NAFLD,[41] inflammatory bowel disease,[42] and type 2 diabetes mellitus.[39,43] Furthermore, the anti-inflammatory effects of Nrg4 have been observed in an animal model of osteoarthritis and colitis.[44,45] Since inflammation is pivotal to coronary atherosclerotic plaque development and destabilization,[46] the assessment of inflammatory markers and their relationships with Nrg4 in patients with CAD could help the discovery of involved pathways and their prevention or management.

The obtained cutoff of the serum Nrg4 level for detecting CAD in our study was higher than the cutoff reported for detecting increased carotid intima-media thickness (0.71 ng/mL) and the presence of carotid plaques (0.69 ng/mL) in a population of obese Chinese adults.[22] Still, it was lower than the cutoff reported to predict coronary heart disease in

patients with type 2 diabetes mellitus (11.175 ng/mL).[47] The Nrg4 level cutoff for diagnosing NAFLD in children and adolescents with obesity was 3.39 ng/mL,[36] approximately near our cutoff. The reported cutoffs of Nrg4 levels are somewhat different, but one finding is consistent in all studies: the decreased levels of Nrg4 in pathologic conditions. Due to the high heterogeneity of the studies conducted in terms of different populations and baseline characteristics of the individuals, further studies are needed to achieve a general cutoff of the serum Nrg4 level to predict diseases.

Overall, according to our results and the above data regarding the role of Nrg4 in cardiovascular health and metabolic homeostasis, this novel adipokine seems to exert protective effects against CAD. As a BAT representative with the ability to regulate BAT activity and modify cardiovascular disease risk factors, Nrg4 should be investigated in the cross-talk between CAD and BAT in future research.

The results of the present study should be interpreted in light of some of its limitations. Our investigations were based on a single-center analysis. Furthermore, given the case-control design of this study, the causality effect could not be established. Therefore, further longitudinal and population-based studies are required to explore the potential causal relationship between Nrg4 and CAD.

Conclusion

In summary, the results of the current study demonstrated that the circulating Nrg4 level was decreased in CAD patients, and it might be a useful biomarker for predicting CAD. These findings underline the significance of Nrg4 as a potential preventive and therapeutic target for CAD, which needs to be assessed in future research.

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