Research Brief

Association between serum netrin-1, netrin-4 and risk of the acute coronary syndrome in patients with type 2 diabetes mellitus-A pilot study

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ABSTRACT

The present study was done to assess the diagnostic utility of serum netrin-1 and netrin-4 for recognising the acute coronary syndrome (ACS) in type 2 diabetes mellitus (T2DM) patients. Forty-two T2DM patients with ACS (Cases) and forty-two T2DM patients without ACS (Controls) were compared. Cases had lower serum netrin-1 and netrin-4 levels than controls and were negatively associated with creatinine kinase-total, creatinine kinase-MB, troponin-T and H-FABP. ROC analysis showed that netrin-1 and netrin-4 had good sensitivity and specificity for ACS prediction in T2DM patients. Serum netrin-1 and netrin-4 levels might be considered complementary markers for ACS diagnosis in T2DM patients.

1. Introduction

Diabetes mellitus and coronary artery disease share various risk factors like obesity, dyslipidaemia, stress, a sedentary lifestyle, etc. The incidence of the acute coronary syndrome (ACS) is two to three times higher in type 2 diabetes mellitus (T2DM) patients than in the general population.[1] Changes in the extracellular matrix have been postulated in the pathogenesis and complications of atherosclerosis.[2] Netrin-1, an extracellular and laminin-related protein, has a role in atherosclerosis.[3]

Netrin-1 causes inhibition of macrophage migration from the artery, thus promoting atherosclerosis.[4] Also, it increases nitric oxide (NO) synthesis and reduces NADPH oxidase-4 expression, thus improving mitochondrial function and finally reducing infarct size.[5] Additionally, it reduces autophagy in a coronary ligation model of myocardial infarction (MI) after supplementation.[6] Netrin-1 is also responsible for atherosclerosis' complications as its levels were reduced in patients with coronary artery

calcification compared to patients without calcification.[7] Also, its expression was downregulated in atherosclerotic plaques (carotid, femoral, and aortic plaques).[8] Because of the controversial effects and lack of information about their utility in ACS diagnosis, the study aimed to assess the ACS diagnostic utility of serum netrin-1 and netrin-4 levels in T2DM patients by determining their association with markers of myocardial infarction, i.e. serum creatinine kinase-MB (CK-MB), troponin-T, and heart-type fatty acid-binding protein (H-FABP).

2. Materials and methods

2.1. Study population

It is a retrospective and case−control study involving 42 T2DM patients (>18 yrs old) with ACS, including Unstable Angina (UA), non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI), presented in the emergency ward as CASES and 42 age, gender, and duration of diabetes matched T2DM patients without ACS as CONTROLS. Approvals of the Institute Research Council and Institute Human Ethics Committee were obtained.

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2.2. Analysis of Anthropometry and biochemical investigations

Personal, past and medical histories of all participants were recorded. Anthropometry [body surface area, waist circumference, body mass index (BMI)] and seated blood pressure were measured for all participants. Under the strict aseptic condition, five millilitre of blood was collected from cases within 24–30 h of ACS occurrence and from controls at their OPD visit. The collected blood was analysed for routine biochemistry investigations [glucose, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine kinase-total (CK-Total), CK-MB, blood urea nitrogen (BUN) and creatinine] by AU480 Biochemistry Autoanalyzer and netrin-1, netrin-4, and H-FABP levels by commercially available enzyme-linked immunosorbent assays from Abbkine (China) and Elabscience Biotechnology (USA).

2.3. Statistical methods

All statistical analyses were performed using SPSS v 17.0 (Chicago, IL, USA). Normally distributed continuous variables were compared using the student's t-test. Pearson's correlation coefficient to determine the association and ROC analysis to determine the study parameters' diagnostic utility were done. Multivariate linear regression analysis was done to examine the independent association between study parameters.

3. Results

The general characteristics of study participants were shown in Table 1. Twenty-nine (69%) cases had STEMI, eight (19%) cases had NSTEMI, and six (12%) cases had UA. Cases had higher weight, height, systolic blood pressure, and serum creatinine levels than statistically significant controls. Cases had significantly higher serum CK-Total, CK-MB, troponin-T, and H-FABP levels compared to controls. Cases had significantly lower serum netrin-1 and netrin-4 levels compared to controls. ROC analysis showed that the level of

serum netrin-1 lesser than 556.07 pg/mL was associated with high ACS risk (AUC = 0.851, sensitivity = 90% and specificity = 74%, LR = 3.46, p < .001, Fig.1). ROC analysis showed that the level of serum netrin-4 lesser than 87.24 ng/mL was associated with high ACS risk (AUC = 0.968, sensitivity = 97% and specificity = 81%, LR = 5.11, p < .001, Fig.1).

Correlation analyses on all study participants showed that serum netrin-1 and netrin-4 levels were positively associated [Table 2] with serum total cholesterol, LDL-C, HDL-C levels and negatively associated with weight, systolic blood pressure, CK-Total, CK-MB, troponin-T, and H-FABP levels. Multivariate linear regression analysis showed that serum netrin-1 levels were negatively correlated with serum CK-MB [OR (95%CI) = − 0.26 (− 0.45 to − 0.05)] and H-FABP levels [OR (95%CI) = − 0.40 (− 8.76 to − 2.68)], and serum netrin-4 levels were negatively correlated with serum CK-MB [OR (95%CI) = − 0.36 (− 863.41 to − 260.85)], troponin-T [OR (95%CI) = − 0.36 (− 189.81 to − 67.26)] and total cholesterol levels [OR (95%CI) = − 0.23 (− 410.55 to − 27.60)].

4. Discussion

Different expressions of netrins in various tissues, their inhibition of macrophage recruitment, and their role in inflammation have made researchers to study netrin's significance in organogenesis, cancer, DM, and atherosclerosis.[9] Foam cells and inflammatory markers alter the extracellular matrix protein in favourable manners, resulting in atherosclerotic plaque formation. Similar changes are expected to occur in netrin, one of the extracellular proteins. There are contradictory findings of beneficial and harmful effects of netrins in atherosclerosis and inflammation.[10]

Lower serum netrin-1 and netrin-4 levels in T2DM patients with ACS than T2DM patients without ACS might be due to the extensive severity of atherosclerosis and inflammation. Similar results were demonstrated in patients with subclinical atherosclerosis and arterial wall inflammation compared to healthy individuals.[11] The beneficial effects of netrin-1 were due to the reduction of monocytes recruitment, expression of adhesion molecules, and

Table 1
Clinical and biochemical properties of the study population.

S. No.	Parameters	T2DM with ACS (n = 42) Mean ± SD	T2DM (n = 42) Mean ± SD	p value
1	Age (years)	58.29 ± 10.24	56.02 ± 7.22	0.246
2	Male/Female, n	31/11	29/13	0.639
3	Smoking, n (%)	12 (29)	8 (19)	0.321
4	Alcoholism, n (%)	10 (24)	8 (19)	0.608
5	Duration of DM (years)	13.2 ± 3.6	12.9 ± 2.6	0.703
6	Body surface area (m^2)	1.83 ± **0.14**	1.72 ± **0.04**	<0.001
7	WC (cm)	85.7 ± 8.1	83.7 ± 7.6	0.252
8	BMI (kg/m^2)	23.5 ± 1.9	23.6 ± 2.0	0.845
9	Systolic blood pressure (mmHg)	132.6 ± 25.1	112.8 ± 11.9	< 0.001
10	Diastolic blood pressure (mmHg)	80.7 ± 11.9	82.0 ± 9.30.80	0.584
11	Fasting blood glucose (mg/dL)	242.48 ± 87.36	219.14 ± 65.20	0.169
12	Total Cholesterol (mg/dL)	160.59 ± 44.85	190.78 ± 47.67	0.004
13	TG (mg/dL)	157.95 ± 57.22	180.38 ± 68.92	0.108
14	LDL-C (mg/dL)	103.57 ± 28.02	134.93 ± 38.44	< 0.001
15	HDL-C (mg/dL)	31.00 ± 7.31	42.07 ± 5.96	< 0.001
16	BUN (mg/dL)	27.55 ± 9.70	27.07 ± 8.32	0.810
17	Creatinine (mg/dL)	0.91 ± 0.29	0.78 ± 0.26	0.044
18	CK-Total (U/L)	191.88 ± 89.99	64.90 ± 18.50	< 0.001
19	CK-MB (U/L)	58.12 ± 22.98	10.43 ± 5.46	< 0.001
20	Troponin-T (pg/mL)	189.44 ± 126.03	8.62 ± 2.84	< 0.001
21	Netrin-1 (pg/mL)	510.47 ± 85.72	623.25 ± 68.55	< 0.001
22	Netrin-4 (ng/mL)	43.96 ± 9.80	142.71 ± 24.01	< 0.001
23	H-FABP (ng/mL)	17.37 ± 4.52	6.81 ± 3.30	< 0.001

T2DM: type 2 diabetes mellitus; ACS; acute coronary syndrome; WC: waist circumference; BMI: body mass index; TG: triacylglycerol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; BUN: blood urea nitrogen; CK-Total: creatinine kinase-total; CK-MB: creatinine kinase-MB; H-FABP: heart-fatty acid-binding protein.
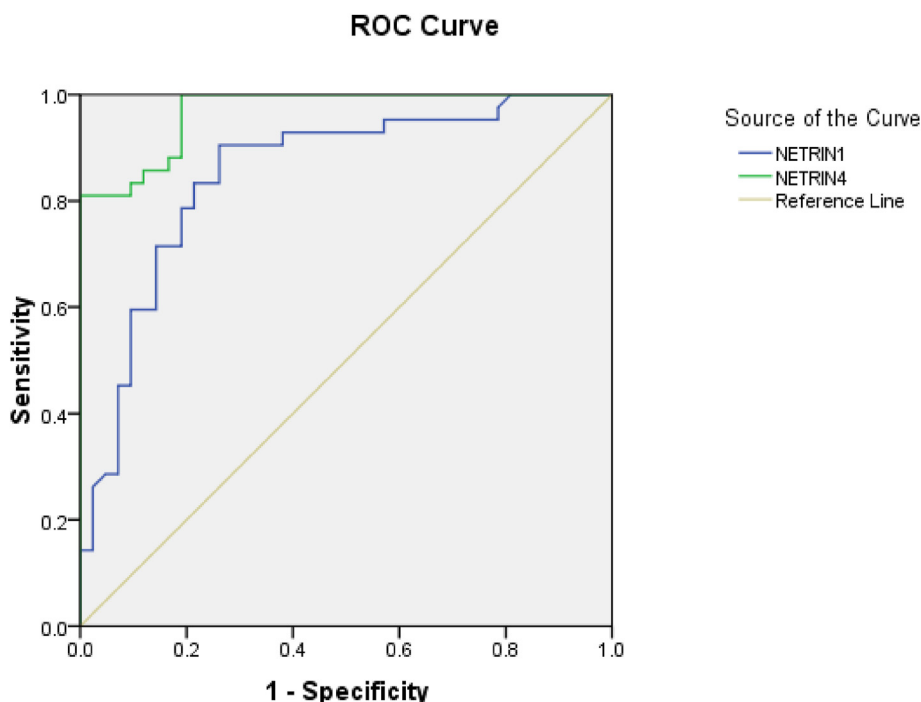
ROC Curve

Fig. 1. ROC curves of serum netrin-1 (pg/mL) and netrin-4 (ng/mL) for prediction of acute coronary syndrome in patients with type 2 diabetes mellitus.

Table 2
Correlation of serum netrin-1, netrin-4 levels with other variables.

Parameters n = 84	Netrin-1		Netrin-4	
	r value	p value	r value	p value
Age	0.025	0.821	− 0.122	0.267
Weight	− 0.251	0.021	− 0.276	0.011
Height	− 0.184	0.940	− 0.450	< 0.001
WC	− 0.130	0.239	− 0.092	0.408
BMI	− 0.168	0.127	0.036	0.748
Systolic Blood Pressure	− 0.257	0.018	− 0.438	< 0.001
Diastolic Blood Pressure	0.062	0.572	0.021	0.851
Duration of DM	− 0.099	0.371	− 0.099	0.369
Fasting blood glucose	− 0.181	0.100	− 0.154	0.162
CK-Total	− 0.507	< 0.001	− 0.675	< 0.001
CK-MB	− 0.510	< 0.001	− 0.790	< 0.001
Troponin-T	− 0.363	0.001	− 0.659	< 0.001
Total Cholesterol	0.224	0.040	0.301	0.005
TG	− 0.035	0.750	0.197	0.073
HDL-C	0.452	< 0.001	0.583	< 0.001
LDL-C	0.252	0.021	0.452	< 0.001
BUN	− 0.107	0.335	− 0.066	0.551
Creatinine	− 0.069	0.534	− 0.208	0.058
H-FABP	− 0.565	< 0.001	− 0.744	< 0.001

DM: diabetes mellitus; WC: waist circumference; BMI: body mass index; CK-Total: creatinine kinase-total; CK-MB: creatinine kinase-MB; TG: triacylglycerol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; BUN: blood urea nitrogen; H-FABP: heart-fatty acid-binding protein.

endothelium-derived cytokine productions, increasing M1 to M2 macrophage phenotype,[12] protection of heart from ischemia-reperfusion injury by increasing NO synthesis[13] and reversal of diabetes-induced vascular dysfunction in diabetic mice by its overexpression.[6] Similarly, netrin-4 was essential for maintaining homeostasis and vascular health.[14] These effects are found beneficial, which reduce the progression and complications of atherosclerosis and justified their reduction in T2DM patients with ACS. However, some studies showed that netrin-1 inhibits foam cell migration resulting in their entrapment, development and instability of atherosclerotic plaque.[11,15] The observed beneficial and harmful effects of netrin-1 might be due to its tissue source and types of receptors through which netrin-1 acts. Based on the correlation, ROC and multivariate linear regression analyses, the diagnostic utility of serum netrin-1 and netrin-4 levels for recognising ACS in T2DM patients may be established, which were comparable with markers of myocardial infarction. Small sample size, retrospective nature and lack of interventions details were significant limitations of the study.

5. Conclusion

The study demonstrates that serum netrin-1 and netrin-4 levels might be considered complementary diagnostic markers for ACS in T2DM patients. However, the research over a large population is warranted to confirm our claim.

'What is Already Known?'
Netrin-1 and netrin-4 expressions are reduced in atherosclerotic plaques
'What this Study Adds?'
Serum netrin-1 and netrin-4 might be considered complementary markers for ACS diagnosis in T2DM patients.

Declaration of competing interest

Nil.

Data availability statement

The data that support the study's finding is available on request from the corresponding author, [P.S. Adole]. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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