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Relationship between Proneurotensin Level and Cardiovascular Disease among Type 2 Diabetic patients

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ABSTRACT

Background: Patients who had cardiovascular disease (CVD) events in the past have fewer traditional risk factors to help them identify their risk. In this regard, measuring circulating biomarkers has been investigated as a potential method for determining event risk. **Objective:** To measure of viability of proneurotensin (pro-NT) as a good prediction for CVD among diabetic patients. **Patients and methods:** In the Internal Medicine Department's Outpatient Clinic and Endocrinology Unit at Zagazig University Hospitals, 84 type 2 diabetes mellitus cases were the subject of this case control research. They were equally subdivided into diabetic patients without coronary artery disease (CAD) and diabetic patients with established CAD. **Results:** Regarding the number of WBCs, there was a highly statistically significant difference between the examined groups. It was shown that diabetic patients with coronary artery disease had considerably higher cholesterol and LDL levels. In terms of proneurotensin, there was a statistically significant difference between the study groups. Pro-NT and cholesterol showed a strong positive link in patients with coronary artery disease, but HDL showed a substantial negative correlation in people without coronary artery disease. Pro-NT levels greater than or equal to 119.1 can be utilised to forecast when the condition will arise.

Conclusion: To our knowledge, our work is the first to show a relationship between pro-NT levels and insulin resistance and their ability to predict the occurrence of CVD.

Keywords: Proneurotensin, Type 2 Diabetes, CVD.

INTRODUCTION

In developed nations, coronary artery disease (CAD) is the leading cause of mortality. According to the American Heart Association's 2018 Heart Disease and Stroke Statistics, 16.5 million persons over the age of 20 are thought to have CAD [(1)]. There are many biological functions that affect the body, including those that have an impact on the cardiovascular system, such as controlling heart rate, myocardial contractility, and vascular tone. Originally derived from intestinal tissue and then from the bovine hypothalamus, neurotensin (NT) is a 13 a-a peptide. Neurotensin's effects are predominantly communicated through three receptors: the G-protein-coupled NTS3 and NT2 receptors, the non-G-protein coupled NTS3 receptor, and the satilin receptor 1 (SORT1), which is crucial for the hepatic production of VLDL. Coronary artery disease development is associated with genetic diversity in the 1p13 region, which contains the SORT1 gene [(2)].

The key function of NT, a hormone and neurotransmitter, in controlling intestinal fatty acid absorption, body fat buildup, and subsequently raising the risk of T2DM and CVD, has been shown in strong epidemiological studies [(3)].

Neurotensin is difficult to measure in blood due to its unpredictability and quick removal from the circulation. The content of proneurotensin was independently predictive for diabetes mellitus and CVD, especially in women, hence immunoassays were devised to detect the pro-peptide fragment of the peptide released in equivalent levels to native neurotensin [(4)].

In a study of **Cimini *et al.*** [(5)] they reported that higher pro-NT level in a T1D patient indicates the onset of a poor metabolic profile, which, after ten years, translates to a high CV risk profile. Pro-NT is a brand-new CV risk factor prediction marker for adults with T1D. Additionally, a high concentration of the peptide pro-NT is linked to an increased risk of mortality, CVD, and diabetes in women. But not necessary in men, a new study suggest this peptide could become a new target for drug therapy as well as new marker for risk prediction [(6)]. Therefore, this study aimed to measure of viability of proneurotensin as a good prediction for CVD among diabetic patients. Also, to identify the relationship between development of CVD, type 2 DM and level of proneurotensin.

PATIENTS AND METHODS

In the Internal Medicine Department's Outpatient Clinic and Endocrinology Unit at Zagazig University Hospitals, from January to August, 2022, 84 type 2 diabetes mellitus cases were the subject of this case control research. It was conducted on total number of 126 subjects with 54.59 mean age ±SD (80 female and 46 male). There were further classified into:

1- Control group: composed of 42 healthy individuals with mean age of 51.8 ±7.4 from 40 to 68 years old; 22 female and 20 male

2- Diabetic group: included 84 patients who were subdivided according to presence or absence of cardiovascular disease into:

a- Diabetic with CVD (n 42): mean age 55.3±6.9 from; 28 female and 14 male.

b- Diabetic without CVD (n 42): mean age 54.2±5.4; 30 female and 12 male.

Inclusion criteria:

Patients with type 2 diabetes, both sexes, aged 18 to 65.

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Exclusion criteria:

Patients with Type1 DM, chronic liver and kidney diseases.

Clinical assessment:

A thorough physical examination and history taking were performed on every client. According to the most recent recommendations, arterial hypertension was defined [7]. Routine investigations in the form of CBC, liver function tests and coagulation profile. FBS, PPS, and glycosylated hemoglobin level (HbA1C) were done (Cobas 6000, USA). Lipid profile by enzymatic colorimetric assay on (Cobas 6000, USA), urea and creatinine were performed.

Following a 10- to 12-hours fast, all measures were made in the morning at a constant room temperature of 23±1°C. Smoking and caffeine use were prohibited for everyone for 8 to 10 hours, and participants only received any antidiabetic or other drugs at the conclusion of the test.

Human Proneurotensin ELISA Kit (ProNT/NMN):

ProNT/NMN in human plasma may be determined quantitatively using an ELISA kit. The competitive inhibition enzyme immunoassay method was used in this test. A microplate had been pre-coated with an anti-ProNT/NMN antibody. With the pre-coated antibody specific to ProNT/NMN, a competitive inhibitory process started between biotin-labeled growth hormone and unlabeled ProNT/NMN. The unbound conjugate was removed after incubation. After that, each microplate well was filled with avidin conjugated to horseradish peroxidase (HRP), which was then incubated. The amount of ProNT/NMN present in the sample had a reverse inverse relationship with the colour intensity that results.

Echocardiography:

We used an echocardiographic machine: Siemens – Acuson X300 (made in India). All standard views: Lt. parasternal long axis, Lt. parasternal short axis and apical four chamber views, dimensions, EF, FS, and presence of wall motion abnormality were measured.

ECG measurement:

At a paper speed of 25 mm/s and a recording rate of 10 mm/mV, all common ECGs were taken. Standard 12-lead ECGs were taken immediately before to catheterization and 24 hours following the procedure.

Ethical approval:

Zagazig Medical Ethics Committee of the Zagazig Faculty of Medicine gave its approval to this study. All participants gave written consent after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

Using SPSS V. 20, all data were examined. Categorical qualitative data were reported as absolute frequencies and relative frequencies, whereas continuous quantitative variables were expressed as mean±SD, median, and range. Shapiro Wilk test was used to determine the normality of continuous data. To compare between more than two groups of normally distributed data, a one-way ANOVA test was employed followed by LSD as a post-hoc test. In order to compare categorical data, the Chi-square test (X^2 test) was used. In order to determine the degree of link between two quantitative variables, Pearson's correlation coefficient was utilised. The sensitivity, specificity, predictive value positive, predictive value negative, and accuracy of the screening test (Pro-NT) were all evaluated using ROC curve. P value was fixed at 0.05 for statistical significance and <0.001 for high significant result.

RESULTS

The current investigation found no statistically significant age or sex differences between the tested groups. Regarding the duration of diabetes mellitus, the prevalence of hypertension, and the mode of therapy employed, there were no statistically significant differences between the analysed groups (Table 1).

Table (1): Baseline characteristics and Clinical history of the studied groups:

Variable		Patients with CAD (n=42)	Patients without CAD (n=42)	Control group (n=42)	P
Age: (years)	Mean ± SD Range	55.3± 6.9 42 – 72	54.2± 5.4 48 - 69	51.8 ± 7.4 40 - 68	0.050#
Sex: Female: Male:		 28 (66.7%) 14 (33.3%)	 30 (71.4%) 12 (28.6%)	 22 (52.4%) 20 (47.6%)	 0.168^
Duration of disease (DM) **Median** **Range**		 9.9 ± 5.7 4 – 30	 10.9 ± 4.7 5 - 30	 NA	 0.064
Hypertension **No:** **Yes:**		 13 (31%) 29 (69%)	 22 (52.4%) 20 (47.6%)	 NA	 0.046^
Type of treatment: **Insulin:** **OHD:**		 31 (73.8%) 11 (26.2%)	 26 (61.9%) 16 (38.1%)	 NA	 0.243^

#: One-way ANOVA TEST. ^: Chi-square test.

Regarding the WBC count, which was shown to be much greater among diabetic individuals with coronary artery disease compared to their counterparts, there was a highly statistically significant difference between the analysed groups. Regarding PLT, it was shown that diabetic individuals without CAD had much lower levels of it. Regarding hemoglobin, however, there was no significant difference between them (Table 2).

Table (2): Blood picture of the studied groups:

Variable	Patients with CAD (n=42)	Patients without CAD (n=42)	Control group (n=42)	P#	LSD
Hemoglobin (g/dL) Mean ± SD	11.6 ± 1.6	10.9 ± 2.6	11.6 ± 2.8	0.568# (NS)	>0.051 >0.052 >0.053
WBCs (mcL) Mean ± SD	10.3 ± 2.4	7.8 ± 1.8	6.3 ± 1.4	**<0.001# (HS)**	**<0.051** **<0.052** **<0.053**
PLT (mcL) Mean ± SD	262.9 ± 64.9	251.8 ± 61.6	296 ± 73.3	**0.04# (S)**	>0.051 >0.052 **<0.053**

#: ANOVA test; S: Significant; HS: Highly significant; P1: Patients with CAD versus patients without CAD; P2: Patients with CAD versus control group; P3: Patients without CAD versus control group.

Between the tested groups, there were highly statistically significant differences in all renal function tests and lipid profiles. When compared to healthy individuals, diabetic patients with coronary artery disease had considerably higher cholesterol and LDL levels (Table 3).

Table (3): Kidney function test and lipid profile of the studied groups:

Variable	Patients with CAD (n=42)	Patients without CAD (n=42)	Control group (n=42)	P#	LSD
Creatinine (mg) Mean ± SD	0.95 ± 0.22	1.05 ± 0.24	0.79 ± 0.18	**0.002 (S)**	>0.051 **<0.052** **<0.053**
Cholesterol (mg/dL) Mean ± SD	178.9± 43.6	156.3 ± 31.2	80.7 ± 8.6	**<0.001 (HS)**	**<0.051** **<0.052** **<0.053**
Triglycerides (mg/dL) Mean ± SD	172.8 ± 41.4	193.9 ± 38.1	84.9 ± 12	**<0.001 (HS)**	>0.051 **<0.052** **<0.053**
HDL (mg/dL) Mean ± SD	32.6 ± 3.6	31.6± 3.6	46.1 ± 10	**<0.001 (HS)**	>0.051 **<0.052** **<0.053**
LDL (mg/dL) Mean ± SD	115.9 ± 26.7	91.3 ± 21.8	58.3 ± 14.2	**<0.001 (HS)**	**<0.051** **<0.052** **<0.053**

#: ANOVA test; S: Significant; HS: Highly significant; P1: Patients with CAD versus patients without CAD; P2:

Patients with CAD versus control group; P3: Patients without CAD versus control group.

Regarding cardiac enzymes (Troponin and CK) and HBA1C, which were all seen to be considerably higher among CAD patients compared to the other groups (Table 4), there were highly statistically significant differences between the analysed groups.

Table (4): Cardiac enzymes and HbA1C of the studied groups:

Variable	Patients with CAD (n=42)	Patients without CAD (n=42)	Control Group (n=42)	P#	LSD
Troponin (ng/mL) Mean ± SD	707.9 ± 171.2	4 ± 1.8	3.3 ± 0.7	**<0.001 (HS)**	**<0.051** **<0.052** >0.053
CKMB (U/L) Mean ± SD	43.6 ± 10.1	0.6 ± 0.14	0.2 ± 0.04	**<0.001 (HS)**	**<0.051** **<0.052** **<0.053**
HBA1C (%) Mean ± SD	8.7 ± 1.5	7.7 ± 0.4	4.7 ± 0.5	**<0.001 (HS)**	**<0.051** **<0.052** **<0.053**

#: ANOVA test; HS: Highly significant; P1: Patients with CAD versus patients without CAD; P2: Patients with CAD versus control group; P3: Patients without CAD versus control group.

Proneurotensin was shown to be statistically substantially greater in diabetic patients with coronary artery disease compared to their counterparts across the analysed groups (Figure 1).

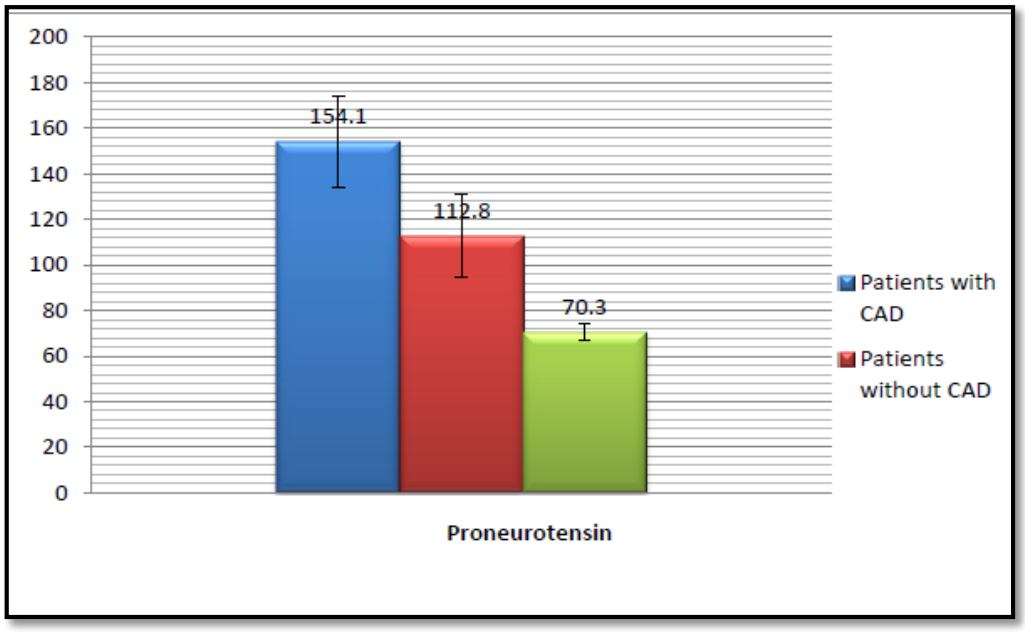

Figure (1): Bar chart showing proneurotensin levels among the studied groups.

Pro-NT and cholesterol showed a substantial positive association in patients with coronary artery disease, while pro-NT and HDL showed a significant negative correlation in patients without coronary artery disease (Table 5).

Table (5): The correlation between proneurotensin and different parameters among the studied groups:

Variable (Pro-NT)	Patients with CAD		Patients without CAD	
	r	p	r	p
Hemoglobin (mg)	0.235	0.134	0.119	0.453
WBCs (mcL)	0.009	0.955	0.036	0.819
PLT (mcL)	0.216	0.169	0.051	0.746
Creatinine (mg)	0.149	0.347	-0.119	0.454
Troponin (ng/mL)	0.172	0.267	0.158	0.318
CKMB (U/L)	0.175	0.267	0.015	0.927
HBA1C (%)	0.157	0.321	0.030	0.849
Cholesterol (mg/dL)	**0.385**	**0.02**	0.221	0.159
Triglycerides (mg/dL)	-0.75	0.638	-0.236	0.133
HDL (mg/dL)	-0.60	0.707	**-0.512**	**0.001**
LDL (mg/dL)	0.269	0.085	-0.158	0.317

Pro-NT level more than or equal to 119.1 can be used as a predictor for the occurrence of CAD **(Table 6; Figure 2)**.

Table (6): Performance of proneurotensin as a predictor of coronary artery disease among the studied groups:

Cutoff Point	AUC	Sens.	Spec.	PVP	PVN	accuracy	P-value
PNT ≥ **119.1**	0.637	71.4%	61.6%	65.2%	68.4%	66.7%	**0.03**

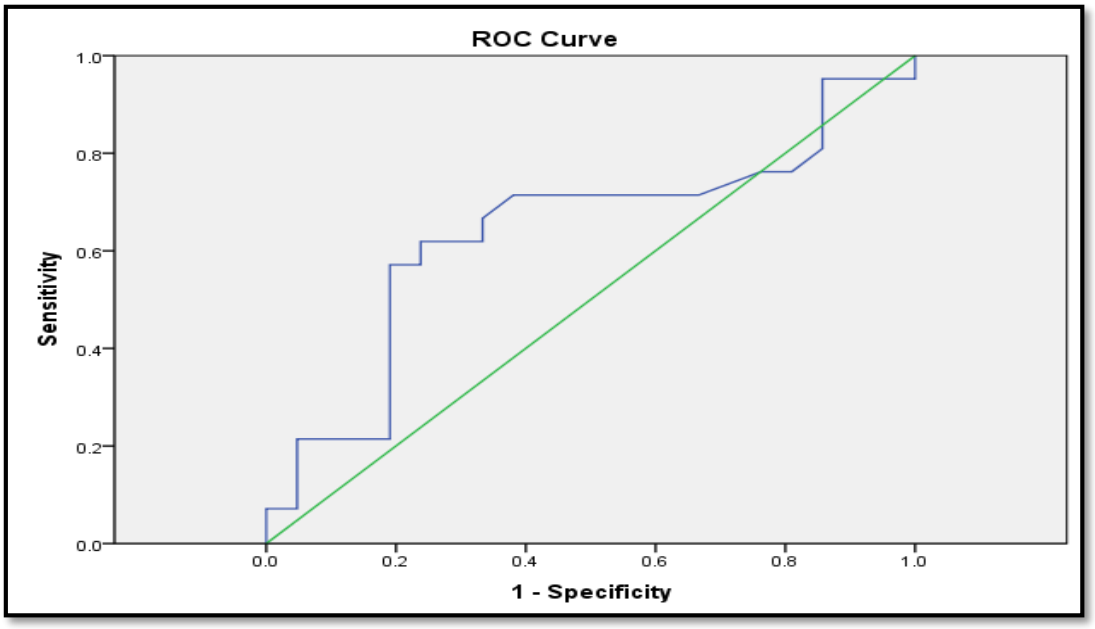

Figure (2): ROC curve showing performance of pro-NT as a predictor of CAD.

DISCUSSION

CVD is the main killer in DM. The greatest significant risk factor for CAD is diabetes mellitus [(8)]. It is possible to predict CAD using a biomarker for diabetic mellitus and vice versa. In actuality, CAD and diabetes have several comparable biomarkers. These indicators can help identify individuals who are at high risk for developing CAD and may make suitable treatment targets [(9)].

The central nervous system, the gastrointestinal tract, and the cardiovascular system all contain neurotensin (NT). The major mechanism by which NT exerts its effects is by binding to the NTSR1, NTSR2, and NTSR3 receptors [(2)]. Numerous cardiovascular activities of NT have been demonstrated through experimental research, including the control of heart rate, systemic blood pressure, myocardial contractility, and coronary vascular tone [(10)].

This case control study held in Zagazig University Hospital, Internal Medicine Department, Endocrinology Unit and Clinic. Patients were subdivided into two groups according to presence of CVD into diabetic patients without CAD and diabetic patients with established CAD**.** This study aimed to measure the validity of proneurotensin as a good prediction for CVD among diabetic patients.

The current study showed that regarding CAD group, the mean age of cases was 55.3 years, 66.7% were females, and 33.3% were males. While in diabetic cases without CAD, the mean age was 54.2 years, 71.4% were females, and 28.6% were males. There were non-significant differences between the studied groups as regarding age and sex. This result was in disagreement with **Fawad *et al.*** [(11)] who tested whether proneurotensin predicts the development of diabetes and CVD in an older population and if gender differences exist in this regard. The 4804 people had a 69-year-old median age, and 69.8% of them were males. Because there were so many instances included in their analysis, there were different outcomes for age and gender.

Also, **Barchetta *et al.*** [(12)] disagreed with our findings as they reported that diabetic cases without CVD; the mean age was 40.5 years, 61.5% were males. The mean age of diabetic cases without CAD was 49.5 years with 55% males and 45% females. **Oh *et al.*** [(13)] reported that regarding CAD group, the age of cases was 65.4 years, 84% were females, and 16% were males. While in group without CAD, the mean age was 52.9 years, 66% were females, and 34% were males.

Concerning the clinical history of cases, the CAD group showed that duration of DM was 8 years, 69% were hypertensive, and 73.8% and 26.2% treated with insulin and OHD, respectively. Moreover, the non-CAD group showed that duration of DM was 9.5 years, 47.6% were hypertensive, and 61.9% and 38.1% treated with insulin and OHD, respectively. Regarding the occurrence of hypertension, the duration of diabetes mellitus, and the method of treatment, there were no statistically significant differences between the analysed groups. Our results were consistent with those of **Wang *et al.*** [(14)]**,** who found that the PCAD group had

more patients with type 2 diabetes mellitus (43.73% vs. 29.59%, P>0.05) and hypertension (76.53% vs. 60.36%, P>0.05) than the NPCAD group did. In comparison to patients in the NPCAD group, significantly more patients in the PCAD group (63.81% vs. 52.68%, 24.84% vs. 12.96%, respectively, both P<0.05) were given hypotensive and hypoglycemic medications. Also, **Oh *et al.*** [13] reported that the CAD group showed that 93.5% were hypertensive. Moreover, the non-CAD group showed that 43.1% were hypertensive.

The CAD group's analysis of the blood images of the patients revealed that the mean hemoglobin level was 11 mg/dl, the mean WBC count was 10.3 mcL, and the mean platelet count was 230. Additionally, the non-CAD group revealed a mean hemoglobin level of 11, a WBC count of 7.8, and a platelet count of 213.5 mcL. Regarding the WBC count, which was shown to be much greater among diabetic individuals with coronary artery disease compared to their counterparts, there was a highly statistically significant difference between the analysed groups. In terms of hemoglobin and PLT, however, there was no statistically significant difference between them.

Respecting the kidney function and lipid profile of cases, the CAD group showed that the median level of creatinine was 0.92, albumin was 3.6, cholesterol was 208.5, triglycerides was 152, HDL was 32, and LDL was 92.5. Moreover, the non-CAD group showed that the median level of creatinine was 0.98, albumin was 3.4, cholesterol was 156, triglycerides was 188, HDL was 32, and LDL was 95. Regarding triglyceride levels, there was a highly statistically significant difference between the tested groups (152 versus 188, respectively), with diabetic individuals with coronary artery disease having much lower levels than their counterparts. Patients with CAD were found to have considerably greater albumin levels. However, there was no statistically significant difference between them in terms of creatinine, cholesterol, HDL, and LDL. This finding agreed with **Barchetta *et al.*** [12] who reported that in diabetic cases without CVD, the mean cholesterol was 213 mg/dl, triglycerides was 118 mg/dl, LDL was 141.9 mg/dl, and HDL was 52 mg/dl.

Moreover, our results disagreed with **Wang *et al.*** [14] who reported that the CAD group showed that the median level of creatinine was 72.73, cholesterol was 4.94, triglycerides was 2.11, HDL was 1.04, and LDL was 2.63. Moreover, the non-CAD group showed that the median level of creatinine was 71.55, cholesterol was 9.14, triglycerides was 2.64, HDL was 1.25, and LDL was 2.29. The difference in results was due to their study was a study considering 490 samples of Chinese people.

Our findings were in accordance with **Oh *et al.*** [13] who reported that CAD group showed that the median level of cholesterol was 164 mg/dl, triglycerides was 135.6 mg/dl, HDL was 38.1 mg/dl, and LDL was 100.6 mg/dl. Moreover, the non-CAD group showed that the median level of cholesterol was 202.1 mg/dl, triglycerides was 131.9 mg/dl, HDL was 52 mg/dl, and LDL was 112.6 mg/dl.

Concerning cardiac enzymes and HbA1C of cases, the CAD group showed that the median level of troponin was 105, CKMB was 7.5, and HBA1C was 8.5. Moreover, the non-CAD group showed that the median level of troponin was 3, CKMB was 0.7, and HBA1C was 7.8. Regarding troponin, CK, and HbA1C, which were all shown to be considerably greater among CAD patients than the other group, there were highly statistically significant differences between the analysed groups. The observed findings were consistent with **Oh *et al.*'s** [13] study that the CAD group's mean HbA1c level was 6.9%. When compared to the non-CAD group, which had a mean cholesterol level of 6.2%. The current findings were consistent with those of **Barchetta *et al.*** [12], who showed that diabetic subjects without CVD had mean HbA1C values of 5.2 and 6.7, respectively.

Our findings revealed that regarding proneurotensin, there was a statistically significant difference between the study groups, with diabetic individuals with coronary artery disease having much greater levels than their counterparts (154.1 vs 112.8, respectively). The current findings were in agreement with **Wang *et al.*** [14] study that the CAD group's mean pro-NT level was 59.52. Additionally, the non-CAD group had a median pro-NT level of 56.57. Also, agreed with **Barchetta *et al.*** [12] who reported that in diabetic cases without CVD, the mean pro-NT was 86.7 pmol/l.

The current findings revealed that, among individuals without coronary artery disease, there was a substantial positive connection between pro-NT and cholesterol and a significant negative correlation with triglycerides.

According to **Fawad *et al.*** [11], persons with above-median levels of proneurotensin compared to those with below-median levels had a substantially higher chance of experiencing their first CVD event (Kaplan-Meier analysis, P =0.045). This connection was significant in females (P=0.044), but not in men (P = 0.777). Proneurotensin and gender had a statistically significant interaction on the result of incident diabetes (P=0.029). Numerous experimental findings suggest a causal relationship between the neurotensin system and the onset of atherosclerosis, vascular calcification, and inflammation, which should motivate more interventional research.

Barchetta *et al.* [12] reported that greater pro-NT levels were associated with the presence of T2D and female sex in the bivariate analyses (r = 0.25, P = 0.001 and 0.15, P = 0.05, respectively), but not with the number of MS components (r = 0.11, P = 0.08), which showed a trend towards a positive association but did not reach statistical significance. Pro-NT, age, and measures of body adiposity as BMI and waist circumference were not linked.

Our findings were in line with **Wang *et al.*** [14] who reported that pro-NT levels were found to be

positively associated with group, female gender, BMI, TC, and urea. But negatively associated with LDL-C in the whole study population. Independent of age, gender, metabolic syndrome subtypes, or obesity, **Barchetta *et al.*** [12] found a strong correlation between the highest pro-NT quartile and greater leptin levels.

On the other side, our results were on the contrary with **Cimini *et al.*** [5] who reported that a trend towards an association with triglycerides levels was seen, though it did not reach statistical significance, and higher pro-NT levels were significantly associated with worse glycemic control, as indicated by higher FBG and HbA1c levels. Pro-NT levels and serum creatinine levels, which serve as indicators of renal function, were not linked. The connection between basal pro-NT and HbA1c levels at the linear multivariate analysis was unrelated to age, sex, or BMI. Their study was longitudinal retrospective study with less cases number.

Recent research from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study demonstrates that higher pro-NT levels at baseline independently predict the onset of metabolic syndrome in a large cohort of over 3700 participants, particularly low HDL levels and impaired glucose regulation [15].

The current findings reported that pro-NT level more than or equal to 119.1 can be used as a predictor for the occurrence of CAD, with specificity of 61.6%, sensitivity of 71.4%, and accuracy of 66.7%. Partially unknown mechanisms underlie the association between pro-NT and increased CV risk. The development of accumulating cardiometabolic risk factors may be influenced by NT through changed glucose metabolism, increased intestinal lipid absorption, altered adipose tissue homeostasis, and altered body fat distribution [11].

CONCLUSION

To our knowledge, our work is the first to show a relationship between pro-NT levels and insulin resistance and their ability to predict the occurrence of CVD. It is necessary to do studies using longitudinal designs and bigger cohorts to look at the potential contribution of pro-NT to the onset, progression, and prognosis of CAD.

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