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Relationship between lipid profile and B-type natriuretic peptide T-381C (rs198389) gene polymorphism in patients with stable coronary artery disease

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ABSTRACT

The research explored the link between Brain Natriuretic Peptides (BNP) gene promoter T-381C polymorphism, serum BNP, and lipid profiles in Kurdish people from Iraq with stable coronary artery disease (CAD). The study was conducted on 62 individuals with CAD and 31 without CAD (control group). DNA was extracted from each individual's sample using the Sanger sequencing method to study the BNP gene's polymorphism. The identified alleles were TT, TC, and CC. The frequency of the TT genotype decreased significantly among the patient group compared to the control group, while the CC genotype's frequency was higher ($p<0.05$). However, there was no significant increase in BNP levels in TC and CC genotypes compared to the TT genotype. Lipid profile values were not significantly different among the genotypes. The study utilized a cut-off value for BNP activity for predicting CAD and found that individuals with a BNP activity value less than the cut-off had significantly greater changes in lipid profile and renal function ($p<0.05$). Stepwise multivariate regression analysis showed that cholesterol was not the only primary determinant of BNP rate in subjects with stable CAD; oxidized low-density lipoprotein (Ox-LDL), a history of heart attacks, and oxidative stress malondialdehyde (MDA) had a significant effect. Homozygous C allele carriers at position 381 of the BNP precursors gene promoter were more likely to exhibit atherosclerosis lesions. We found that BNP rs198389 was not correlated with lipid profile and kidney disease.

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Introduction

Cardiovascular diseases cause the majority of noncommunicable disease-related deaths. It affects 17.7 million people annually, primarily in low- to middle-income countries (1). Cardiovascular disease has become the most common cause of death in Iraq (2).

Coronary artery disease (CAD) is a common heart disorder characterized by the narrowing of major blood vessels that supply the heart, known as coronary arteries, due to atherosclerotic plaque buildup within the inner layer of the vessel wall (3). It is the leading cause of mortality and morbidity worldwide (4). Dyslipidemia and lipid oxidation represent a wide range of lipid abnormalities that are essential contributors to atherosclerosis development and progression, ultimately resulting in CAD (5).

The natriuretic peptides are a group consisting of three structurally associated hormones, which include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), that play critical roles in the cardiovascular system's function (6).

BNP, primarily produced by the ventricular myocardium, is released in response to volume expansion, pressure overloading, and increasing diastolic pressure (7). Serum BNP levels are correlated with the extent of ventricular dysfunction (8). Endothelial dysfunction, which impairs arterial vasodilation, is an early stage of atherosclerotic damage; it frequently happens by being exposed to cardiovascular-related risk factors such as smoking, hypertension, diabetes, high blood cholesterol, and obesity. The correlation between lipid parameters and CAD has been extensively explored, in which elevated levels of total cholesterol, low-densities lipoprotein cholesterol (LDL), and triglycerides (TG), in addition to a decrease in levels of high-density lipoprotein cholesterol (HDL), have been identified as significant risk factors (9,10). However, the interplay between dyslipidemia and BNP gene polymorphisms in patients with stable CAD remains poorly understood. On the other hand, the low plasma levels of HDL cause oxidation of ox-LDL, which causes a proliferation of inflammation mediators and the production of reactive oxygen species (ROS), which may influence cardiomyocyte functions (11,12).

The potential use of BNP as a cardiac biomarker is secondary to its activating by the myocardium stretch and ischemia, as demonstrated in heart failure (HF) and myocardial infarction (MI). Nevertheless, age and gender significantly regulate the plasma BNP (13–15).

BNP/NPPB genes are located on chromosome one in humans, which contains numerous polymorphisms related to cardiovascular conditions and their risk factors. Many single-nucleotide polymorphisms (SNPs) have been identified for that region, some of which have been currently correlated with elevated BNP and ANP plasma levels, decreased blood pressure, and incidence of hypertension (16). In three separate studies, the SNP rs198389

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(also known as BNP T-381C) in the promoter part of the BNP gene was found to be correlated with elevated BNP levels (17–19). Furthermore, it was found that in vitro, the C allele correlated to more significant promoter activity (17). The present research aims to determine the association between the BNP gene polymorphism (T-381C) and BNP levels, lipid profile, and other particular parameters in individuals with stable CAD.

Materials and Methods

Patient population

The research study on stable coronary artery disease was conducted in Erbil, Iraq, at the Surgical Specialty Hospital-Cardiac Center, from January to June 2022. Among the 300 patients who had undergone coronary angiography in the Department of Cardiology for diagnostic purposes, 93 male participants were selected for the study.

The patient population (Group I) consisted of 62 patients who had stable coronary artery disease with stenosis above fifty percent (50%) in at least one of their coronary arteries; the other 31 participants who did not have coronary artery stenosis were assigned to the non-CAD control group (Group II).

To ensure the homogeneity of the study population, patients with other heart conditions, participants with autoimmunity, acute disease of the liver or kidneys, malignancies, or any other form of chronic medical condition were excluded from the study. Individuals, who experienced unstable angina, including STEMI and non-STEMI, were also excluded from the study.

Sample collecting

After an overnight fast, venipuncture was performed to collect 10 mL of whole blood samples, then separated into two halves. The first half was collected in an EDTA tube and processed for hematological and molecular assessments. The second portion was transferred to gel tubes and centrifuged to separate the serum. The serum was then kept at -20 degrees Celsius until further use.

Biochemical assay

After centrifuging non-heparinized blood, serum brain nitric peptide activity was measured using a sandwich enzyme immunoassay technique (SL0372Hu-China) with a 1.2pg/mL sensitivity. The intra- and inter-assay coefficient of variation was less than 10% for all assays. A Cobas e411: 1242-22 analyzer (Roche/ Germany) was utilized for analyzing the serum biochemical parameters.

Coronary angiography

Diagnostic coronary angiography was performed on each patient to determine the severity and extent of CAD through the right femoral approach. Experienced cardiologists evaluated coronary angiograms to assess the severity of the CAD, who were unaware of the patient's clinical history and biochemical results. The severity of the stenosis has been categorized using the Coronary Artery Disease Reporting and Data System (CAD-RADS) classification (20). The study population was divided into two separate groups; the first group consisted of 62 patients who were diagnosed with coronary artery disease (CAD-RADS 3 or more) with significant coronary artery stenosis (≥50%); the second group consisted of 32 subjects who showed no evidence of CAD (CAD-RADS 0) as determined by coronary angiography.

DNA extraction

The GeneAll® Exgene™ Genomic DNA Extraction for Clinic Cell SV mini kit was used to extract DNA from whole blood specimens to obtain BNP gene polymorphisms. Following GeneAll® (Songpa-gu, Seoul, KOREA) manufacturer's instruction sets, 50-µL of elution buffer was utilized for DNA extraction. Then genomic extraction was frozen at –20°C before a PCR test.

Genotyping of the BNP gene rs198389 loci

The polymerase chain reaction (PCR) technique was used to amplify the fragment of target DNA. The primer sequence, reaction, system and conditions of the PCR amplification reaction are shown below; the target DNA sequence was detected by Sanger sequencing. A programmable thermal cycler PCR system was used for amplification. The forward primer: 5′-CTGTGAGTCACCCCGTGCTC-3′ and reverse primer: 5′-GGCAGGAACGCGCTGGAGAC-3′; fifty µL reaction mixtures were used for preparing the PCR cocktail, including 25 µL of 2^x PCR master mix (AMPLIQON, Denmark), 1.0 µL of each the primer (10 pmol), and 1.5 µL of template genomic DNA. The total volume was completed to 50 µL with nuclease-free water.

The PCR started with an initial denaturation step at 95°C for 5 minutes, 35 cycles at 94°C for 30 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for 30 seconds, as well as a final extension step at 72°C for 10 minutes. The amplified products were then subjected to electrophoresis on 1.5% agarose gel to verify the fragment size (21), and the PCR product length was 186 bp.

Statistical analysis

Graph Pad Prism version 9 and MedCalc version 18 were used for the data analysis. Chi-square statistics analyzed the demographic characteristics. The Kruskal Wallis test calculated Mean±SEM, p-value≤0.05 was considered significant, and the person correlation test was used to evaluate between-group comparisons for categorical variables. Additionally, the predictive significance of the study determined severity via Receiver Operator Characteristic (ROC) Curve analysis and the results were expressed as area under the curve (AUC), cut-off value, specificity and sensitivity for BNP level. The Hardy-Weinberg equilibrium was estimated using the H-W calculator for two alleles. We used stepwise multiple regression modeling to assess factors affecting serum BNP levels in all patients with stable CAD.

Results

Subject characteristics

Tables 1 and 2 show the clinical features of 93 male subjects, distinguishing between those diagnosed with CAD and those without CAD. The prevalence of high age, a high body mass index (BMI), smoking habit, elevated blood pressure, a personal history of diabetes mellitus, and personal history of heart attack, and a family history of hypertension and coronary artery disease were significantly higher among those with CAD patients than in the non-CAD group. In addition, significant differences were observed in various lipid parameters between the

Table 1. Baseline characteristics of the population.

Variable		CAD n=62 (%)	non-CADN (%) n=31	*p*
Mean ± SE of Age (years)		53 ±1.118	48±1.962	0.030
Smoking	Yes	17(27.4 %)	5 (16.12%)	0.027
	No	45(72.6%)	26 (83.87%)	
BMI (Kg/m^2)		29.37±0.596	26.31±0.468	0.0003
SBP (mm Hg)		136.8±2.628	114.2±1.721	0.0001
DBP (mm Hg)		84.94±2.058	77.53±1.118	0.0102
Physical activity	Yes	20(32.25%)	11 (35.48%)	0.879
	No	42(67.74%)	21 (64.51%)	
Fast food intake	Yes	22(35.48%)	11 (35.48%)	0.762
	No	40(64.51%)	20 (64.51%)	
Soft drink	Yes	10 (16.12%)	6 (19.35%)	0.599
	No	52 (83.87%)	25 (80.64%)	
Personal Diabetic	Yes	12(19.35%)	0 (0)	0.000
	No	50(80.64%)	31 (100%)	
Personal stork	Yes	3 (4.83%)	0 (0)	0.104
	No	59(95.16 %)	31 (100%)	
Personal heart attack	Yes	12(19.35%)	0 (0)	0.000
	No	50(80.64%)	31 (100%)	
Family history of Diabetic	Yes	32(51.61%)	14 (45.16%)	0.379
	No	30(48.38%)	17 (54.83%)	
Family history of hypertension	Yes	35(56.45%)	12 (38.70%)	0.001
	No	30(48.38%)	19 (61.29%)	
Family history of hyperlipidemia	Yes	14(22.58%)	9 (29%)	0.615
	No	48(77.41%)	22 (70.96%)	
Family history of heart attack	Yes	13(20.96%)	5 (16.12%)	0.132
	No	49(79.03%)	26 (83.87%)	
Family history of coronary artery	Yes	21(33.87%)	3 (9.6%)	0.000
	No	41(66.12%)	28 (90.32%)	

Table 2. Biochemical parameters of CAD and non-CAD individuals.

Variable	CAD n=62 (%)	non-CAD (%) n=31	*p*
Total cholesterol (mg/dL)	148.5±4.462	139.2±3.327	0.582
Triglycerides (mg/dL)	174.1± 8.774	131.4± 2.584	0.0026
HDL-c (mg/dL)	30.83 ±0.824	40.53 ±1.424	0.0001
LDL-c (mg/dL)	90.25±3.887	85.31±3.409	0.6897
Cholesterol/HDL	4.982±0.183	3.633±0.1.6	0.0001
TG/HDL	6.060±0.408	3.435±0.169	0.0001
BNP pg/mL	55.93±2.552	49.05±0836	0.6897
OX-LDL pg/mL	180.7±9.52	191.7±5.04	0.0006
Urea (mg/dL)	37.06±1.524	32.65 ±1.577	0.1301
Creatine (mg/dL)	0.880±0.025	0.8523± 0.026	0.4762
AZGP1 ng/mL	52.52±7.593	81.94±2.174	0.0001
MDA ng/mL	4.487±0.372	3.223±0.142	0.0112
SOD ng/mL	2.630±0.118	4.632±0.762	0.0001
T-AOC U/mL	4.919±0.170	7.494±1.101	0.0001
GPX pmol/mL	16.46±0.666	27.95±3.218	0.0001

two groups. In the CAD group, TG, total cholesterol to HDL-ratio (cholesterol/HDL), and the triglycerides to HDL ratio (TG/HDL) were all significantly elevated. The overall cholesterol level and LDL levels also increased, but non considerably. In contrast, HDL was lower in the CAD group than in the non-CAD group.

In addition, the levels of BNP were slightly elevated in the CAD group compared to the non-CAD group. Moreover, Ox-LDL levels showed a significant decrease among the CAD group. In addition to cardiovascular markers, markers of kidney function, including urea and creatinine, were slightly elevated in the CAD group compared to the

non-CAD group. In the CAD group, antioxidant capacity, including 2-glycoprotein 1 (AZGP1), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and glutathione peroxidase (GPX), were found to be significantly less common than in the non-CAD-group. MDA, an indicator of oxidative stress, was found to be higher in the CAD group than in the non-CAD group.

ROC curve analysis for BNP activity

The evaluation of the ROC curve estimated the cut-off levels for BNP activities used to distinguish between individuals with and without CAD. The area under the ROC curve for BNP activities as a predictor of CAD was 0.526 ($p<0.05$). Following the analysis of the ROC curve, the BNP activity level of 54.842 pg/mL had sensitivity and specificity in predicting CAD (sensitivity = 32.2 6% and specificity = 96.77 %). Also, the study population has been divided into two groups based on the cut-off value of the BNP activity levels. Numeral biomarkers, which include total cholesterol, TG, cholesterol/HDL-ratio, triglyceride/HDL-ratio, urea, and creatinine, increased significantly ($p<0.05$) in individuals with BNP activity levels below the cut-off value. However, age and BMI levels were slightly elevated in BNP groups below the cut-off value, and no-significant decrease in HDL and ox-LDL amounts among those in low cut-off point groups (Table 3).

Multivariate analysis

Factors affecting serum BNP levels in all patients of CAD were assessed using stepwise multiple regression modeling (Table 4). All continuous variables that did not follow a normal distribution were log-transformed. Our stepwise multiple regression analysis showed that several independent variables significantly affected serum BNP levels in patients with stable CAD. In Model 1, total cholesterol significantly predicted serum BNP levels (βeta=0.998, $p<0.05$). However, introducing OX-LDL in Model 2 significantly reduced the effect of cholesterol on BNP levels (βeta=0.519, $p<0.05$), indicating that OX-LDL may be a more important predictor of BNP levels in CAD patients than cholesterol.

Ox-LDL has a crucial role in the pathogenesis of CAD. Indeed, the effect of Ox-LDL on serum BNP levels in the second model was almost the same as that of total cholesterol. Moreover, the impact of Ox-LDL on serum BNP levels did not decrease in the subsequent models but remained a significant predictor of serum BNP levels even after the inclusion of other independent variables. The inclusion of the personal history of heart attack and MDA in the third and fourth models also had a significant impact on serum BNP levels, leading to a further reduction in the effect of cholesterol on serum BNP levels (βeta=0.443, $p<0.05$) and (βeta=0.313, $p<0.05$) respectively.

BNP rs198389 genotype

The amplification of the BNP gene was carried out, and the amplicons were gel electrophoresed, as shown in Figure 1. The rs198389 polymorphism of BNP was genotyped. As illustrated in Figure 2, the heterozygous and homozygous variants of BNP rs198389 were determined

Table 3. Risk factors predicted by the cut-off value of BNP activity in the study population.

Categorical Variables	BNP>54.842	BNP ≤ 54.842	*p*
Total cholesterol (mg/dL)	141.3±3.333	158.7±8.032	0.0416
TG (mg/dL)	154.1±7.184	180.5 ±12.59	0.033
HDL (mg/dL)	34.65±1.151	32.23±1.493	0.3074
LDL (mg/dL)	85.72±2.958	97.08±7.262	0.155
Cholesterol/HDL	4.351±0.164	5.117±0.336	0.0483
TG/HDL	4.961±0.363	5.988±0.549	0.0472
OX-LDL pg/mL	179.8±8.699	170.9±7.680	0.8807
Urea (mg/dL)	33.94±1.203	41.38±2.839	0.0332
Creatinine (mg/dL)	0.857±0.020	0.917±0.050	0.3605
Age (year)	50.81±1.160	52.10±2.093	0.529
BMI (kg/m^2)	29.53±1.03	28.01±0.49	0.103

Table 4. Stepwise multiple regression analysis on serum BNP as a dependent variable in a whole study population.

	Model	B	beta	Partial correlation	95%CI		Adjusted R^2	F	P
					Lower	Upper			
1	**Cholesterol**	0.935	0.998	0.998	0.923	0.946	0.996	2556	0.000
2	**Cholesterol**	0.486	0.519	0.568	0.339	0.632	0.997	37.205	0.000
	Ox-LDL	0.431	0.481	0.539	0.291	0.571			
3	**Cholesterol**	0.415	0.443	0.52	0.284	0.58	0.998	13.626	0.000
	Ox-LDL	0.489	0.546	0.604	0.334	0.615			
	Personal heart attack	0.088	0.022	0.363	0.015	0.109			
4	**Cholesterol**	0.293	0.313	0.3335	0.118	0.492	0.998	5.393	0.022
	Ox-LDL	0.407	0.454	0.497	0.302	0.583			
	Personal heart attack	0.094	0.023	0.391	0.015	0.108			
	MDA	0.202	0.221	0.239	0.011	0.308			

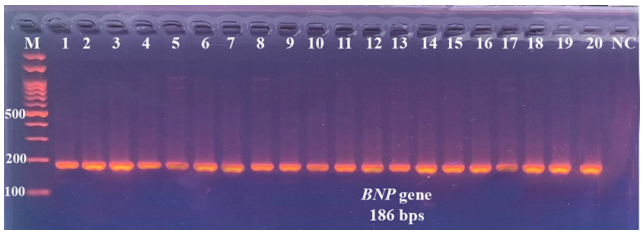

Figure 1. Gel-electrophoresis documentation. Lane L: a standard 100-bp marker, Lane 1 to 20 BNP gene (186 bp). Lanes 21: negative control.

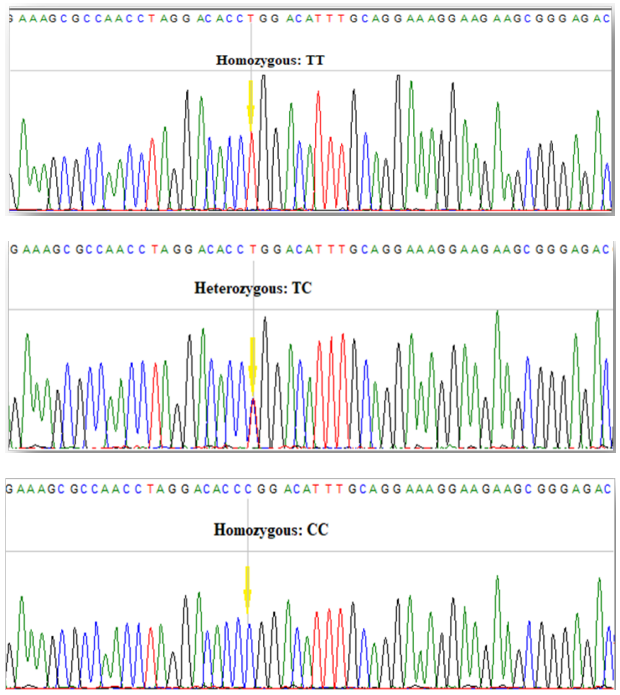

Figure 2. DNA sequencing chromatograph for the BNP gene rs198389 (A) reference allele, homozygous genotype: TT; (B) heterozygous genotype: TC; (C) homozygous genotype: CC.

to be TC and CC, while the TT genotype was considered a reference allele.

BNP rs198389 genotype polymorphism with CAD

Results showed that the wild homogeneous TT genotype was less common in stable CAD patients than non-CAD with significant change. The OR: 0.23, CI 95%: 0.08 to 0.64. They indicated a significant protective factor against getting the disease. The mutant heterogeneous TC was a risk factor for the disease with OR: 1.73, CI 95%: 0.65 to 4.81. While the mutant homogenous CC genotype was also the risk factor with nearly three folded impacts on getting the disease, OR: 2.86, CI95%: 0.98 to 9.33. The dominant genotype of stable CAD patients and non-CAD has a significant impact as a protective effect against the developing the disease, $p<0.05$, while the recessive genotype variants TC+CC has an impact as a risk factor for the disease progression with a 4.4 fold effect, and $p<0.05$.

Regarding the allele frequency, the T allele had a protective effect against the disease with the OR: 0.34, CI 95%: 0.17 to 0.68. While the C allele is the risk factor for getting the disease with almost three-folded impacts, OR: 2.91, CI95%: 1.47 to 5.84. Table (5)

The interaction between the BNP rs198389 polymorphism and CAD risk factors.

The combined impacts of BNP genotype and specific CAD risk factors were determined; Coronary artery disease is influenced by factors such as dyslipidemia, kidney dysfunction, and BNP SNPs. In this research, we examined the impact of specific risk factors in individuals with different genotypes, specifically focusing on the C allele (variants TC and CC) and the reference allele (TT) of the BNP rs198389 polymorphism (Figure 3). Those participants with CAD and the TT or TC and CC genotype exhibited a significant increase in triglycerides, cholesterol/HDL-ratio, and TG/HDL-ratio, urea compared to the non-CAD group with the TT or TT ad TC genotype. Moreover, individuals with CAD and the TT or TC and CC genotype demonstrated significantly lower HDL levels than ' 'the participant's non-CAD group with the TT or TC and CC genotype. Additionally, Total Cholesterol was non-significantly lower in CAD individuals with the TC and CC genotypes compared to the CAD group with the TT genotype.

Furthermore, our study revealed a non-significantly elevation in levels of BNP and OX-LDL in CAD participants with the TT or TC and CC genotypes compared to the non-CAD group with the TT or TC and TT genotype. However, the two groups observed no significant differences in creatinine levels. High lipid profile, kidney dysfunction, increased BNP, and OX-LDL were risks for developing CAD. These changes were observed in the reference and mutated genotypes among individuals with CAD and those without CAD. Thus, we did not identify any significant interactions between the BNP rs198389 polymorphism and risk factors.

Discussion

The main findings of the present study are; first, our re-

Table 5. The frequency distribution of genotype BNP T-381C promoter region between CAD patient and control BNP gene with odd ratio.

Genotypes	Patients	Control	OR	CI 95%	*P* value	X^2
	N=62	N=31				
TT	12	16	0.23	0.08 to 0.64	0.002	10.11
TC	28	10	1.73	0.65 to 4.81	0.16	1.4
CC	22	5	2.86	0.98 to 9.33	0.042	3.717
TT	12	16	0.23	0.08 to 0.64	0.002	10.11
TC+CC	50	15	4.44	1.56 to 12.72	0.002	10.11
T	52	42	0.34	0.17 to 0.68	0.001	10.95
C	72	20	2.91	1.47 to 5.84	0.001	10.95

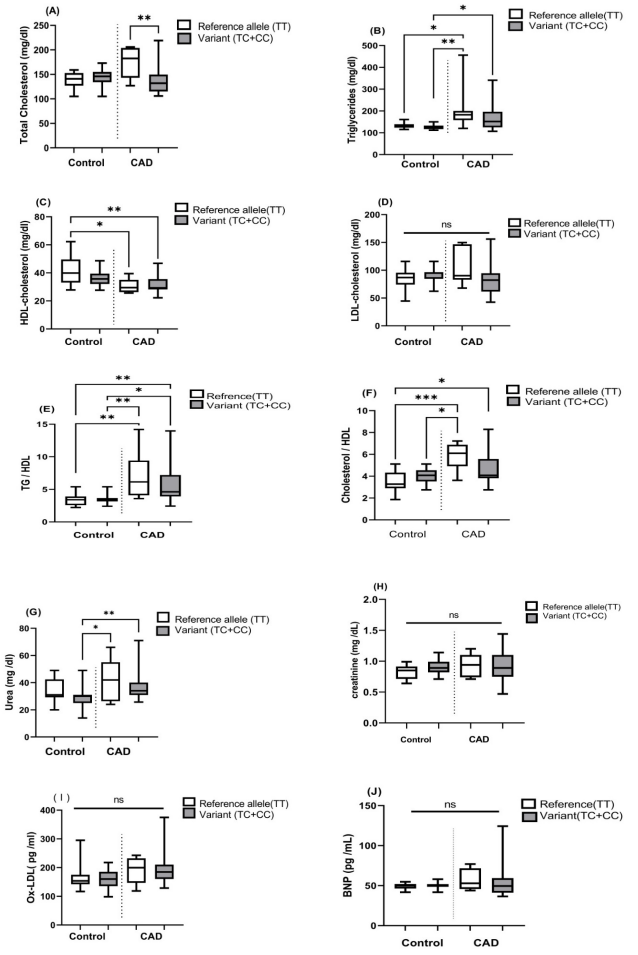

Figure 3. Relationships between the BNP rs198389 polymorphism genotype in stable coronary artery disease risk factors. CAD (reference allele TT; variant TC CC) groups.

sults verified previous findings regarding the relationship between traditional risk factors and CAD. Specifically, we observed a higher prevalence of BMI, smoking habit, hypertension, advanced age, personal history of diabetes mellitus, personal history of heart attack, and family history of hypertension in the CAD group compared to the non-CAD group. These findings underscore the importance of comprehensive risk assessment and management of these modifiable risk factors in preventing and controlling CAD (22).

Second, BNP activity levels in a group with the below cut-off BNP≤ 54.842 displayed elevated levels of total cholesterol, TG, cholesterol/HDL-ratio, TG/HDL-ratio, urea, and creatinine. Low BNP activity levels could be related to lipid disorders and impaired kidney function, both of which have been identified as risk factors for cardiovascular disease. Several organs and tissues express natriuretic peptide (NP) receptors, including blood vessels, kidneys, skeletal muscle, and adipose tissue (23). BNP inhibits cholesterol synthesis in human adrenocortical cells, especially when stimulated by angiotensin II (24). According to Potter *et al.* (25), NP receptors, which include the BNP receptor, are predominantly expressed in the adipose tissue and the kidneys.

Several factors are correlated with B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide (NT-proBNP), including sex, high age, and renal dysfunction (26). Additionally, BMI and NT-proBNP levels were identified as potential factors influencing the associations between cardiac natriuretic peptides and lipid profiles, particularly total cholesterol. Notably, the strength of this association was found to be stronger in older subjects than younger ones. This observation can be partly explained by the fact that older patients are more likely to have higher levels of natriuretic peptides due to an increased prevalence of cardiovascular comorbidities and age-related changes in cardiac structure and function (27). Furthermore, older individuals commonly exhibit lower total cholesterol levels, which may be influenced by lipid-lowering therapy or clinical conditions such as malnutrition and frailty (28,29). On the other hand, increased expression of BNP or genetic/pharmacological cyclic guanosine 3′,5′-monophosphate (cGMP) enhancement in multiple animal studies model stimulates adipose tissue browning and lipid oxidation, which promoted the biosynthesis of mitochondria and fat oxidation in skeletal muscles, which prevented obesity and glucose intolerance (30–32). Van Kimmenade RRJ et al. observed that cardiac NPs directly impact the major determinants of LDL levels, including LDL receptor (LDLR) and (PCSK9). PCSK9 receptor is expressed in the adipose tissues of humans and has to be regulated in opposing ways by insulin and ANP (32,33). An increase in BNP level with age is partly related to a drop in the estimated glomerular filtration rate (eGFR). This age-related rise in BNP could be a protective mechanism promoting favorable lipid metabolism. In obese individuals, nevertheless, the concentration of natriuretic peptides is lower. One possible explanation for this is increases in clearance as natriuretic peptides are involved in lipolysis, and BNP receptors have been detected in adipose tissue (34,35).

Third, a multivariate stepwise regression model was employed to identify which clinical and anthropometric variables were independently correlated with BNP. Numerous anthropometric and biochemical variables have been individually introduced to the model. Among these variables, total cholesterol, OX-LDL, personal history of heart attack, and MDA revealed a direct association with BNP among all of these variables. Interestingly, OX-LDL was a more significant predictor of BNP levels in patients with CAD. Furthermore, ox-LDL's effect on BNP levels remained significant even when other independent variables were accounted for in subsequent models. Notably, including heart attack history and MDA in the analysis models showed substantial effects on serum BNP levels.

Acute exposure of adult rat ventricular cardiomyocytes to ox-LDL alters intracellular calcium handling. The findings of this study suggest that increased levels of ox-LDL could lead to ventricular dysfunction before a coronary event, especially in people with a high risk of cardiovascular disease, even at young ages. Ox-LDL also inhibits systolic Ca2+ release and induces abnormal diastolic Ca2+ release, which raises the risk of arrhythmias. These detrimental effects on Ca2+ cycling in cardiomyocytes may lead to abnormal intracellular Ca2+ dynamics, impairing cardiac function resulting from abnormal intracellular Ca2+ cycling (36).

Studies on the young cohort showed ox-LDL levels elevated with cardiovascular risk over a lifetime. The one exception was individuals with stable CAD, which had lowered ox-LDL levels than those with low lifetime cardiovascular risk despite having a high BMI and increased prevalence of males and hypertension. Individuals with stable CAD are observed to be at high risk for cardiovascu-

lar disease, but this risk might be reduced through pharmacological and lifestyle management (36,37). Young individuals with stable CAD expertise had the same systemic oxidative stress as those with low cardiovascular risk and a healthy lifestyle. All participants in this research with stable CAD had taken statins, which have been shown to reduce ox-LDL levels (38). Thus, the lipid-lowering and antioxidant capabilities of statins; might contribute to maintaining low ox-LDL levels in individuals with stable CAD that have been well-controlled (39,40). The relationship between NT-pro-BNP and ox-LDL suggests that ox-LDL may be directly associated with cardiac function before and after the onset of cardiovascular disease. Ox-LDL has been linked to reduced cardiac function in the population and patients with congestive heart failure (41,42). The association between NT-proBNP levels and ox-LD-Lwhich typically correlates with oxidative stress, may be influenced by conventional cardiovascular risk factors. In addition, it has been stated that LDL levels directly affect ox-LDL levels, whereas hypertension is associated with volume overload and controls NT-proBNP levels. The multivariate regression analysis was estimated to determine whether NT-proBNP has been affected by ox-LDL independently of LDL, systolic blood pressure, and traditional cardiovascular risk factors. Positive correlations were found between NT-proBNP levels, ox-LDL, and systolic blood pressure, indicating that each variable independently affected NT-proBNP levels. Even with controlling for LDL and traditional cardiovascular risk factors, the relationship between NT-proBNP and ox-LDL remains significant (43-45).

Fourth, BNP rs198389 genotype polymorphism and relationship with CAD risk factors, the wild homogeneous TT genotype was less common in stable CAD patients than non-CAD with significant change, which is the protective factor against getting the disease. The mutant heterogeneous TC and homogenous CC genotypes were risk factors for getting the disease. Pfister *et al.* (46) determined that genotypes of the rs198389 (T-381C polymorphism) were correlated with BNP levels. There was no relationship between these genotypes and the risk of heart failure (HF). Costello-Boerrigter (47) identified non-significant variants in genotype frequencies of the BNP gene polymorphism (T-381C) between different study groups, which includes patients with HF and CAD ($p>0.05$). Nonetheless, a correlation was found between genotypes and BNP and NT-proBNP levels. Particularly, the CC genotype was correlated with higher BNP and NT-proBNP levels than the TT and TC genotypes (CC > TC > TT, $p<0.05$ for all assays). Takeishi *et al.* (18) found in a separate study that participants who carried the homozygous C allele had more BNP levels compared to individuals with the homozygous T allele and those who were heterozygous, and the study showed the fact that the -381C allele was associated with higher BNP levels and increased BNP promoter activity within reporter gene assays in a large Japanese adult general population.

In summary, this study investigated the findings that BNP activity levels below the established cut-off value, along with elevated levels of lipid profiles and oxidative stress markers, are associated with an increased risk of developing CAD. Ox-LDL plays a more crucial role in the pathogenesis of CAD. On the other hand, the TC and CC genotypes were identified as risk factors for CAD development. While the T allele was associated with a protective effect, and the C allele increased the risk of developing CAD. No significant interactions were observed between this study's BNP rs198389 polymorphism and risk factors.

Conflict of competence

The authors declare that they have no conflict of interest.

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Contribution of all authors to the papers

All authors have contributed significantly to this research. (G.A. Sh.) took responsibility for collecting samples, laboratory investigations, statistical assessment, and manuscript composition. (M.S. Sh.) and (K.A. M.) played roles in the conception, and design, with the interpretation of the research's results and offered valuable input and feedback throughout the development of the manuscript. All the authors carefully reviewed and approved the final draught of the manuscript.

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