Original Research

Brain-derived neurotrophic factor gene polymorphism in post-ST-elevation myocardial infarction patients undergoing primary percutaneous intervention

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ABSTRACT

Introduction:The goal of this study was to elucidate a link of brain-derived neurotrophic factor (BDNF) Val66Met gene with combined 6-month clinical end points in post-myocardial infarction patients. **Methods**: 256 post-myocardial infarction patients who underwent primary percutaneous coronary intervention were enrolled in the study. Variants of Val66Met gene BDNF were identified by real-time polymerase chain reaction at baseline. **Results**: The combined clinical end points (major cardiovascular events and hospitalization) were determined in 61 (23.8%) post-STEMI patients; consequently, 195 (76.2%) patients did not meet the events. Univariate linear regression revealed that predictors for combined clinical end points were peak TnI levels, NT-proBNP, SYNTAX score, TIMI score, obesity, left ventricular ejection fraction, and genotype 66ValMet+66MetMet in BDNF gene. The cumulative clinical outcomes (major adverse cardiac events and admission) were determined in 61 (23.8%) patients. Kaplan-Meier curves demonstrated that 66ValVal genotype of BDNF gene was significantly associated with the low number of combined end points. **Conclusion**: The Val66Met polymorphism in BDNF gene independently predicted 6-month combined clinical end points in post-myocardial infarction patients.
Key words: ST-segment elevation myocardial infarction, single nucleotide polymorphism Val66Met, brain derived neurotrophic factor, outcomes

INTRODUCTION

Recurrent major adverse cardiac events (MACEs) and heart failure (HF) remain the most common causes of premature cardiovascular (CV) mortality amid post-ST-segment elevation myocardial infarction (STEMI) patients, regardless of wide implementation of early re-vascularization strategies including primary percutaneous coronary intervention (PPCI)[1,2]. In fact, conventional pure approaches based on clinical assessment, transthoracic echocardiography and biomarkers of myocardial necrosis are insufficient today to catch vulnerable post-STEMI patients after successful PPCI[3]. Although there are several biomarkers (natriuretic peptides, high sensitive cardiac troponins, soluble ST2, *etc.*) that are designed for risk stratification among patients with STEMI[4–6], the risk assessment and prediction of poor clinical outcomes after successful competed PPCI in post-STEMI patients are uncertain[7]. There are several limitations that could decrease the predictive value of conventional biomarkers in post-STEMI individuals, such as older age, morbid obesity, diabetes mellitus (DM), preserved or normal left ventricular (LV) ejection fraction (EF), co-existing kidney insufficiency and atrial fibrillation[8–10].

Neurotrophins are a superfamily of regulatory proteins which regulate proliferation, differentiation, survival and plasticity of neurons[11]. Brain-derived neurotropic factor (BDNF) was previously found as one of the neurotrophins with proliferative, cholinergic, serotoninergic and dopaminergic activities, and is predominantly synthesized in central and peripheral neurons[12]. Later, an expression of mRNA BDNF was found in myocardium, vessel vasculature, skeletal muscles, parenchymal organs (including lung, spleen and kidney), visceral epithelial cells, and mature and progenitor endothelial cells[13–15]. Previous animal and clinical studies have revealed that BDNF through an activation of nuclear factor kB receptors mediates endothelial cell survival and neoangiogenesis, reduces p75-mediated apoptosis of cardiac myocytes, enhances endothelial function, regulates blood flow in ischemic myocardium, and improves LV function after ischemic injury, thereby providing cardioprotective effects[16,17].

Observational and clinical investigations have shown strong inverted associations between BNDF levels in peripheral blood and cognitive dysfunction in HF

patients, and CV risk in individuals with known DM, hypertension, cardiomyopathy, stable coronary artery disease and STEMI [18–21]. However, circulating levels of BDNF exhibited a close correlation with single nucleotide polymorphism (SNP) in BDNF gene that was associated with a replacement of valine to methionine in the 66 position of codon (Val66Met). It has been suggested that this SNP Val66Met affects the intracellular processing of the peptide and leads to a decline in the secretion of BDNF, resulting in lowered susceptibility to ischemia and myocardial injury [22,23]. The aim of the study was to elucidate a link of BDNF Val66Met gene with cumulative 6-month clinical end points in post-myocardial infarction patients.

MATERIAL AND METHODS

Patient population

From 2016 August to 2019 February, 320 patients underwent selective coronary angiography due to suspected acute STEMI. Acute STEMI was diagnosed in accordance to the European Society of Cardiology (ECS) recommendation (2017) [24]. The study flow chart is shown in **Figure 1**. We identified 256 acute STEMI individuals who underwent PPCI with an implantation of bare-metal stent "COMMANDER" manufactured by "Alvimedica" (Turkey) in culprit artery (1 to 4 stents onto ischemic-related arteries) and discharged with post-PPCI TIMI III score from the hospital. All patients were treated with current adjuvant care [24].

Coronary angiography

We used radial or femoral vascular access and two-to-three orthogonal projections to receive angiograms at baseline. A digital X-ray system "Integris Allura-9" (Philips Healthcare, Eindhoven, Netherlands) was used to visualize coronary anatomyto visualize coronary anatomy. Ultravist-370 (Baier Pharma, GmbH, Germany) was the contrast giving automatically through the injector.

Ethical declaration

All patients enrolled in the study gave voluntary informed consent to participate. The study was approved by the local ethics committee (Protocol №8, 29.08.2016). The study had been carried out in accordance with the 1964 Helsinki declaration.

Sample size calculation

We calculated sample size by taking into consideration the effect size estimation (0.99), type II error (0.2), type I error (0.05), expected mortality rate of 7%, and one-year mortality rate of 14% [25]. The sample size was 250 individuals.

Determination of risk factors and co-morbidities

DM was diagnosed as serum level of fasting glucose of > 7.0 mmol/L, 2-h postprandial glucose serum level of > 11.1 mmol/L, or current therapy of anti-diabetic drugs [26]. Hypercholesterolemia (HCE) hypertension was diagnosed in accordance with current recommendation [27,28]. HF was diagnosed according to current ECS clinical guidelines [29]. Information of other diseases and medical history were obtained by investigators through a review of medical history or direct contact with the patient's general practitioner.

Echocardiography examination

Transthoracic B-mode echocardiography and Tissue Doppler Imaging were carried out using 3.5 MHz phase probe with "Aplio 500" (TUS-A500, Toshiba Inc., Japan) at baseline. LVEF were measured by Simpson's method. LV global longitudinal strain (e') and early transmitral velocity (E) were measured by tissue Doppler imaging technique and impulse transmitral Doppler regime, respectively.

Determination of STEMI prognosis

We used the TIMI score and the GRACE score to validate prognostic capacity after STEMI [30,31].

SYNTAX score determination

SYNTAX score (SS) was calculated by an experienced interventional cardiologist accordingly [32].

Determination of endpoints

The primary endpoint was MACEs in combination with hospitalization that occurred during 6-month period post-discharge period. MACEs included CV death, recurrent angina, and newly diagnosed HF. The endpoint was ascertained by personal or phone contact with a general practitioner or staff of the hospital in which the patient had been admitted.

Calculation of glomerular filtration rate

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate glomerular filtration rate (GFR) [33].

Collection of blood samples

Venous blood samples were collected from the patients using vacutainers at baseline and at the end of the study. After 30-minute centrifugation, the serum
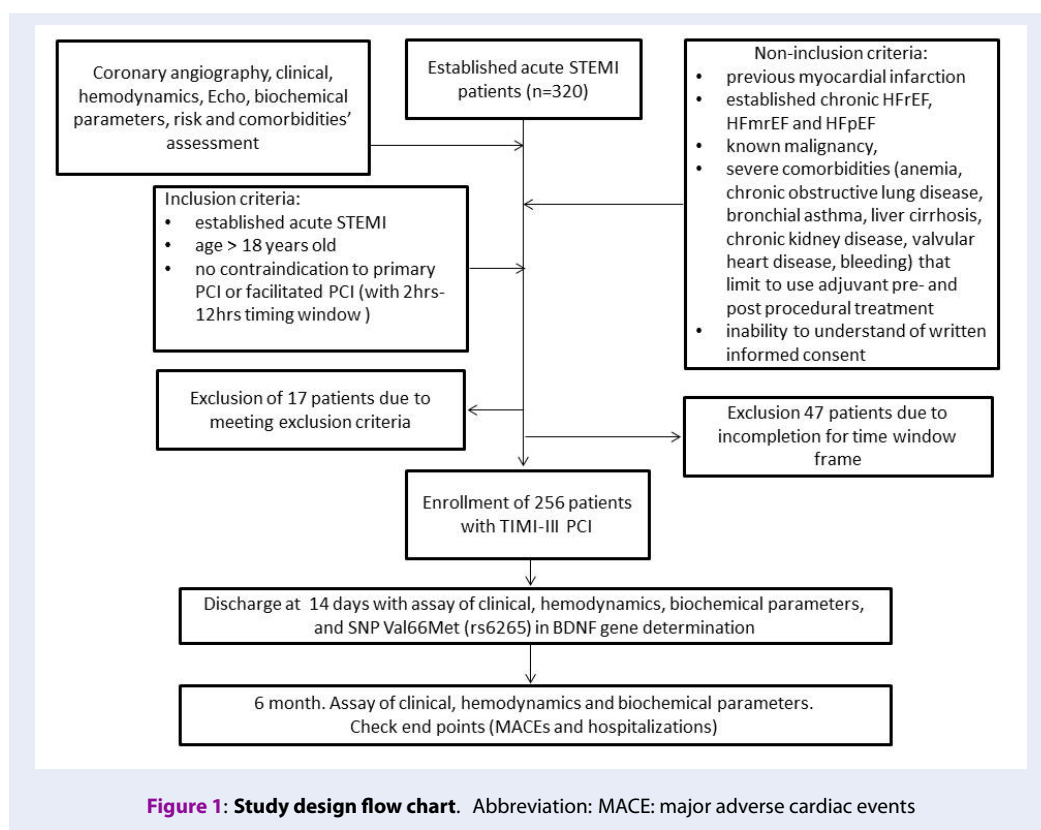
Figure 1: **Study design flow chart**. Abbreviation: MACE: major adverse cardiac events

was collected and then stored at -70^{0}C until transfer to the laboratory of GI "L.T.Malaya TNI NAMSU". Hematology and biochemistry, including lipid profiles and fasting glucose, were provided for each patient.

Serum levels of troponin I (TnI) were measured by chemo-luminescent immunoassay method on the biochemical analysator (Humalyser 2000, Mannheim, Germany). The average of the TnI level was 0.5-50 ng/mL.

N-terminal fragment of brain natriuretic peptide (NT-proBNP) was evaluated by use of a commercially available kit manufactured by R&D Systems GmbH (Wiesbaden-Nordenstadt, Germany). The average of the NT-proBNP level was 10-12000 pg/mL.

SNP Val66Met (rs6265) in BDNF gene determination

The extraction of DNA was executed per the conventional protocol for a commercial set (TacMan TMSNP Genotyping Assays; Thermo Fisher Scientific Assay IDC_11592758_1). The BDNF genetic variants were evaluated by real-time polymerase chain reaction (PCR). Primers used in the BDNF Val66Met (rs6265) polymorphism assay were as follows: CCTACAGTTCCACCAGGTGAGAAGAGTG (forward), TCATGGACATGTTTGCAGCATCTAG-GTA (reverse).

Statistics

Baseline characteristics are presented as mean $\pm$ standard deviation (SD) or median (IQR) for continuous variables and proportions for categorical variables. Chi-square test or Mann-Whitney U test and T-test were used to compare the categorical or normally-distributed data, respectively. Hardy–Weinberg Equilibrium was conducted to estimate allele frequencies. Spearman correlation analysis was performed to investigate the relationship between circulating levels of cardiac biomarkers, co-morbidities, and BDNF gene SNP. Univariate and multiple variate linear regression analyses were done; factors that predict the combined endpoint were determined. The beta coefficient, standard error (SE), odds ratio (OR), and 95% confidence interval (CI) for predictors were evaluated. We also checked whether five common subject-level gene models (recessive, multiplicative, additive, dominant, and over-dominant models) were related to the explanatory variables[34]. We also used area under curve (AUC), integrated discrimination indices (IDI), and net-reclassification improvement (NRI) for prediction performance analyses. Significant differences

between variables were set at p-value < 0.05.

RESULTS

The combined clinical endpoints (MACEs and hospitalization) were determined in 61 (23.8%) post-STEMI patients; consequently, 195 (76.2%) patients did not meet the events. General clinical characteristics of the entire patient population enrolled in the study and both cohorts (with MACEs and free MACEs) are reported in **Table 1**. The entire population consisted of participants with mean age of 58.76 years old, with male/female proportion of 77.3%/22.7%, with CV risk factors including hypertension (52%), type 2 DM (T2DM) (19.5%), hypercholesterolemia (63.7%), obesity (37.5%), and smoking (34.9%). We did not find significant differences between both cohorts of the post-STEMI patients in terms of demographics, glomerular filtration rate, and CV risk factor presentation, except in the incidence of T2DM and hypertension, which were more frequently seen in patients who met MACEs than in individuals who were free of MACEs (P = 0.032 and P = 0.0008, respectively). Therefore, participants with MACEs were older that those who did not meet MACEs (P = 0.027).

The patients who met MACEs exhibited higher peak serum levels of troponin I and circulating levels of NT-proBNP than those who had no MACEs. Significant differences in lipid profiles among both patient cohorts were not found.

The observed frequency of Val66Met BDNF genotype in post-STEMI patients was as follows: 66ValVal — 74.2% and 66ValMet+66MetMet — 25.8%. Genotype frequency matched to Hardy–Weinberg equilibrium ($\chi^2 = 0.17$, P > 0.05). There were not differences between both cohorts in terms of phenotype of BDNF (P = 0.272).

All post-STEMI patients were treated according to contemporary clinical protocol and there were not significant differences in concomitant medications, except for metformin which was prescribed frequently in the patient cohort with MACEs (P = 0.046). We found that TIMI risk in patients with MACEs was significantly higher in comparison with individuals who had no combined endpoint (P = 0.046) (**Table 2**). Nevertheless, the number of participants in both cohorts having 22 – 32 SYNTAX score points and ≤ 22 SYNTAX score points did not differ, but the proportion of the patients with MACEs having > 32 SYNTAX score points occurred frequently when compared with those who did not meet the endpoint. Additionally, total GRACE score points were similar in both patient cohorts (P = 0.294).

Significant differences between both cohorts in terms of localization of STEMI were not found, even though one vessel injury frequently occurred in MACEs-free patients. In contrast, patients having MACEs represented frequent circumflex artery injury (p = 0.021). Hemodynamic characteristics in post-STEMI patients at baseline are reported in **Table 3**. The patients having MACEs had lower LVEF (P = 0.005), higher left atrium volume (P = 0.021), and E/eʿ ratio (P = 0.042) than those who had no MACEs.

Spearman correlation analysis between STEMI severity, circulating levels of cardiac biomarkers, co-morbidities and BDNF gene SNP

There were statistically significant correlations of circulating levels of NT-pro-BNP with TIMI score (r = −0.33; p = 0.001), LVEF at baseline (r = −0.42; p = 0.003), obesity (r = −0.26; p = 0.012), age (r = 0.27; p = 0.022), and 66ValVal genotype of BDNF gene (r = −0.41; p = 0.003). The SYNTAX score was inversely correlated with LVEF at baseline (r = −0.34; p = 0.001) and 66ValVal genotype of BDNF gene (r = −0.26; p = 0.001), and positively correlated with T2DM (r = 0.32; p = 0.002).

Univariate and multivariate linear regressions

Univariate linear regression revealed that predictors for the combined clinical endpoints were peak TnI levels, NT-proBNP, SYNTAX score, TIMI score, obesity, LVEF, and genotype 66ValMet+66MetMet in BDNF gene (**Table 4**). Unadjusted multivariate linear regression showed that the peak of TnI levels, NT-proBNP, SYNTAX score, TIMI score, and 66ValMet+66MetMet genotype in BDNF gene all remained independent predictors for the combined clinical endpoints. After adjustment for SYNTAX score and TIMI score, genotype 66ValMet+66MetMet in BDNF gene (OR = 1.5476; 95% CI = 1.1277 – 4.1426; P = 0.0246) and NT-proBNP (OR = 1.7546; 95% CI = 1.0219 – 3.1002; P = 0.046) independently predicted cumulative clinical endpoint.

Comparison of the predictive abilities of the models

We compared both models (genotype 66ValMet+66MetMet in BDNF gene and NT-proBNP) for STEMI individuals (**Table 5**). The genotype 66ValMet+66MetMet in BDNF gene was better than NT-proBNP and standard model, while the only adjusted models were entered in the analysis.

Table 1: Clinical characteristic of STEMI patients enrolled in the study

Variables	Entire STEMI population (n = 256)	Patients who met MACEs (n = 61)	Patients who did not meet MACEs (n = 195)	χ^2,p
Age, years, M (SD)	58.76±9.85	61.07±9.39	58.13±10.02	0.027
Male, n (%)	198(77.3)	42(68.9)	156(80.0)	0.070
Female, n (%)	58(22.7)	19(31.1)	39(20.0)	
Hypertension, n (%)	133(52.0)	39(63.9)	94(48.2)	0.032
T2DM, n (%)	50(19.5)	21(34.4)	29(14.9)	0.0008
Smoking, n (%)	89(34.9)	27(44.3)	62(31.8)	0.074
HCE, n (%)	163(63.7)	42(68.9)	121(62.1)	0.335
BMI > 30 kg/m^2, n (%)	96(37.5)	24(39.3)	72(36.9)	0.733
GFR, ml/min/1.73 m^2	104.67±27.56	103.68±27.77	107.50±26.96	0.389
Circulating biomarkers				
Peak TnI, ng/ml	18.4 [5.44-87.3]	21.56 [11.55-99.45]	13.18 [5.97-68.5]	0.038
NT-proBNP, pg/mL	246.81 [26.78 – 610.97]	415.12 [74.45-1305,42]	202.43 [54.48-802.60]	0.001
TC, mmol/l	4.97[4.00-5.75]	5.13[4.26-5.87]	4.89[3.97-5.68]	0.192
HDL, mmol/l	1.09 [0.90-1.28]	1.12 [0.94-1.28]	1.08 [0.90-1.28]	0.534
LDL, mmol/l	3.00[2.11-3.71]	3.20[2.45-3.98]	2.86[2.06-3.63]	0.078
TG, mmol/l	1.53 [1.17-2.02]	1.48 [1.17-1,83]	1.57 [1.17-2.06]	0.455
SNP polymorphism of BDNF gene				
66ValVal, n (%)	190(74.2)	42(68.9)	148(75.9)	0.272
66ValMet+66MetMet, n (%)	66(25.8)	19(31.1)	47(24.1)	
Concomitant medications				
Beta-blockers, n (%)	256 (100)	61 (100)	195 (100)	1.00
ACEI / ARBs, n (%)	245 (95.7)	58 (95.1)	187 (95.9)	0.98
Clopidogrel /Ticagrelor, n (%)	252 (98.4)	58 (95.1)	194 (99.5)	0.99
Statins, n (%)	256 (100)	61 (100)	195 (100)	1.00
MCRAs, n (%)	178 (69.5)	43 (70.5)	135 (69.2)	0.94
Metformin, n (%)	32 (12.5)	10 (16.4)	22 (11.3)	0.046
Loop diuretics, n (%)	71 (27.7)	20 (32.7)	51 (26.2)	0.12

Abbreviations:
BMI: bodymass index; HCE: hypercholesterolemia; LDL: low-density lipoprotein; HDL: high-density lipoprotein; T2DM: type 2 diabetes mellitus; TG: triglycerides; Tn: cardiac troponin; ACEI: angiotensin-converting enzyme inhibitor; ARBs: angiotensin-II receptor antagonist; MCRA: mineralocorticoid receptor antagonist

Table 2: STEMI localization and injured coronary arteries in patient population

Variables	Entire STEMI population (n = 256)	Patients who met MACEs (n = 61)	Patients who did not meet MACEs (n = 195)	χ^2,p
STEMI scores				
TIMI risk score, point	6 [4-7]	8 [5-9]	6 [4-8]	0.046
Total SYNTAX score, point	28.7±6.15	27.54±6.41	25.65±8.82	0.134
> 32 points, n (%)	76 (42.9)	41 (54.6)	35 (34.3)	0.011
22 - 32 points, n (%)	79 (44.6)	38 (50.7)	41 (40.2)	0.167
≤ 22 points, n (%)	22 (12.4)	10 (13.3)	12 (11.8)	0.765
Total GRACE Score, points	150 (120-172)	143 (117-170)	152 (119-176)	0.294
STEMI localization				
Anterior, n (%)	126(49.2)	30(49.2)	96(49.2)	0.995
Posterior, n (%)	113(44.1)	22 (36.1)	91(50.8)	
Lateral, n (%)	17 (6.6)	9 (14.8)	8 (4.1)	
Culprit coronary arteries				
One vessel injury, n (%)	96(37.5)	15(24.6)	81(41.5)	0.017
Two vessels injury, n (%)	69 (26.9)	19(31.1)	51(26.2)	0.445
Three and more vessels injury, n (%)	70 (27.3)	22(36.1)	48(24.6)	0.080
Quantity of stenosis	2.0 [1.0-4.0]	2.0 [1.0-4.0]	2.0 [1.0-4.0]	1.0
Left anterior descending injury, n (%)	185(72.3)	46(75.4)	139(71.3)	0.530
Right coronary artery injury, n (%)	142(55.5)	42(68.9)	107(54.9)	0.053
Circumflex artery injury, n (%)	106(41.4)	33(54.1)	73(37.4)	0.021
Left coronary artery injury, n (%)	24(9.4)	8(13.1)	16(8.2)	0.92

Table 3: Hemodynamic characteristics in post-STEMI patients at baseline

Variables	Entire STEMI population (n = 256)	Patients who met MACEs (n = 61)	Patients who did not meet MACEs (n = 195)	P value
HR, per minute	76±15	79±16	76±15	0.079
Systolic BP, mm Hg	134±25	123±22	138±25	0.003
Diastolic BP, mm Hg	80±12	77±12	81±12	0.019
LV EDV, ml	136±37	137±45	136±35	0.930
LV ESV, ml	64±28	69±36	63±25	0.516
LVEF, %	51±10	48±11	52±10	0.005
LA, cm	4.10±0.51	3.68±0.54	4.04±0.53	0.477
LAV, ml	56±7	57±10	54±9	0.021
E/e', unit	11.6±4.28	12.89±5.34	11.31±4.86	0.042

Abbreviations:
HR: heat rate; LAD: left atrium diameter; LAV: left atrium volume; LVEDV: left ventricular end diastolic volume; LVESV: left ventricular end systolic volume; LVEF: left ventricular ejection fraction; BP: blood pressure

Table 4: The factors contributing to 6-month combined clinical end point in post- STEMI patients: The results of univariate and multivariate linear regressions

Variables	Univariate linear regressive analysis ($\chi^2 = 22.917$ P = 0.0182)				Multivariate linear regressive analysis ($\chi^2 = 15.244$ P = 0.0042)			
	β-coefficient	OR	95% C	P value	β-coefficient	OR	95% C	P value
Age	0.03101	1.0315	1.0000 – 1.0640	0.0503	-			
Peak TnI	0.74033	2.0966	1.0945 – 4.4990	0.0470	0.86154	1.6132	1.0751 – 4.2210	0.0480
NT-proBNP	0.8244	2.4746	1.1233 – 4.6833	0.0410	0.92370	1.8144	1.0330 – 3.1122	0.0420
SYNTAX score	1.17560	1.9428	1.2493 – 3.5422	0.0244	1.41380	1.6844	1.1830 – 2.3655	0.0234
TIMI score	1.37250	1.8970	0.9720 – 2.880	0.0410	1.17280	1.0940	1.010 – 1.3240	0.0420
BMI > 30 kg/m^2	1.12320	2.1448	0.4607 – 3.8995	0.0383	1.02	1.9560	0.0774 – 3.4539	0.0526
Type 2 diabetes mellitus	-0.3267	0.7213	0.5020 – 1.0364	0.0773	-			
Genotype 66ValMet+66MetMet in BDNF gene	0.74033	2.0966	1.0945 – 4.4990	0.0470	0.68301	1.9798	1.1545 – 4.1065	0.0395
Smoking	0.088830	1.0929	0.5289 – 2.2583	0.8104	-			
Anterior STEMI	0.37969	1.4618	0.8738 – 2.4455	0.1481	-			
LVEF	-0.035797	0.9648	0.9346 – 0.9961	0.0276	-0.036369	0.9643	0.9319 – 0.9978	0.0574
Constant	-2.789400	-	-	-	-			

Abbreviations:
LVEF: left ventricularejection fraction; STEMI: ST-segment elevation myocardial infarction; OR: odds ratio; CI: confidence interval

Table 5: Comparisons of predictive models with integrated discrimination indices and net-reclassification improvement

Models	Depending variable: combined end point								
	AUC			NRI			IDI		
	M	95% CI	P value	M	95% CI	P value	M	95% CI	P value
Standard model (TIMI score)	0.547	0.450 – 0.620	-	Referen	-	-	Refere	-	-
Genotype 66ValMet+66MetMet in BDNF gene + Standard model vs Standard model	0.709	0.610 – 0.810	0.023	0.31	0.27 – 0.36	0.044	0.068	0.057 – 0.077	0.049
NT-proBNP + Standard model vs Standard model	0.697	0.592 – 0.795	0.048	0.30	0.22 – 0.41	0.057	0.046	0.020 – 0.061	0.180

Abbreviations:

AUC: Area Under Curve; IDI: integrated discrimination indices; NRI: net-reclassification improvement; CI: confidence interval

Determination of genetic model variants

We found that the model based on the genotype 66ValMet+66MetMet in BDNF gene carried out an additive value because it was defined by the following equation: OR2 = 2 − 1 / OR1 (**Table 6**). This means that the regression analysis was the best fitted to the model by applying the variables which predicted cumulative clinical endpoint.

Kaplan-Meyer analysis for combined end-point accumulation trends in post-STEMI patients

Kaplan-Meier curves demonstrated that post-STEMI patients having 66ValVal genotype of BDNF gene had the lowest accumulation of combined endpoint when compared with those who had the combination of 66ValMet and 66MetMet genotypes (Cox-criterion, P = 0.019; log-rank criterion, P = 0.03) (**Figure 2**).

DISCUSSION

The results of our study showed negative associations between the 66ValMet + 66MetMet polymorphisms in the BDNF gene and 6-month endpoints in post-STEMI patients. The prognostic significance of BDNF 66ValMet polymorphism was investigated in post-STEMI patients first, while these relations were previously determined in individuals with acute coronary syndrome[35]. Therefore, there was an association between the BDNF levels and severity of HF NYHA functional class among individuals with HF with reduced LVEF (HFrEF)[21], atherosclerosis severity in stable CAD patients[36], and patients with unstable angina[37]. The exact causes for the decline in circulating BDNF levels among patients with known CV diseases (including unstable angina, acute coronary syndrome, STEMI and HF) are uncertain. Probably, BDNF can be released from skeletal muscle, and skeletal muscle dysfunction (rather than loss of skeletal muscle mass) in HF patients may explain lowered levels of circulating BDNF in HFrEF. However, it does not determine declining levels of the peptide in post-STEMI patients with adverse cardiac remodeling, HF with preserved EF, nor recurrent CV events[38-41]. Whether BDNF production is under control by myocardial function and exercise is not well-understood. In this context, lowered levels of BDNF have been ensured by SNP in the 66ValMet BDNF gene, which controls synthesis of the peptide[42].

Previous animals and clinical trials have revealed that the BDNF levels were modulated by BDNF gene polymorphism and that CV actions of circulating BDNF likely correspond to this SNP[43,44]. Moreover, low circulating levels of BDNF were associated with adverse cardiac remodeling after STEMI and higher levels of NTproBNP[45]. Nevertheless, there are investigations that have yielded no close association between the genetic variants of BDNF and the serum levels of this peptide[43,45]. There is a hypothesis that the BDNF Val66Met genotype can affect cardiac function and CV risk through other mechanisms. We found an association between circulating levels of NT-proBNP and BDNF gene polymorphism; however, there is no plausible evidence for the interrelation between BDNF gene polymorphism and cardiac function, severity of atherosclerosis, and CV risk factors (not including T2DM). Importantly, previous clinical

Table 6: Determination of genetic model variant

	ValVal	ValMet	MetMet
Patients who met MACEs (n = 61)	42	14	5
Patients who did not meet MACEs (n = 195)	148	44	3
OR1	1.12	-	-
OR2	5.23	-	-

Notes: OR1 = oddMm / oddMM; OR2 = oddmm / oddMm.
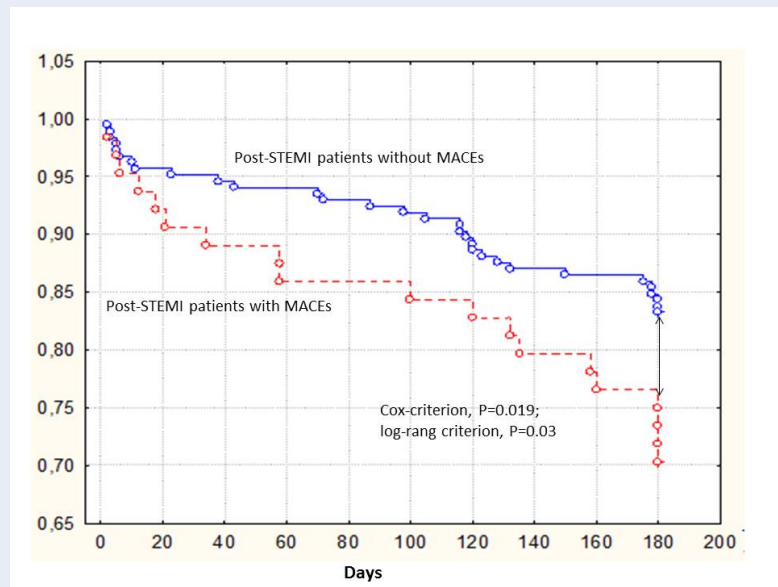
Figure 2: **Kaplan-Meier curve accumulation of combined end point after 6 month observation depending on polymorphism Val66Met in BDNF gene**. **Notes**: 1st group: genotype 66ValVal, 2nd group: 66Val-Met+66MetMet.

studies have shown associations between metabolic disease (such as morbid obesity and T2DM) and circulating levels of the BDNF, but not with the BDNF gene polymorphism [46,47]. Some researchers have proposed that adipocytokines can be triggers for the decline of BDNF levels, but BDNF Val66Met polymorphism has shown no significant association with circulating levels of leptin [47]. However, in our investigations, patients who were enrolled in these studies were not qualified as those having stable CAD or STEMI. We suggest that BDNF gene polymorphism is an independent factor contributing to MACEs and readmission to the hospital through altered cardioprotective effect. Since BDNF specifically binds to the tropomyosin-related kinase receptor B, it acts as modulator of various intracellular signaling pathways (*e.g.* Akt pathway, transforming growth factor-beta/ Smad pathway), which suppress inflammation and oxidative stress, upregulate the viability of ischemic tissue, promote differentiation of endothelial progenitor cells, and initiate neovascularization and angiogenesis in response to hypoxic and ischemia stimuli [48–51]. Interestingly, the predictive values for both the BDNF Val66Met gene polymorphism and serum levels of NT-proBNP were almost similar after adjustment for severity of atherosclerosis and STEMI. Although the results of the study demonstrated that the BDNF Val66Met gene polymorphism predicted the combined endpoint in post-STEMI patients, there is no explanation for the exact molecular mechanisms which support this effect. We believe this fact is required to be investigated in the future to clearly understand primary ways that BDNF Val66Met gene polymorphism affects the clinical outcomes of post-STEMI patients after successful PPCI.

Our study had several limitations. The first limitation was associated with the small sample size, it is important to calculate sample size such that the sta-

tistical difference between both cohorts can be discerned. However, a large clinical study is required to obtain more information regarding the BDNF gene polymorphism as a predictor for poor clinical outcomes in post-STEMI patients. The second limitation is the lack of optical coherent tomography to rule in correct expanding of stents. The third limitation relates to respectively lowered number of secondary endpoints including hospitalization due to HF, recurrent unstable angina, and STEMI and PPCI. We did not perform analysis of secondary endpoint accumulation.

CONCLUSION

We found that 66ValMet + 66MetMet BDNF gene polymorphisms independently predicts the combined 6-month endpoint in post-STEMI patients after treatment with PPCI.

ABBREVIATIONS

ACEI: angiotensin-converting enzyme inhibitor
ARBs: angiotensin-II receptor antagonist
BDNF: brain-derived neurotrophic factor
BMI: body mass index
BP: blood pressure
CI: confidence interval
CV: cardiovascular
EF: ejection fraction
GFR: glomerular filtration rate
HCE: hypercholesterolemia
HDL: high-density lipoprotein
HF: heart failure
HR: heat rate
LAD: left atrium diameter
LAV: left atrium volume
LDL: low-density lipoprotein
LV: left ventricular
LVEDV: left ventricuar end diastolic volume
LVEF: left ventricular ejection fraction
LVESV: left ventricular end systolic volume
MACEs: major cardiovascular events
MCRA: mineralocorticoid receptor antagonist
OR: odds ratio
PPCI: primary percutaneous coronary intervention
STEMI: ST-segment elevation myocardial infarction
T2DM: type 2 diabetes mellitus
TG: triglycerides
Tn: cardiac troponin

AUTHOR'S CONTRIBUTIONS

Olga V. Petyunina: conception of the study, protocol and study design developing, patient enrollment, data collection, statistical analysis, and writing of the article. Mykola P. Kopytsya: data collection, statistical analysis, and writing of the article. Alexander E. Berezin: data collection, statistical analysis, writing of the article and critical revision for intellectual content. All authors read and approved the final manuscript.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the amended Declaration of Helsinki. The institutional review board approved the study (Protocol №8, 29.08.2016), and all participants provided written informed consent.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Karayiannides S, Norhammar A, Frøbert O, James SK, Lagerqvist B, Lundman P. Prognosis in Patients With Diabetes Mellitus and STEMI Undergoing Primary PCI. J Am Coll Cardiol. 2018;72(12):1427–1428. PMID: 30213337. Available from: https://doi.org/10.1016/j.jacc.2018.06.061.

2. Paradies V, Zheng H, Chan MHH, Chan MYY, Foo DC, Lee CW, et al. Impact of time of onset of symptom of ST-segment elevation myocardial infarction on 1-year rehospitalization for heart failure and mortality. Am Heart J. 2020;224:1–9. PMID: 32259591. Available from: https://doi.org/10.1016/j.ahj.2020.03.011.

3. Chiara A, Clagnan E, Valent F. Epidemiology and mortality in an Italian region after the adoption of the universal definition of myocardial infarction. J Cardiovasc Med (Hagerstown). 2020;21(1):34–39. PMID: 31834103. Available from: https://doi.org/10.2459/JCM.0000000000000893.

4. Berezin AE. Circulating Biomarkers in Heart Failure. Adv Exp Med Biol. 2018;1067:89–108. PMID: 29352578. Available from: https://doi.org/10.1007/5584_2017_140.

5. El-Ahmadi A, Abassi MS, Andersson HB, Engstrøm T, Clemmensen P, Helqvist S, et al. Acute kidney injury - A frequent and serious complication after primary percutaneous coronary intervention in patients with ST-segment elevation myocardial infarction. PLoS One. 2019;14(12):e0226625. PMID: 31860670. Available from: https://doi.org/10.1371/journal.pone.0226625.

6. Halilčević M, Begić E, Džubur A, Šabanović-Bajramović N, Mekić M, Ejubović M, Džubur A, Štimjanin E. Cardiac biomarkers and left ventricular systolic function in acute myocardial infarction with ST- segment elevation in diabetes mellitus type 2 patients. Med Glas (Zenica). 2020;17(2). Available from: 10.17392/1094-20.

7. Zhang C, Liu H, Wang H, Tao Q, Lin X, Ge S, Zhai Z. The Predictive Value of Potential Hematological Biomarkers in Acute Coronary Syndrome. Clin Lab. 2019;65(10). Available from: https://doi.org/10.7754/Clin.Lab.2019.181232.

8. Lino DOC, Freitas IA, Meneses GC, Martins AMC, Daher EF, Rocha JHC, Silva Junior GB. Interleukin-6 and adhesion molecules VCAM-1 and ICAM-1 as biomarkers of post-acute myocardial infarction heart failure. Braz J Med Biol Res. 2019;52(12):e8658. PMID: 31778438. Available from: https://doi.org/10.1590/1414-431x20198658.

9. Mzoughi K, Chouaieb S, Zairi I, Fredj S, Ben Kilani M, Berriri S, Zili M, Kraiem S. Prognostic value of ST2 in myocardial infarction. Tunis Med. 2019;97(2):335–343.

10. McGilligan V, Watterson S, Rjoob K, Chemaly M, Bond R, Iftikhar A, et al. An exploratory analysis investigating blood protein biomarkers to augment ECG diagnosis of ACS. J Electrocardiol. 2019;57S:S92–S97. PMID: 31519392. Available from: https://doi.org/10.1016/j.jelectrocard.2019.09.002.

11. Yano H, Chao MV. Neurotrophin receptor structure and interactions. Pharm Acta Helv. 2000;74:253–260. Available from: https://doi.org/10.1016/S0031-6865(99)00036-9.

12. Skaper SD. The biology of neurotrophins, signalling pathways, and functional peptide mimetics of neurotrophins and their receptors. CNS Neurol Disord Drug Targets. 2008;7(1):46–62. PMID: 18289031. Available from: https://doi.org/10.2174/187152708783885174.

13. Kermani P, Hempstead B. Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. Trends Cardiovasc Med. 2007;17(4):140–143. PMID: 17482097. Available from: https://doi.org/10.1016/j.tcm.2007.03.002.

14. Nakano I, Kinugawa S, Hori H, Fukushima A, Yokota T, Takada S, et al. Serum Brain-Derived Neurotrophic Factor Levels Are Associated with Skeletal Muscle Function but Not with Muscle Mass in Patients with Heart Failure. Int Heart J. 2020;61(1):96–102. PMID: 31956152. Available from: https://doi.org/10.1536/ihj.19-400.

15. Emanueli C, Meloni M, Hasan W, Habecker BA. The biology of neurotrophins: cardiovascular function. Handb Exp Pharmacol. 2014;220:309–328. PMID: 24668478. Available from: https://doi.org/10.1007/978-3-642-45106-5_12.

16. Donovan MJ, Lin MI, Wiegn P, Ringstedt T, Kraemer R, Hahn R, et al. Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. Development. 2000;127(21):4531–4540.

17. László A, Lénárt L, Illésy L, Fekete A, Nemcsik J. The role of neurotrophins in psychopathology and cardiovascular diseases: psychosomatic connections. J Neural Transm (Vienna). 2019;126(3):265–278. PMID: 30767081. Available from: https://doi.org/10.1007/s00702-019-01973-6.

18. Manni L, Nikolova V, Vyagova D, Chaldakov GN, Aloe L. Reduced plasma levels of NGF and BDNF in patients with acute coronary syndromes. Int J Cardiol. 2005;102(1):169–171. PMID: 15939120. Available from: https://doi.org/10.1016/j.ijcard.2004.10.041.

19. Jiang H, Liu Y, Zhang Y, Chen ZY. Association of plasma brain-derived neurotrophic factor and cardiovascular risk factors and prognosis in angina pectoris. Biochem Biophys Res Commun. 2011;415(1):99–103. PMID: 22020095.

20. Costa HS, Lima MMO, Figueiredo PHS, Martinelli PM, Camargos ER, Chaves AT, et al. Prognostic value of serum brain-derived neurotrophic factor levels in patients with Chagas cardiomyopathy. Mem Inst Oswaldo Cruz. 2018;113:e180224. Available from: https://doi.org/10.1590/0074-02760180224.

21. Barman HA, Şahin I, Atıcı A, Durmaz E, Yurtseven E, Ikitimur B, et al. Prognostic significance of brain-derived neurotrophic factor levels in patients with heart failure and reduced left ventricular ejection fraction. Anatolian journal of cardiology. 2019;22(6):309–316. Available from: https://doi.org/10.14744/AnatolJCardiol.2019.37941.

22. Liu YQ, Su GB, Duan CH, Wang JH, Liu HM, Feng N, et al. Brain derived neurotrophic factor gene polymorphisms are associated with coronary artery disease related depression and antidepressant response. Mol Med Rep. 2014;10(6):3247–3253. PMID: 25324022. Available from: https://doi.org/10.3892/mmr.2014.2638.

23. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A,et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003;112(2):257–269. Available from: https://doi.org/10.1016/S0092-8674(03)00035-7.

24. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H. et al. ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. 2018;39(2):119–177. PMID: 28886621. Available from: https://doi.org/10.1093/eurheartj/ehx393.

25. Kirby A, Gebski V, Keech AC. Determining the sample size in a clinical trial. Med J Aust. 2002;177(5):256–257. PMID: 12197821. Available from: https://doi.org/10.5694/j.1326-5377.2002.tb04759.x.

26. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman ME, et al. Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. Diabetes Care. 2011;34(6):e61–e99. PMID: 21617108. Available from: https://doi.org/10.2337/dc11-9998.

27. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H. et al. ESC Scientific Document Group. 2016 ESC/EAS Guidelines for the Management of Dyslipidemias: The Task Force for the Management of Dyslipidemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Atherosclerosis. 2016;253:281–344. PMID: 27567407. Available from: https://doi.org/10.1093/eurheartj/ehw272.

28. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi

M, Burnier M. et al. ESC Scientific Document Group. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018;39(33):3021–3104. PMID: 30165516. Available from: https://doi.org/10.1093/eurheartj/ehy339.

29. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS. et al. ESC Scientific Document Group. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: the task force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail. 2016;18:891–975. PMID: 27206819. Available from: https://doi.org/10.1093/eurheartj/ehw128.

30. Morrow DA, Antman EM, Charlesworth A, Cairns R, Murphy SA, de Lemos JA, et al. TIMI Risk Score for ST-Elevation Myocardial Infarction: A Convenient, Bedside, Clinical Score for Risk Assessment at Presentation. An Intravenous nPA for Treatment of Infarcting Myocardium Early II Trial Substudy. Circulation. 2000;102:2031–2037. PMID: 11044416. Available from: https://doi.org/10.1161/01.CIR.102.17.2031.

31. Eagle KA, Lim MJ, Dabbous OH, Pieper KS, Goldberg RJ, Van WF, Goodman SG, Granger CB, Steg PG, Gore JM, Budaj A, Avezum A, Flather MD, Fox KA. A validated prediction model for all forms of acute coronary syndrome: estimating the risk of 6-month post-discharge death in an international registry. JAMA. 2004;291:2727–2733. PMID: 15187054. Available from: https://doi.org/10.1001/jama.291.22.2727.

32. Hayase M, Yoda S, Hatta T, Hori Y, Monno K, Fujito H, et al. Prognostic Significance of the Residual SYNTAX Score and Ischemic Reduction Detected with Nuclear Cardiology for Prediction of Major Cardiac Events after Revascularization. Intern Med. 2020;PMID: 32161215. Available from: https://doi.org/10.2169/internalmedicine.3397-19.

33. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al, for the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med. 2009;150:604–612. PMID: 19414839. Available from: https://doi.org/10.7326/0003-4819-150-9-200905050-00006.

34. Horita N, Kaneko T. Genetic model selection for a case-control study and a meta-analysis. Meta Gene. 2015;5:1–8. PMID: 26042205. Available from: https://doi.org/10.1016/j.mgene.2015.04.003.

35. Kim JM, Stewart R, Kim SY, Kim JW, Kang HJ, Lee JY, et al. Interaction between BDNF val66met polymorphism and personality on long-term cardiac outcomes in patients with acute coronary syndrome. PLoS One. 2019;14(12):e0226802. PMID: 31887219. Available from: https://doi.org/10.1371/journal.pone.0226802.

36. Jiang R, Babyak MA, Brummett BH, Hauser ER, Shah SH, Becker RC, et al. Brain-derived neurotrophic factor rs6265 (Val66Met) polymorphism is associated with disease severity and incidence of cardiovascular events in a patient cohort. Am Heart J. 2017;190:40–45. PMID: 28760212. Available from: https://doi.org/10.1016/j.ahj.2017.05.002.

37. Jiang H, Wang R, Liu Y, Zhang Y, Chen ZY. BDNF Val66Met polymorphism is associated with unstable angina. Clin Chim Acta. 2009;400(1-2):3–7. PMID: 19013140. Available from: https://doi.org/10.1016/j.cca.2008.10.017.

38. Caporali A, Emanueli C. Cardiovascular actions of neurotrophins. Physiol Rev. 2009;89(1):279–308. PMID: 19126759. Available from: https://doi.org/10.1152/physrev.00007.2008.

39. Sookaromdee P, Wiwanitkit V. Brain-derived neurotrophic factor as biomarker. Anatol J Cardiol. 2020;23(3):192. PMID: 32120366. Available from: https://doi.org/10.14744/AnatolJCardiol.2020.84699.

40. Kadowaki S, Shishido T, Honda Y, Narumi T, Otaki Y, Kinoshita D, et al. Additive clinical value of serum brain-derived neurotrophic factor for prediction of chronic heart failure outcome. Heart Vessels. 2016;31:535–544. PMID: 25616498. Available from: https://doi.org/10.1007/s00380-015-0628-6.

41. Nakano I, Kinugawa S, Hori H, Fukushima A, Yokota T, Takada S, et al. Serum Brain-Derived Neurotrophic Factor Levels Are Associated with Skeletal Muscle Function but Not with Muscle Mass in Patients with Heart Failure. Int Heart J. 2020;61(1):96–102. PMID: 31956152. Available from: https://doi.org/10.1536/ihj.19-400.

42. Sustar A, Perkovic MN, Erjavec GN, Strac DS, Pivac N. Association between reduced brain-derived neurotrophic factor concentration & coronary heart disease. Indian J Med Res. 2019;150(1):43–49. PMID: 31571628. Available from: https://doi.org/10.4103/ijmr.IJMR_1566_17.

43. Suriyaprom K, Tungtrongchitr R, Thawnashom K, Pimainog Y. BDNF val66Met polymorphism and serum concentrations of BDNF with smoking in Thai males. Genet Mol Res. 2013;12:4925–4933. PMID: 24301752. Available from: https://doi.org/10.4238/2013.October.24.3.

44. Terracciano A, Piras MG, Lobina M, Mulas A, Meirelles O, Sutin AR, et al. Genetics of serum BDNF: Meta-analysis of the val66Met and genome-wide association study. World J Biol Psychiatry. 2013;14:583–589. PMID: 22047184. Available from: https://doi.org/10.3109/15622975.2011.616533.

45. Bahls M, Könemann S, Markus MRP, Wenzel K, Friedrich N, Nauck M, et al. Brain-derived neurotrophic factor is related with adverse cardiac remodeling and high NTproBNP. Sci Rep. 2019;9(1):15421. PMID: 31659205. Available from: https://doi.org/10.1038/s41598-019-51776-8.

46. Krabbe KS, Nielsen AR, Krogh-Madsen R, Plomgaard P, Rasmussen P, Erikstrup C, et al. Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. Diabetologia. 2007;50:431–438. PMID: 17151862. Available from: https://doi.org/10.1007/s00125-006-0537-4.

47. Suriyaprom K, Tungtrongchitr R, Thawnasom K. Measurement of the levels of leptin, BDNF associated with polymorphisms LEP G2548A, LEPR Gln223Arg and BDNF Val66Met in Thai with metabolic syndrome. Diabetol Metab Syndr. 2014;6:6. PMID: 24444121. Available from: https://doi.org/10.1186/1758-5996-6-6.

48. Feng N, Huke S, Zhu G, Tocchetti CG, Shi S, Aiba T, et al. Constitutive BDNF/TrkB signaling is required for normal cardiac contraction and relaxation. Proc Natl Acad Sci USA. 2015;112(6):1880–1885. PMID: 25583515. Available from: https://doi.org/10.1073/pnas.1417949112.

49. Hong JH, Park HM, Byun KH, Lee BH, Kang WC, Jeong GB. BDNF expression of macrophages and angiogenesis after myocardial infarction. Int J Cardiol. 2014;176(3):1405–1408. PMID: 25129264. Available from: https://doi.org/10.1016/j.ijcard.2014.08.019.

50. Halade GV, Ma Y, Ramirez TA, Zhang J, Dai Q, Hensler JG, Lopez EF, et al. Reduced BDNF attenuates inflammation and angiogenesis to improve survival and cardiac function following myocardial infarction in mice. Am J Physiol Heart Circ Physiol;305(12):H1830–H1842. PMID: 24142413. Available from: https://doi.org/10.1152/ajpheart.00224.2013.

51. Okada S, Yokoyama M, Toko H, Tateno K, Moriya J, Shimizu I, et al. Brain-derived neurotrophic factor protects against cardiac dysfunction after myocardial infarction via a central nervous system-mediated pathway. Arterioscler Thromb Vasc Biol. 2012;32(8):1902–1909. PMID: 22556331. Available from: https://doi.org/10.1161/ATVBAHA.112.248930.