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Assessment of serum levels of bone morphogenetic proteins as sensitive biomarkers for early prediction of acute myocardial infarction

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ABSTRACT

Although acute myocardial infarction (AMI) is a life-threatening disease, rapid diagnosis may reduce mortality risk. Despite being highly specific for AMI, cardiac troponin I (cTnI) does not distinguish between the etiologically diverse of myocardial injury that may be due to MI or non-MI causes. Bone morphogenetic proteins (BMPs), which are important modulators for cardiac morphogenesis, are members of the transforming growth factor-beta superfamily. This work intended to evaluate the utility of 2 members of BMPs (BMP2 and BMP4) as diagnostic biomarkers for AMI. This study included 110 AMI patients and 30 healthy volunteers. AMI patients were subdivided into 39 patients without hypercholesterolemia or diabetes mellitus (AMI without HC or DM), 33 patients with hypercholesterolemia (AMI with HC), and 38 patients with diabetes mellitus (AMI with DM). BMP2 and BMP4 levels were assessed in all subjects. The results showed that serum levels of both BMPs showed significant elevation in all AMI patients compared to healthy controls. In contrast, serum BMP2 level only was significantly elevated in AMI patients with DM compared to those without HC or DM and therefore was able to discriminate between the two subgroups, unlike cTnI and BMP4. Notably, the diagnostic efficacy of the two BMPs was improved when combined. In conclusion, the two BMPs are good diagnostic biomarkers for AMI. Nevertheless, BMP2 had a higher diagnostic performance than BMP4 in discriminating AMI with DM from AMI without HC or DM with AUC 0.919 (p=0.001) and the highest PPV (100%), meanwhile, BMP4's AUC is 0.891 (p=0.001) and a PPV (85.7%).

1. Inrtoduction

Acute myocardial infarction (AMI) is the leading cause of death in the United States. AMI occurs due to underlying coronary artery disease [1]. The occlusion of the coronary artery causes a deprivation of the myocardium from the oxygen supply, which eventually leads to myocardial cell death and necrosis [1, 2]. Bone morphogenetic proteins (BMPs) are a group of low molecular weight glycoproteins that play a critical role in the development and growth of several tissues and organs including the brain and bone during embryogenesis [3]. To date, more than 30 BMPs have been identified [4]. The BMPs members are classified into 3 subclasses according to sequence identity; one subclass consists of human BMP2 and BMP4, a second subclass comprises human BMP5, BMP6, BMP7, and BMP8, and a third subclass comprises human BMP3 [5].

The BMPs gene family can modulate the activation of cardiac genes hence they are playing a significant role during mammalian cardiogenesis [6]. BMPs can exert their signaling pathway via interactions with cognate Type I and Type II serine/threonine kinase receptors [7]. Following AMI, BMPs, which expressed by cardiomyocytes, trigger the release of pro-inflammatory factors that may increase the infarct size [8]. BMPs, particularly BMP2 and BMP4, are excellent subjects of scientific interest [9]. These proteins actively participate in gene expression and regulate cell differentiation and proliferation, including that of cardiac cells [10]. Currently, experimental findings demonstrated the significant values of BMP2 and BMP4 in AMI. They reported that increased BMP2 treatment and the deletion of the BMP4 gene, which is linked to substantial abnormalities in heart development [11], can diminish the infarction zone in the AMI model [6].

BMP2 and BMP4 are localized temporally and spatially to the myocardium overlying the atrioventricular canal (AVC) [12]. Previously, it was found that BMP2 reflects the standard stream of inflammation after AMI [13, 14]. Further, recent study showed that BMPs especially BMP4, which expressed by the systematic arterial endothelium, could trigger endothelial dysfunction and inflammation in a way that is dependent on nuclear factor-kappa B (NF-κB) and nicotinamide adenine dinucleotide phosphate oxidase [15]. Moreover, BMP4 promotes the activation of leukocytes, as well as aggravates the severity of atherosclerosis [15].

All of these evidences indicate that BMPs elevation exerts anti-inflammatory effects at the lesion site of defected heart. However, unclear vision persists regarding the clinical connection between circulating BMPs levels and AMI. Therefore, the purpose of this research was to explore the utility of BMP2 and BMP4 as novel diagnostic biomarkers for AMI patients.

2. Subjects and methods

2.1. Study population and samples collection.

This study included 110 AMI patients admitted to Ain Shams Specialized Hospital, Ain Shams University, Cairo; Egypt. AMI was diagnosed according to WHO criteria including chest pain lasting more than 20 minutes in the previous 24 hours and/or recent ECG abnormalities (new Q-waves and/or ST-segment deviations in two or more contiguous leads on 12 -lead ECG).). Later, AMI was confirmed based on the elevation of CK-MB >2 folds above the upper limit of normal or cardiac troponin I (cTnI) levels >0.1 ng/ml. AMI patients were sub-classified into three subgroups; AMI without hypercholesterolemia or diabetes mellitus (AMI without HC or DM, n=39), AMI with HC (n=33), and AMI with DM (n=38). The control group included 30 healthy subjects without a history of cardiovascular disease or hyperglycemia.

Exclusion criterion included patients with acute HF caused by acute valves dysfunction, malignant arrhythmia, and severe infection. Patients with systemic diseases, such as autoimmune disease, blood disease, active bleeding, and malignant tumor, were also excluded. Blood samples were the first obtainable samples from patients after admission. From all participants, 5 ml of venous blood was drawn and divided into 2 parts; the first (4 ml) was collected into dry tubes, allowed to clot, and centrifuged at 2000 xg for five minutes to get sera for estimating random blood sugar (RBS) and cardiac markers levels, while the other (1 ml) was collected into EDTA-coated tubes for the determination of glycated hemoglobin (HbA1c) level. In addition, 2ml of fasting venous blood was collected for lipid profile estimation.

2.2. Biochemical analysis

Levels of cardiac biomarkers including cTnI and creatine kinase-MB (CK-MB) were estimated in sera using an automated sandwich chemiluminescent immunoassay (Advia Centaur® XP, Siemens Healthcare Diagnostics Inc., NY, USA). Available commercial kits purchased from Bio diagnostics (Giza, Egypt) were used to determine blood sugar (RBS), total cholesterol (TC), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C) levels on Roche Hitachi 912 chemistry analyzer (Roche Diagnostics, In, USA). Using Friedewald's formula to calculate low-density lipoprotein-cholesterol (LDL-C) concentration. The D-10 Hemoglobin Testing System based on high-performance liquid chromatography (Bio-Rad Laboratories, Inc., CA, USA) was used to determine the level of HbA1c.

2.3. Assays for BMP2 and BMP4

Serum levels of BMP2 (Cat# CSB-E04507h) and BMP4 (Cat# CSB-E17298h) were determined according to the manufacturer's instructions of commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, TX, USA).

The assays' LLD (Lower Limit of Detection) was 15.6 pg/ml for BMP2 and 3.9 pg/ml for BMP4. The intra-assay coefficient of variation (CV) of the assays was <8%, while the inter-assay CV was <10%. The tests were performed on ChroMate® microplate reader (Awareness Technology Inc., FL, and USA).

2.4. Statistical analysis

SPSS version 23.0 (IBM Corp, NY, and USA) was used to perform the statistical analyses. The assumption of Gaussian distribution was tested with the Shapiro-Wilk test; the Gaussian distributed data were expressed as mean±SE, non-Gaussian distributed data were expressed as median and interquartile range (25^{th} and 75^{th} percentile), and categorical variables are expressed as frequencies (percentages). Continuous variables were compared using one-way ANOVA followed by Tukey's post hoc for multiple comparisons or Kruskal-Wallis test followed by Dunn post hoc for multiple comparisons as appropriate.

Chi square test (χ^2 test) was used to compare the differences between categorical variables. The Spearman's rho correlation analysis was used to assess the relation between two variables. Unconditional logistic regression analysis was performed to investigate how strongly the circulating levels of BMP2 and BMP4 associate with the susceptibility to AMI. The strength of the association was measured by crude odd ratio (OR), adjusted OR, and their corresponding 95% confidence interval (CI). Receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic value of BMP2 and BMP4. Of note that a *p-value* <0.05 was regarded as statistically significant and all *p*-values were 2-sided.

3. Results

3.1. General characteristics of the studied groups

The basic characteristics of the study population are presented in Table 1. There was no statistically significant difference among the studied groups in age, sex, and smoking habits. Considering co-morbidity, HbA1c level was significantly elevated in the AMI with DM subgroup only compared to controls (p<0.001). There was a significant elevation in cardiac biomarkers, cTnI, and CK-MB, in all patient subgroups, as expected, compared to the control group (p<0.001). Among the lipid profile, a significant increase in TC, TG, and LDL-C levels was observed, along with a significant decrease in HDL-C, in all patient subgroups compared to controls (p<0.001). According to the pairwise comparison, HbA1c and RBS showed a significant elevation in the AMI with DM subgroup compared to AMI with HC and AMI without HC or DM subgroups (p<0.001).

A significant elevation in cTnI level was shown in AMI with HC compared to AMI with DM and AMI without HC or DM subgroups. On the other hand, its level showed non-significance variation between AMI with DM and AMI without HC or DM subgroups (p>0.05). Among the lipid profile, there was a significant elevation in the levels of TC, TG, and LDL-C in the AMI with HC subgroup compared to AMI with DM and AMI without HC or DM subgroups (p<0.001). In contrast, HDL-C decreased significantly in AMI with DM and AMI with HC subgroups compared to the AMI without HC or DM subgroup (p<0.001), whereas it showed non-significance variation between AMI with HC and AMI with DM subgroups (p>0.05).

3.2. Levels of BMP2 and BMP4

Fig. 1 shows that serum levels of BMP2 and BMP4 were significant increased in all patient subgroups compared to healthy controls (p<0.001). Moreover, a significant increase in serum BMP2 level was observed in AMI with HC and AMI with DM subgroups in comparison with the AMI without HC or DM subgroup (p=0.032 and p<0.001, respectively). The same result was found when the AMI with DM subgroup was compared to the AMI with HC subgroup (p=0.033). Additionally, BMP4 was significantly elevated in the AMI without HC or DM subgroup compared to AMI with HC and AMI with DM subgroups (p<0.001) while its levels show non-significance variation between AMI with HC and AMI with DM subgroups (p>0.05).

3.3. Correlation between BMP2, BMP4, and various factors

Table 2 shows the correlation analysis between BMPs with other biochemical parameters. The results showed a significant negative correlation between BMP2 and TC, while BMP4 correlated positively and significantly with HDL-C, in the control group. When considering all AMI patients, the increased BMP2 concentration correlated positively and significantly with HbA1c and negatively with BMP4. The latter also showed a positive significant correlation with HDL-C, meanwhile it showed a negative correlation with cTnI, HbA1c, TG, and LDL-C.

3.4. BMP2 and BMP4 as risk factors for AMI

Table 3 displays the findings of the binary logistic regression analyses that were done to investigate the association of the circulating levels of BMP2 and BMP4 with AMI risk, regardless whether the AMI patients were with or without HC and DM. The findings showed that the two measures' serum levels were linked to a higher risk of AMI. However, after adjusting for age, sex, smoking habits, presence of diabetes and the serum levels of HbA1C, TG, HDL-C and LDL-C as potential confounders the results still significant just for BMP4.

3.5. Efficacy of BMP2 and BMP4 as potential diagnostic biomarkers for AMI

Figs. 2-4. show the ROC curves describing the potential efficacy of cTnI, BMP2, and BMP4 to discriminate between healthy controls and AMI without HC or DM, AMI with HC, and AMI with DM subgroups, respectively. The results revealed that although cTnI had the strongest diagnostic value, both BMPs were found to have high diagnostic efficacy too. The combination of BMP2 and BMP4 improved their diagnostic efficacy as evidenced by increased AUC. For discriminating between AMI patients without HC or DM and AMI patients with DM, the ROC curves describing the diagnostic efficacy of BMP2, cTnI, and BMP4 revealed that BMP2 had the highest diagnostic efficacy with AUC of 0.919 at an optimal cut-off value of 349.5 pg/ml ($p<0.001$).

4. Discussion

This study was designed to gain a more comprehensive understanding of the diagnostic potential of two selected BMPs, BMP2 and BMP4, as biomarkers for early prediction of AMI. The current study showed that serum levels of BMP2 and BMP4 were significantly increased in all AMI patients compared to healthy controls. These data concur with a recent study that revealed the elevation of BMP2 and BMP4 in AMI patients at admission [16]. The authors speculated that this could be attributed to the ability of BMP2 to increase the expression of other BMPs. It is familiar that M2 macrophages actively release BMPs, which promote and direct inflammatory processes for cardiac remodeling after AMI [6, 17]. The present work revealed that the serum BMP2 level was significantly increased in AMI patients with DM.

Meanwhile, the serum level of BMP4 was significantly decreased in AMI patients with HC and AMI patients with DM compared to AMI patients without HC or DM. It was demonstrated that BMP2 serves a crucial function in both physiological and pathological vascular processes [18]. An earlier investigation revealed that BMP2 expression increased in response to elevated glucose levels concurrently with NF-κB pathway activation [19]. Also, a positive correlation was found between the plasma BMP2 levels and HbA1c have, indicating that chronically high glucose levels may increase BMP2 expression in people with type 2 DM and coronary artery disease [20].

Lin et al., [21] revealed that high glucose concentration increases cyclic adenosine monophosphate (cAMP) and enhances the phosphorylation of extracellular signal-regulated kinase (ERK) by activation of the cAMP/PKA pathway. Furthermore, Grisan et al., [22] reported that cholesterol supplementation on isolated wild-type acinar cells was associated with cAMP generation and induced the downstream phosphorylation and activation of PKA signaling. These studies suggested that high glucose and high cholesterol levels may enhance and activate the cAMP/PKA signaling pathway. In the vascular endothelium, the cAMP/PKA pathway is an important negative regulator of BMP4 expression and the inhibition of the former attenuates stress-induced down-regulation of the latter [23]. These findings may explain the results of the present study regarding why serum BMP4 levels in AMI with HC and AMI with DM subgroups were significantly decreased compared to the AMI without HC or DM subgroup.

This study demonstrated that BMP4 showed a significant negative correlation with cTnI in AMI patients, and disagrees with results of Pallotta et al. [9] study that revealed that BMP4 signaling increased the up-regulation of basal expression levels of the cardiac transcription factor Nkx2-5. This activation could, in turn, boost the differentiation of cells that were not fully differentiated and thus ultimately resulting in an increased number of Troponin C-positive cells. Also, BMP4 was negatively correlated with the serum level of TG, which concurs with the results of a previous study that revealed that BMP4 significantly lowers serum TG [24].

BMP4 induces cardiomyocyte apoptosis whereas BMP2 protects against cardiomyocyte apoptosis [25]. Izumi et al. [26] supported the notion that the BMP2/Smad1 signaling system plays an important role in the regulation of the myocardium. Further, the authors demonstrate the anti-apoptotic effect of BMP2 on cardiomyocytes suggesting its potential as a therapeutic agent for cardiac heart failure. In addition, an association between the increased level of BMP2 and high levels of matrix metalloproteinase-9 and high sensitive C-reactive protein was observed on day 1, day 7, and a six-month follow-up after AMI which most likely indicates that BMP2 is involved in the processes of early left ventricular remodeling and reflects the standard stream of inflammation after AMI [13, 27].

On contrary, BMP4 is a mechanosensitive and pro-inflammatory protein that induces endothelium dysfunction promoting cardiomyocyte apoptosis after ischemia-reperfusion injury-induced myocardial infarction [28, 29]. Furthermore, it was demonstrated that BMP4 promotes cellular apoptosis and plays a crucial role in the pathogenesis of myocardial infarction *via* activation of JNK MAPK pathway [25]. All of these data may explain the finding of the present study that showed an association between serum levels of BMP4 only with increased risk of AMI after adjusting age, sex, smoking habits, TG, HDL-C, LDL-C and presence of DM as potential confounders.

ROC analysis was performed to illustrate the efficacy of BMP2 and BMP4 as potential diagnostic biomarkers for AMI. The results showed that both BMP2 and BMP4 had a high diagnostic potential for the diagnosis of AMI with and without HC, and their combination improved this efficacy. On the other hand, BMP2 had a higher diagnostic performance to discriminate between AMI with and without DM compared to BMP4 and cTnI. Collectively, the present work sheds light on the potential utility of BMP2 and BMP4 as biomarkers for the diagnosis of AMI. Both BMPs, especially BMP2, possess diagnostic efficacy for AMI patients with and without DM. In addition, BMP2 succeeded to discriminate between AMI patients with DM from those without, unlike cTnI and BMP4.

5. Abbreviations

AMI: acute myocardial infarction; **cTnI**: cardiac troponin I; **BMP2**: bone morphogenetic protein 2; **BMP4**: bone morphogenetic protein 4; **DM**: diabetes mellitus; **HC**: hypercholesterolemia; **AVC**: atrioventricular canal; NF-κB: nuclear factor-kappa B; **CK-MB**: creatine kinase-MB; **RBS**: random blood sugar; **HbA1c**: glycated hemoglobin A1c; **LLD**: lower limit of detection; **cAMP**: cyclic adenosine monophosphate; **PKA**: protein kinase A; **ERK**: extracellular signal regulated kinase; **JNK**: The c-Jun NH2-terminal kinase; **MAPK**: mitogen-activated protein kinase.

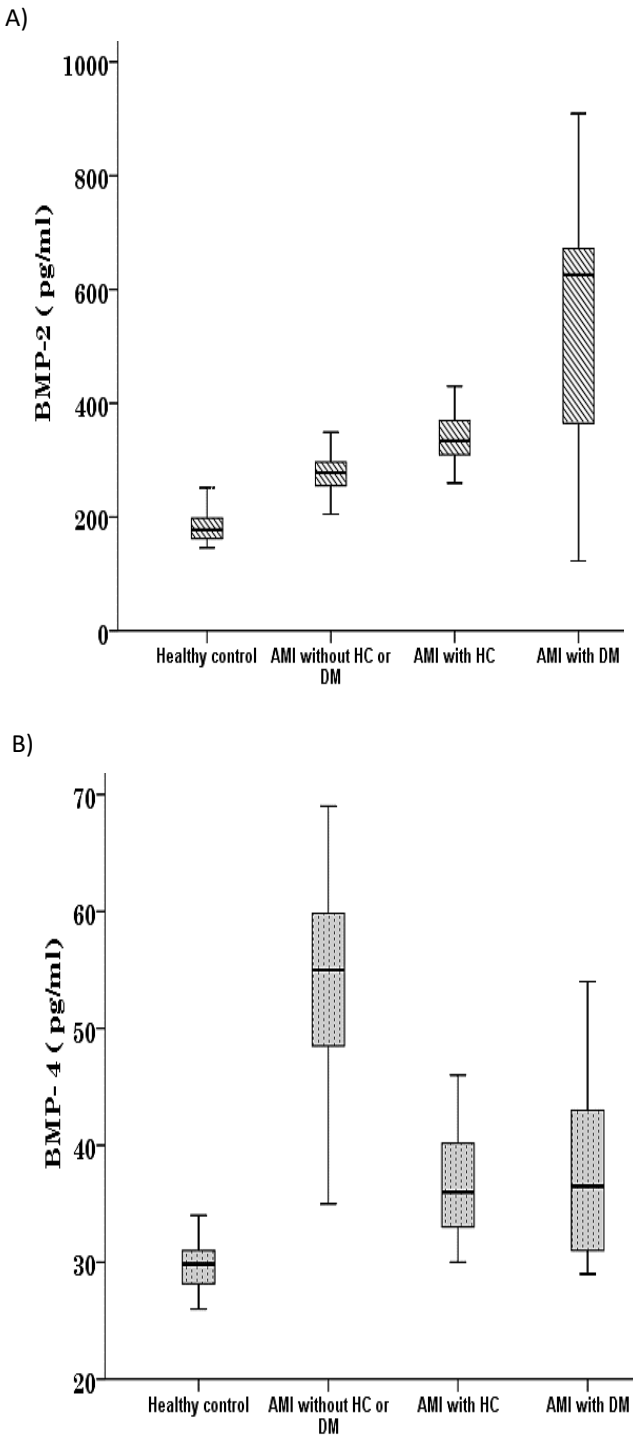
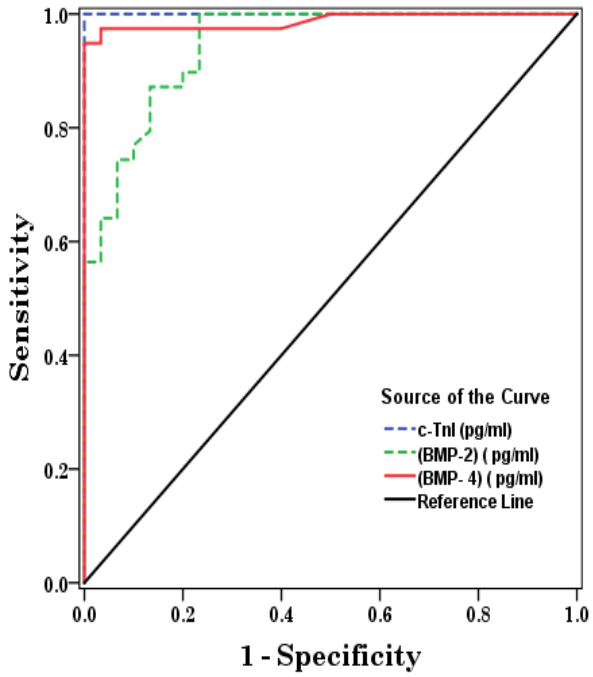


Fig. 1 Serum levels of (A) BMP2 and (B) BMP4 in the studied groups. BMP2: Bone morphogenetic protein-2, BMP4: Bone morphogenetic protein-4. AMI: Acute myocardial infarction, HC: Hypercholesterolemia, DM: Diabetes mellitus. ap<0.05 vs. control group, b p<0.05 vs. AMI without HC or DM subgroup, and c p<0.05 vs. AMI with HC subgroup.

A)



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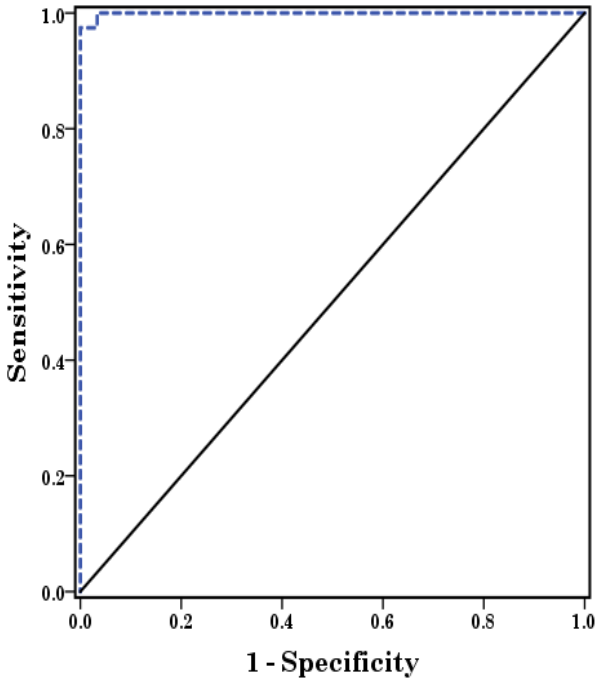
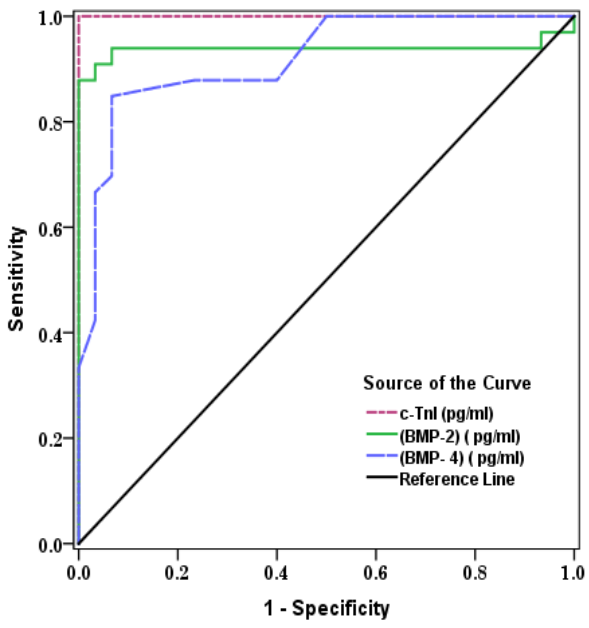


Variable(s)	AUC	p-value	cutoff	95% CI		Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Accuracy (%)
				Lower bound	Upper bound					
c-TnI (pg/ml)	1.000	0.001	138.00	1.000	1.000	100%	100%	100%	100%	100%
BMP2 (pg/ml)	0.946	0.001	201.50	0.897	0.994	100%	76.7%	84.7%	100%	90%
BMP4 (pg/ml)	0.988	0.001	39.00	0.964	1.000	94.9%	100%	100%	93.7%	97%
Combined BMP2 and BMP4	0.999	0.001	0.758	0.996	1.000	97.4%	100%	100%	96.7%	98.5%

Fig. 2 Receiver operating characteristic (ROC) curves of (A) cTnI, BMP2 and BMP4, and (B) Combined BMP2 and BMP4 to discriminate between AMI patients without HC or DM and healthy controls. AMI: Acute myocardial infarction, HC: Hypercholesterolemia, DM: Diabetes mellitus, AUC: Area under curve, CI: Confidence interval, Sens.: Sensitivity, Spec.: Specificity, PPV: Positive predictive value, NPV: Negative predictive value.

A)



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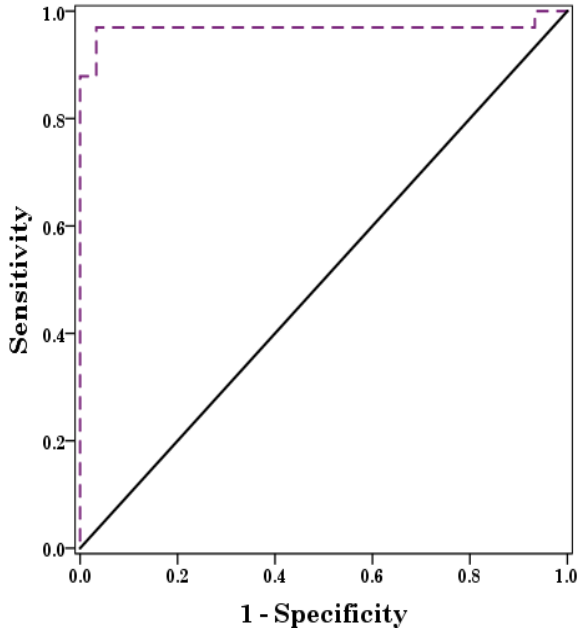
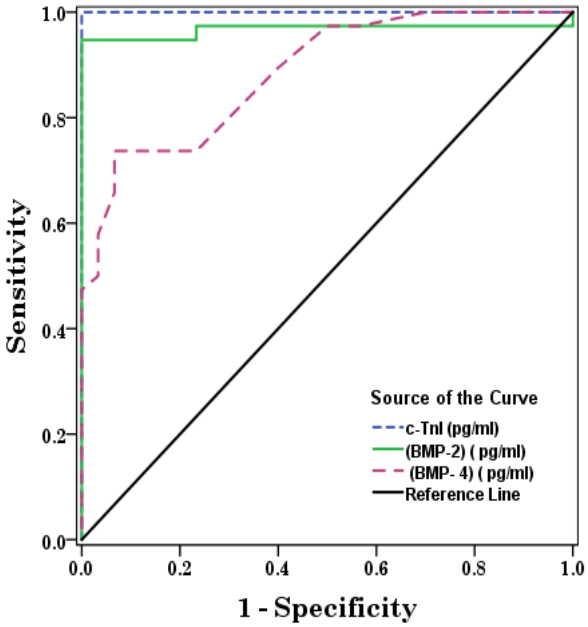


Variable(s)	AUC	p-value	Cut-off	95% CI		Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Accuracy (%)
				Lower bound	Upper bound					
c-TnI (pg/ml)	1.000	0.001	408.00	1.000	1.000	100%	100%	100%	100%	100%
BMP2 (pg/ml)	0.938	0.001	275.58	0.859	1.000	87.9%	100%	100%	88%	93.6%
BMP4 (pg/ml)	0.920	0.001	32.50	0.853	0.987	84.8%	93.3%	93.3%	84.8%	88.8%
Combined BMP2 and BMP4	0.969	0.001	0.47	0.914	1.000	93.9%	96.7%	96.8%	93.5%	95%

Fig. 3 Receiver operating characteristic (ROC) curves of (A) cTnI, BMP2 and BMP4, and (B) Combined BMP2 and BMP4 to discriminate between AMI patients with HC and healthy controls. AMI: Acute myocardial infarction, HC: Hypercholesterolemia, AUC: Area under curve, CI: Confidence interval, Sens.: Sensitivity, Spec.: Specificity, PPV: Positive predictive value, NPV: Negative predictive value.

A)



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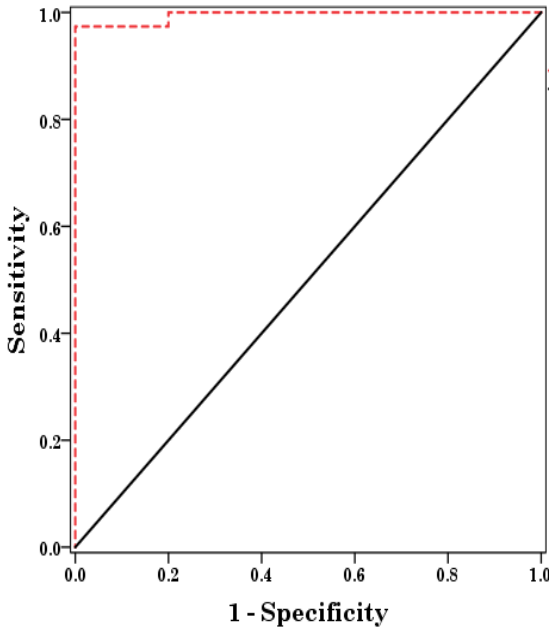
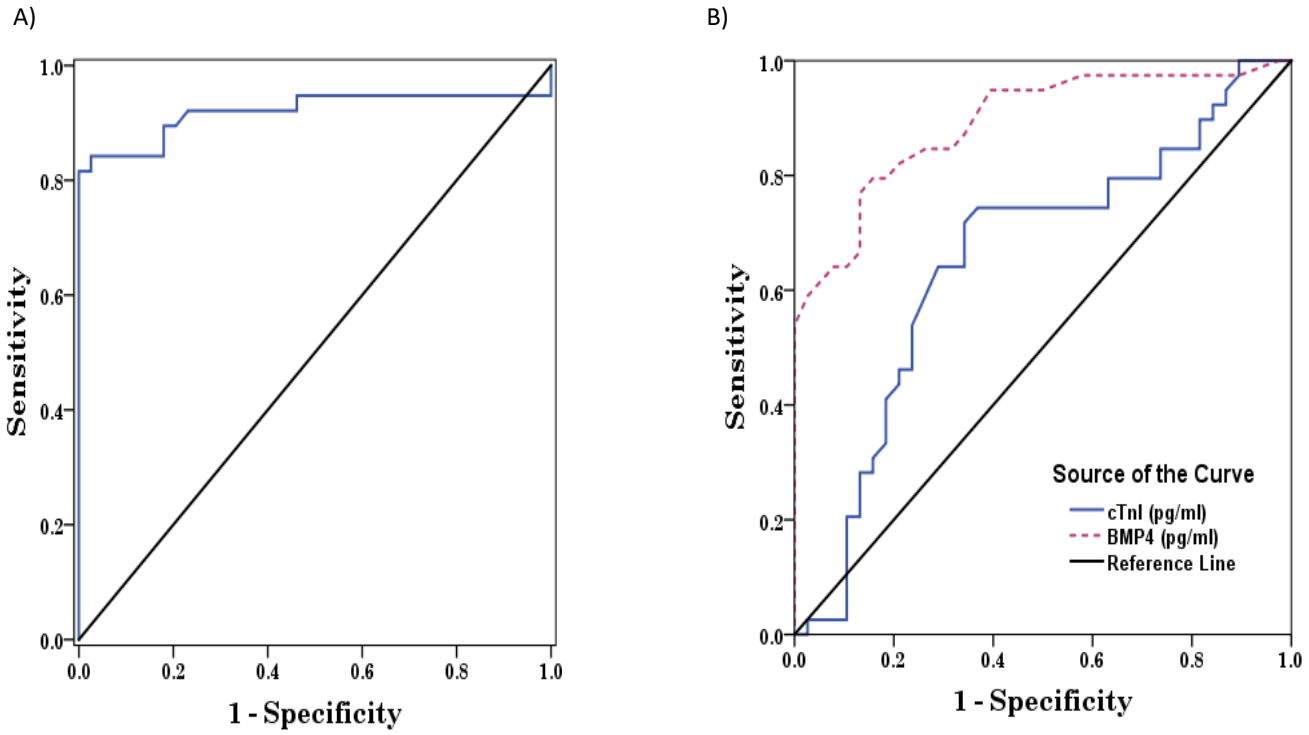


Variable(s)	AUC	p-value	Cut-off	95% CI		Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Accuracy (%)
				Lower bound	Upper bound					
c-TnI (pg/ml)	1.000	0.001	44.00	1.000	1.000	100%	100%	100%	100%	100%
BMP2 (pg/ml)	0.968	0.001	276.00	0.915	1.000	94.7%	100%	100%	93.7%	97%
BMP4 (pg/ml)	0.886	0.001	32.50	0.810	0.961	73.7%	100%	100%	75%	85%
Combined BMP2 and BMP4	0.995	0.001	0.742	0.983	1.000	97.4%	100%	100%	96.7%	98.5%

Fig. 4 Receiver operating characteristic (ROC) curves of (A) cTnI, BMP2 and BMP4, and (B) Combined BMP2 and BMP4 to discriminate between AMI patients with DM and healthy controls. AMI: Acute myocardial infarction, DM: Diabetes mellitus, AUC: Area under curve, CI: Confidence interval, Sens.: Sensitivity, Spec.: Specificity, PPV: Positive predictive value, NPV: Negative predictive value.



Variable(s)	AUC	p-value	Cut-off	95% CI		Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Accuracy (%)
				Lower bound	Upper bound					
cTnI (pg/ml)	0.655	0.019	499.50	0.529	0.781	64.1%	68.4%	67.5%	65%	66%
BMP2 (pg/ml)	0.919	0.001	349.5	0.844	0.955	81.6%	100%	100%	84.7%	91%
BMP4(pg/ml)	0.891	0.001	47	0.818	0.963	76.9%	68.8%	85.7%	78.5%	81.8%

Fig. 5 Receiver operating characteristic (ROC) curves of (A) BMP2 and (B) cTnI and BMP4 to discriminate between AMI patients without HC or DM and AMI patients with DM. AMI: Acute myocardial infarction, HC: Hypercholesterolemia, DM: Diabetes mellitus, AUC: Area under curve, CI: Confidence interval, Sens.: Sensitivity, Spec.: Specificity, PPV: Positive predictive value, NPV: Negative predictive value.

Table 1. General characteristics of the studied groups

	Control group (n= 30)	AMI without HC or DM (n= 39)	AMI with HC (n=33)	AMI with DM (n=38)	*p*-value
Age (year)	52±1.4	54±1.2	52± 1.7	57±1.6	NS
Gender					
Male	22 (73.3%)	33 (84.6 %)	24(72.7%)	25 (65.8%)	NS
Female	8 (26.7%)	6 (15.4%)	9 (27.3%)	13 (34.2%)	
Smoking					
Smoker	15 (50%)	21 (53.7%)	20 (60.6%)	21 (55.3%)	NS
Non smoker	15 (50%)	18 (46.2%)	13 (39.4%)	17 (44.7%)	
HbA1c (%)	5.4±0.04	5.698±0.05	5.5±0.062	9.0±0.28[a,b,c]	<0.001
RBS (mg/dl)	110.9±2.79	138.66±1.3[a]	139.12±1.7[a]	271.6±4.9[a,b,c]	<0.001
cTnI (pg/ml)	0.07±0.00	506.66±19.8[a]	1257.9±35.4[a,b]	452.4±27.3[a,c]	<0.001
CK-MB (U/L)	16.6±0.74	172.5±19.4[a]	77.13±6.7[a,b]	198.4±11.3[a,c]	<0.001
TC (mg/dl)	160.4±1.6	175±21[a]	245±3.79[a,b]	171.3±3.1[a,c]	<0.001
TG (mg/dl)	116.3±2.2	147±7[a]	192.6±2.9[a,b]	170.8±6.8[a,b,c]	<0.001
HDL-C (mg/dl)	51.8±1.2	43.8±1.85[a]	36.5±1.1[a,b]	35.47±1.04[a,b]	<0.001
LDL-C (mg/dl)	86.9±2.4	102±3.8[a]	168.7± 3.6[a,b]	101.7±3.8[a,c]	<0.05

Data are expressed as mean ±SE for Gaussian variables and frequencies (percentages) for categorical variables. AMI: Acute myocardial infarction, HC: Hypercholesterolemia, DM: Diabetes mellitus, HbA1c: Glycated hemoglobin A1, RBS: Random blood sugar, cTnI: cardiac Troponin I, CK-MB: Creatine kinase-MB, TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol. [a]$p<0.05$ *vs.* control group, [b] $p<0.05$ *vs.* AMI without HC or DM subgroup, and [c] $p<0.05$ *vs.* AMI with HC subgroup.

Table 2. Correlation analysis of BMP2 and BMP4 levels with biochemical parameters in controls and AMI patients

Variable	Control group				All AMI patients			
	BMP2		BMP4		BMP2		BMP4	
	r	*p*	r	*p*	r	*p*	r	*p*
cTnI (pg/ml)	0.179	0.345	-0.231	0.219	-0.115	0.233	**-0.283**	**0.017**
CK-MB (U/L)	0.28	0.134	-0.161	0.394	0.143	0.137	0.127	0.186
HbA1c (%)	0.037	0.8	-0.053	0.78	0.454**	<0.001	**-0.193***	**0.043**
TC (mg/dl)	**-0.404***	**0.027**	0.102	0.591	-0.048	0.618	-0.169	0.77
TG (mg/dl)	-0.139	0.464	-0.036	0.851	0.097	0.307	**-0.284****	**0.003**
HDL-C (mg/dl)	0.24	0.2	**0.412***	**0.024**	-0.166	0.08	**0.295****	**0.002**
LDL-C (mg/dl)	-0.351	0.057	0.008	0.968	0.018	0.85	**-0.220***	**0.021**
BMP2 (pg/ml)	-	-	0.113	0.552	-	-	**-0.442****	**<0.001**
BMP4 (pg/ml)	0.113	0.552	-	-	**-0.442****	**<0.001**	-	-

AMI: Acute myocardial infarction, cTnI: Troponin I, CK-MB: Creatine kinase-MB, TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, HbA1c: Glycated haemoglobin A1, BMP2: Bone morphogenetic protein-2, BMP4: Bone morphogenetic protein-4.

Table 3. Binary logistic regression analysis of BMP2 and BMP4 as factors possibly affecting myocardial infarction

Variable	Crude OR (95% CI)	p-value	†Adjusted OR (95% CI)	p-value
Serum BMP2 (pg/ml)	1.035 (1.022-1.048)	0.001	1.267 (0.877-1.8)	NS
Serum BMP4 (pg/ml)	1.638 (1.27-1.2.09)	0.001	1.781 (1.344-2.361)	<0.001

BMP2: Bone morphogenetic protein-2, BMP4: Bone morphogenetic protein-4, OR: Odd ratio, 95% CI: 95% confidence interval, †: Adjusted for age, sex, smoking habits, presence of diabetes, HbA1c TG, HDL-C, and LDL-C as potential confounders

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