The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 1976-1982

Evaluation of Glycogen Phosphorylase Activity in A Sample of Iraqi Patients with Myocardial Infarction

Mena Safaa aldeen jafar*[1], Mohammed I. Hamzah[2], Muayed B. Hamed [3]
[1,3]Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq.
[2]College of Medicine/Al-Nahrain University/Iraq
*Corresponding author: **Mena Safaa Aldeen Jafar, email:**mena.safaa.1992@gmail.com, Tel.: 009647822001017

ABSTRACT

Background: Myocardial ischemia, the most common kind of cardiovascular disease, and the leading cause of mortality worldwide. It is caused mostly by cardiac muscle necrosis caused by an abrupt coronary artery occlusion. Smoking and obesity are two major risk factors for myocardial infarction. Chest pain and dyspnea are common symptoms caused by a reduction in blood flow to the myocardium.

Objective: glycogen (GP) modulates glucose metabolism by mobilizing intracellular glycogen. Heart and brain tissues express it, which generates energy during muscle contraction. Myocardial ischemia activates Glycogen phosphorylase BB, which promotes glycogen breakdown.

Subjects and Methods: A case-control study was used in this investigation. These investigations comprise 160 Iraqi volunteers ranging in age from 25 to 75 years (50 with ST- elevation, 50 without ST- elevation, and 60 in the normal healthy control group).

Results: Glycogen phosphorylase BB (GPBB) Activity by spectrophotometer technique. There were significant differences in GPBB activity among the groups the mean of GPBB for Control (3.25± 0.97) was significantly smaller than for both STEMI (12.54± 2.85), $p < .001$ and non ST-elevation myocardial infarction (NSTEMI) (6.51± 0.73), $p < .001$. The mean of GPBB for ST-elevation myocardial infarction (STEMI) (12.54± 2.88) was significantly larger than for NSTEMI (6.51± 0.73), $p < .001$. A significant negative correlation was observed between GPBB and high sensitive troponin I (hs-troponin I) and a negative correlation was observed between GPBB and TC in the NSTEMI group.

Conclusion: higher serum glycogen phosphorylase levels with higher troponin I levels were both observed in myocardial infarction patients and there was a significant correlation between them.

Keywords: glycogen phosphorylase BB, myocardial infarction.

INTRODUCTION

When the heart is in a state of unstable ischemia, a condition known as myocardial necrosis or acute myocardial infarction can take place. In clinical settings, diagnosis and evaluation might involve a number of different approaches, such as a clinical exam, electrocardiogram (ECG), biochemical tests, invasive and noninvasive imaging, and pathological evaluation. ST-segment elevation on the electrocardiogram is used to classify acute myocardial infarction into six subtypes, including infarction caused by coronary atherothrombosis (type 1), infarction caused by a supply-demand mismatch other than acute atherothrombosis (type 2), infarction leading to sudden death without the opportunity for biomarker or ECG confirmation (type 3), and infarction related to percutaneous coronary artery bypass surgery (type 5) [(1)].

Historically, patients diagnosed with coronary artery disease (CAD) have been "risk categorized" based on factors including blood pressure, cholesterol levels, and smoking behaviors. A comprehensive analysis of these factors is required in order to make an accurate forecast regarding the incidence of cardiovascular events in the future. A cardiovascular event that is known as an acute myocardial infarction is the cause of sudden cardiac death in the vast majority of cases Acute myocardial infarction (AMI) [(2)]. Patients who have acute myocardial infarction may present with symptoms such as typical ischemic-type chest pain, dyspnea, nausea, unexplained weakness, or a combination of these symptoms. If AMI is suspected, the patient should be referred immediately to an emergency room, where an electrocardiogram (ECG) will be obtained and evaluated for ischemic changes within ten minutes of the patient's arrival, and blood will be sent for cardiac troponin testing and another cardiac marker [(1)].

The epidemiological parameters of acute myocardial infarction have undergone significant change over the past three to four decades. Since 1987, the number of persons who have had to be hospitalized to hospitals in the United States because of acute MI has decreased by between four and five percent each year. The American Heart Association estimates that around 550,000 new cases of AMI and 200,000 recurrent episodes of AMI are diagnosed each year [(3)].

There are several different things that can put someone at risk for AMI. While some cannot be changed or treated in any way, some can be (cannot be changed). AMI is more likely to occur in those who have sedentary lifestyles and who have a number of cardiac risk factors. This highlights the importance of regular physical activity. It has been estimated that regular exercise can cut

Received: 20/08/2022
Accepted: 23/10/2022

the risk of coronary heart disease by between 20 and 30 percent. People who do not drink alcohol on a regular basis are at an increased risk of having a myocardial infarction within the next hour if they consume alcohol, which is related with an increased risk overall. Smoking has been associated to an increased risk of sudden cardiac death, an early onset of atherosclerosis, and an increased risk of myocardial infarction. Even in people who are otherwise healthy, early STEMI can be caused by smoking. Because of a number of factors, such as dyslipidemia, hypertension, diabetes mellitus, obesity, and other diseases, cigarette smoking is associated with an increased risk of acute myocardial infarction (AMI).[4].

Glycogen phosphorylase (GP) is an enzyme responsible for regulating glucose metabolism by mobilizing glycogen within cells. It's responsible for generating energy during muscle contraction and is highly expressed in heart and brain tissues. Separation of monosaccharide glucose-1-phosphate from polysaccharide glucose-1-phosphate is the first step in glycogenolysis (glycogen breakdown), which is catalyzed by GP. GPMM is the skeletal muscle isoenzyme, GPLL is the liver isoenzyme, and GPBB is the brain isoenzyme (found in the brain and cardiac muscle). Glycogen breakdown is increased when the GPBB enzyme is stimulated during myocardial ischemia. After ischemic myocardial damage, GPBB is released into the bloodstream between 2 and 4 hours later. Myocardial ischemia and necrosis are characterized by an early rise in GPBB blood levels, which is caused by a combination of mechanisms including rapid glycogenolysis and increased cell membrane permeability [5,6].

The sarcoplasmic reticulum glycogenolytic complex of cardiomyocytes contains GPBB, the main enzyme in glycogenolysis. The degree of association between GP and the sarcoplasmic reticulum glycogenolytic complex is determined by the myocardial metabolic status, which is shown to be especially susceptible to ischemia-induced glycogenolysis. Phosphorylation releases GPBB from its bound state, creating a soluble cytosolic form. Increased cellular permeability in the presence of hypoxia contributes to the occurrence of ischemic heart damage, and a strong GP concentration gradient in the sarcoplasmic reticulum compartment promotes GP efflux into the circulation via the T-tubule system [7,8].

PATIENTS AND METHODS

The participants included 100 MI patients and 60 controls aged 25-75 years, this is done during the period from 13/12/2021 to 18/4/2022. Patients with Renal impairment, pulmonary embolism, sepsis, stroke, diabetes mellitus, heart failure, any infectious disease, or thyroid dysfunction, were excluded from the study. Each participant was subjected to a physical examination. All individuals had their height and weight measured.

The body mass index (BMI) was calculated in kilograms per square meter. MI was diagnosed using the ECG and troponin test and some other cardiac marker, approximately 5mL of venous blood was withdrawn from all participants and converted into a serum-separated tube (Gel Tube) for glycogen phosphorylase measurement. Serum sample preparation was done by letting the whole blood sit for 20-30 minutes at room temperature (25°C) then the sample was centrifuged at 2000-3000 rpm for 20 minutes. The separated serum was stored in deep freeze (-20ºC) for subsequent measurement.

Ethical considerations:

The College of Medicine/Al-Nahrain University Department of Chemistry and Biochemistry, the gynecological outpatient clinic at Al-Immamain Al-kadhmain medical city, and Ibn Al-Nafees hospital's Ethics Committee approved the study. After ensuring confidentiality, all human research agreed with the World Medical Association's Declaration of Helsink. Participants gave informed written consent.

RESULTS

This study showed no significant difference in BMI between MI for the STEMI group were on average (31.61± 25.91), and for the NSTEMI group were on average (28.04± 3.63) and control (25.50 ±4.25) groups.

There were no significant differences in age by group levels, $F(2, 155) = 0.79$, $p = .454$, For Control, the age had an average of 58.63 (SD = 11.99). For STEMI, the age had an average of (60.10 ±13.15). For NSTEMI, the age had an average of (61.51 ±10.34) ANOVA result, $F(2, 155) = 926.49$, $p < .001$ indicating that there were significant differences in CKMB between the levels of the group. The R^2 value of 0.92 indicates that the group accounts for roughly 92% of the variance in CKMB. Compared to STEMI (40.14± 4.43), the mean of CKMB for Control (3.37± 0.35) was significantly lower ($p < .001$). The mean of CKMB for Control (3.37± 0.35) was also significantly lower than for NSTEMI (38.16±7.96), $p < .001$.

There were significant differences in GPBB activity among the groups, $F(2, 155) = 379.07$, $p < .001$, The R^2 was 0.83 indicating group explains approximately 83% of the variance in GPBB. The mean of GPBB for Control (3.25± 0.97) was significantly smaller than for both STEMI (12.54± 2.85), $p < .001$ and NSTEMI (6.51± 0.73), $p < .001$. The mean of GPBB for STEMI (12.54± 2.88) was significantly larger than for NSTEMI (6.51± 0.73), $p < .001$.

Table 1: Comparison of the study parameters between MI patients and controls

Variable	MI		Control	P	F
	STEMI	NSTEMI			
	M± SD		M± SD		
Age	60.10 ±13.15	61.51 ±10.34	58.63± 11.89	0. 454	0.79
BMI	31.61± 25.91	28.04± 3.63	25.50 ±4.25	0.103	2.31
GPBB activity	12.54± 2.85	6.51± 0.73	3.25± 0.97	< .001	234.67
CKMB	40.14± 4.38	38.16± 7.88	3.37± 0.34	< .001	926.49

GPBB, CK-MB, BMI, and Age were all analyzed using Spearman correlation. GPBB levels in both control and STIME groups of participants showed significant negative correlation with CK-MB or BMI but a positive correlation in NSTIME group. The correlation results are shown in Tables 2, 3 and 4. ROC analysis provided the reliability of the methods used and the cut-off values for GPBB and CK-MB as shown in Table 5 with Figures 5 and 6.

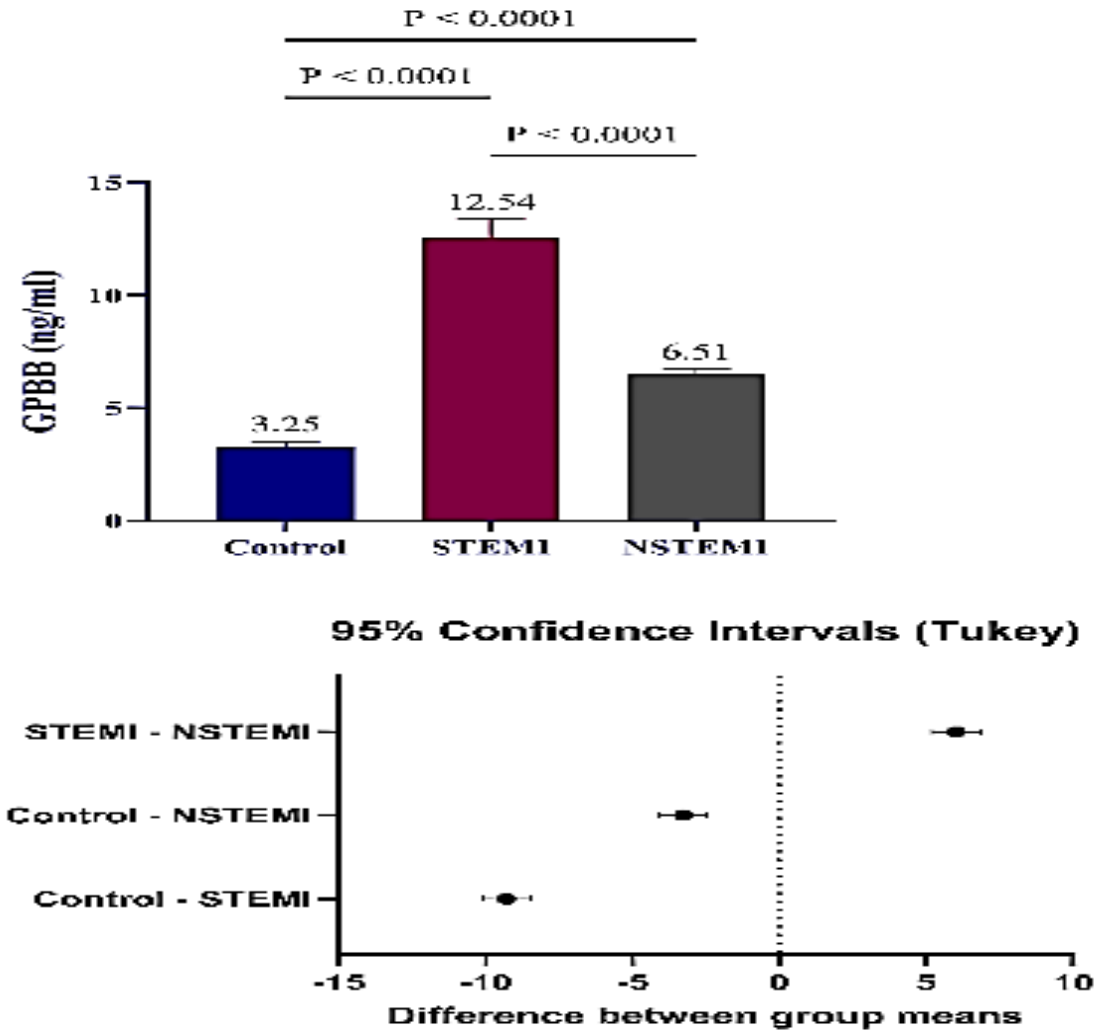

Figure 1: The mean of GPBB Significant difference

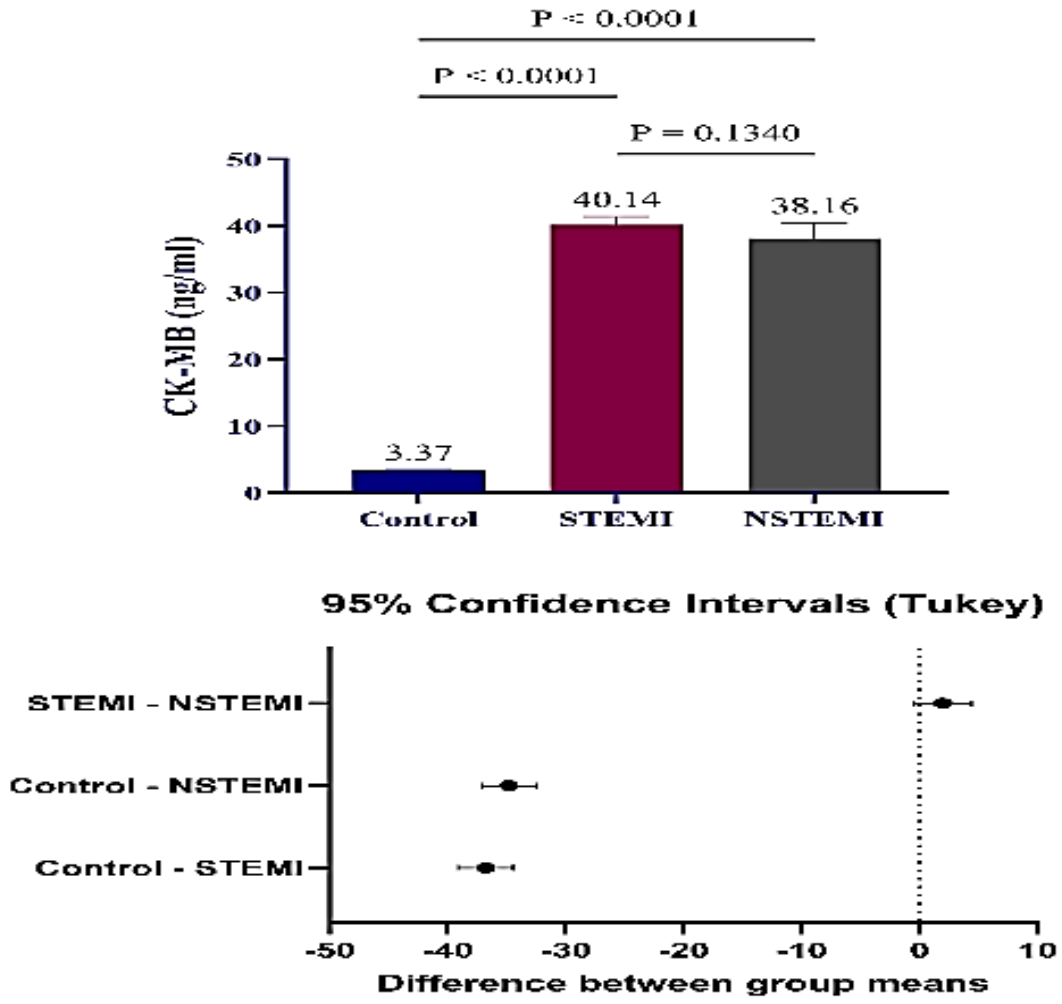

Figure 2: The mean of CK-MB significant difference.

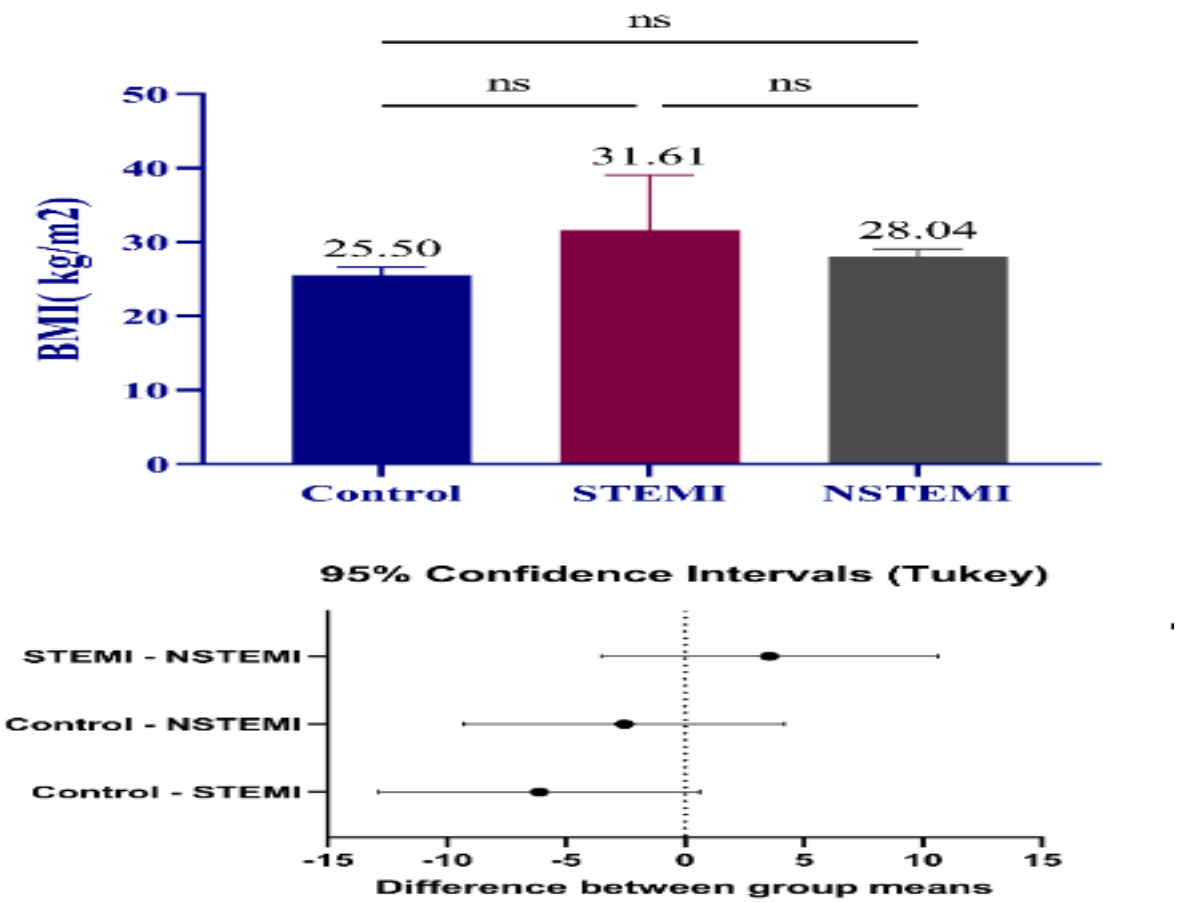

Figure 3: The mean of BMI.

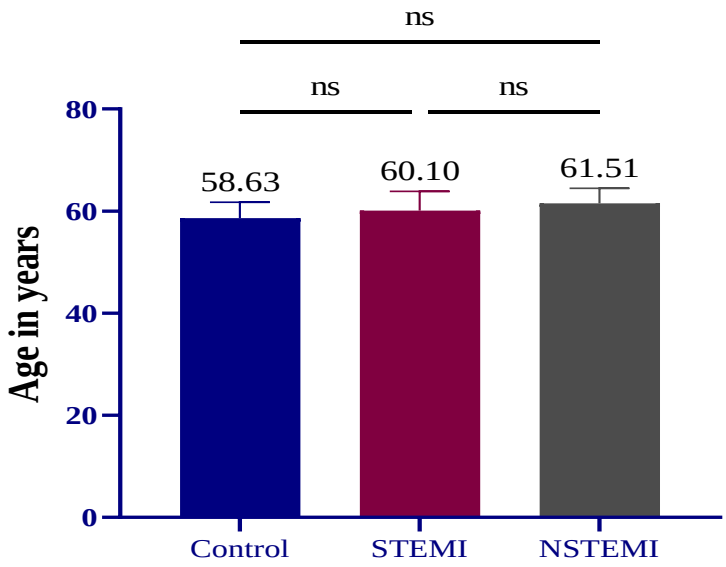

Figure 4: The mean of Age.

Table 2. Spearman Correlation Results among GPBB, CK-MB, BMI, and Age in control Group.

Combination	*R*	95.00% CI	*p*
GPBB-CKMB	-0.01	(-0.27, .24)	0.922
GPBB-age	-0.00	(-0.25, 0.25)	1.000
GPBB-BMI	-0.06	(-0.31, 0.20)	0.643
CKMB-age	-0.09	(-0.33, 0 .17)	0.513
CKMB-BMI	0.18	(-0.07, 0.42)	0.162

Table 3. Spearman Correlation Results among GPBB, CK-MB, BMI, and Age in STIME Group.

Combination	R	95.00% CI	p
GPBB-CKMB	.02	(-0.26, 0.30)	0.876
GPBB-age	-.04	(-0.25, 0.25)	1.000
GPBB-BMI	-.01	(-0.31, `0.20)	0.643
CKMB-age	.30	(0.02, 0.54)	0.037
CKMB-BMI	-.09	(-0.36, 0.20)	0.556

Table 4. Spearman Correlation Results among GPBB, CK-MB, BMI, and Age in NSTIME Group

Combination	R	95.00% CI	p
GPBB-CKMB	-0.22	(-0.47, 0.07)	0.135
GPBB-age	-0.07	(-0.34, 0.22)	0.647
GPBB-BMI	0.06	(-00.22, 0.34)	0.676
CKMB-age	-0.01	(-0.29, 0.27)	0.947
CKMB-BMI	0.13	(-0.16, 0.40)	0.378

Table 4: the cut-off values for GPBB and CK-MB

	Variable	AUC	SE	95% CI	P	cutoff	Sensitivity	Specificity
STEMI vs Control	GPBB	1.000	0.00	1.00 to 1.00	>3.96	100	100	1.000
	CK-MB	1.000	0.00	1.00 to 1.00	>5.17	100	100	1.000
NSTEMI vs Control	GPBB	1.000	0.00	1.00 to 1.00	>3.96	100	100	1.000
	CK-MB	0.997	0.0023	0.993 to 1.000	>5.17	95.92	100	0.997
STEMI vs NSTEMI	GPBB	0.678	0.054	0.572 to 0.785	>35.24	95.92	38.78	0.678
	CK-MB	1.000	0.00	1.00 to 1.00	>7.9	100	100	1.000

DISCUSSION

According to the findings of this study, the activation of GPBB during myocardial ischemia results in an increase in glycogen breakdown. Within the first four hours following the onset of chest pain, the T-tubules system is responsible for the majority of the GPBB isoenzyme being released into the bloodstream. Ischemia and necrosis of the myocardium are characterized by an increase in cellular membrane permeability and a quicker glycogenolysis, both of which lead to the rapid release of GPBB into the bloodstream. These characteristics are indicative of myocardial infarction and necrosis[(9)]. The cardiac contraction requires energy, which can be provided by GPBB, which is highly produced in cardiomyocytes and contains 843 amino acids. Normally, GPBB is secreted into the bloodstream by cardiomyocytes after cardiac damage when the sarcoplasmic reticulum fuses with cardiomyocytes to form a single unit [(10)]. The mean value for serum GPBB Activity in the control group was (3.25± 0.97). The mean value of serum GPBB Activity in the STEMI patient group was (12.54± 2.85). The mean value of GPBB Activity in the NSTEMI patient group was (6.51± 0.73). There was a significant difference between the control and patient groups (p < .001). This study agrees with [(11-15)]. Average cardiac marker values The levels of GPBB, myoglobin, and CKMB were shown to be greater in patients who had been diagnosed with AMI. When compared to myoglobin and CKMB at the first 4 hours, GPBB was found to be the most sensitive and specific biomarker to diagnose myocardial infarction. This was the conclusion reached by the researchers.

In this study, the CK-MB measurement is used to confirm the diagnosis of AMI in suspected cases. This study discovered that CKMB levels were significantly higher in STEMI and NSTEMI patients. According to [(16)], high CK-MB levels were significant and positively associated with infarction. This demonstrates that the CK-

MB values of STEMI and NSTEMI patients differ significantly [17] The postmortem diagnosis of cardiac death always includes both CK-MB and cTn as supporting evidence due to the numerous forensic medicine articles that have established their diagnostic functions[18]. There were 32 (20.13%) obese patients, and there were no significant differences between patients and monitoring collection (p=0.103), because the differences in BMI between the levels of the group were all statistically similar. These findings Obesity has a number of negative effects on cardiovascular disease (CVD). Obesity was linked to slower aging and significantly increased the risk of cardiovascular death in this study, Obesity is a risk factor for CVD [19, 20]. Severe obesity was not always linked to an increased risk, especially in women. In women and men with evidence of obesity, we discovered that severe obesity was associated with an increased risk of incident pulmonary disease and cancer [21].

CONCLUSIONS

Increased levels of GPBB Activity in the early stages of patient MI compared to the control group could be used as diagnostic biomarkers. Increased GPBB Activity concentrations in MI patients compared to control groups have been implicated in the pathophysiology of myocardial infarction (MI). Measuring GPBB activity in patients with MI at an early stage can help predict and prevent angina complications. Both serum levels of GPBB Activity increased significantly in patients with acute myocardial infarction compared to controls, supporting the role of GPBB in AMI patients and suggesting that GPBB plays an important role as a cardiovascular protective factor.

Conflict of interest: no conflict.
Sources of funding: by co-authors.
Author contribution: Authors are contributed equally in the study.

REFRENCES

1. **Anderson J, Morrow A (2017):** Acute Myocardial Infarction. New England Journal of Medicine, 21(376): 2053-2064.
2. **Ohtsuki T, Satoh K, Shimizu T *et al.* (2019):** Identification of Adipsin as a Novel Prognostic Biomarker in Patients with Coronary Artery Disease. Journal of the American Heart Association, 23(8): e013716
3. **Sidney S, Quesenberry C, Jaffe M *et al.* (2016):** Recent trends in cardiovascular mortality in the United States and public health goals. JAMA cardiology, 1(5):594-599.
4. **Rathore V, Singh N, Mahat R *et al.* (2018):** Risk factors for acute myocardial infarction: a review. EJMI., 2(1):1-7.
5. **Chaulin A, Duplyakov D (2020):** Biomarkers of acute myocardial infarction: diagnostic and prognostic value. Part 1. Journal of Clinical Practice, 11(3): 75-84.
6. **Garg C, Singh N, Rathore V *et al.* (2020):** Comparative Study Of Glycogen Phosphorylase Bb And Creatine Kinase Mb At First Hour After Of Acute Myocardial Infarction. Journal of Advanced Scientific Research, 11(02):159-163.
7. **Ghimire A, Giri S, Khanal N *et al.* (2022):** Diagnostic accuracy of glycogen phosphorylase BB for myocardial infarction: A systematic review and meta-analysis. Diagnostic accuracy of glycogen phosphorylase BB for myocardial infarction: A systematic review and meta-analysis - PubMed (nih.gov)
8. **Lee J, Romero R, Dong Z *et al.* (2012):** Glycogen phosphorylase isoenzyme BB plasma concentration is elevated in pregnancy and preterm preeclampsia. Hypertension, 59(2): 274–282.
9. **Bozkurt S, Kaya E, Okutucu S *et al.* (2011):** The diagnostic and prognostic value of first hour glycogen phosphorylase isoenzyme BB level in acute coronary syndrome. Cardiology Journal, 18(5):496-502.
10. **Singh N, Rathore V, Mahat R *et al.* (2018):** Glycogen phosphorylase BB: a more sensitive and specific marker than other cardiac markers for early diagnosis of acute myocardial infarction. Indian Journal of Clinical Biochemistry, 33(3):356-360.
11. **Huang C, Xiao S, Xia Z *et al.* (2021):** The Diagnostic Value of Plasma miRNA-497, cTnI, FABP3 and GPBB in Pediatric Sepsis Complicated with Myocardial Injury. Therapeutics and Clinical Risk Management, 17(1):563.
12. **Singh N, Rathore V, Mahat R *et al.* (2018):** Glycogen phosphorylase BB: a more sensitive and specific marker than other cardiac markers for early diagnosis of acute myocardial infarction. Indian Journal of Clinical Biochemistry, 33(3):356-360.
13. **Garg C, Rastogi P, Shah R *et al.*(2020):** Relation of hs-CRP and Glycogen phosphorylase BB in Acute Myocardial Infarction Patients. Journal of Cardiovascular Medicine and Cardiology, 7(3):272-275.
14. **Di J, Zhang Z, Xin S (2018):** Glycogen phosphorylase isoenzyme Bb, myoglobin and BNP in ANT-induced cardiotoxicity. Open life sciences, 13(1):561-568.
15. **Pantovic S, Slavic V, Roganovic, M (2018):** Heat shock protein 27 and glycogen phosphorylase isoenzyme BB as markers of myocardial stunning in male water polo players. Biomedical Research, 29(15):3069-3073.
16. **Procopio A, De Rosa S, Covello C *et al.* (2019):** Mathematical Model of the Release of the cTnT and CK-MB cardiac biomarkers in patients with acute myocardial infarction. In 2019 18th European Control Conference (ECC), 1653-1658).
17. **Kurniawan P, Setiawan A, Limantoro C *et al.* (2021):** The Differences in Troponin I and Ck-Mb Values in Acute Myocardial Infarction Patients with St Elevation and Without St Elevation. Diponegoro Medical Journal (Jurnal Kedokteran Diponegoro), 10(2):138-144.
18. **Xu C, Zhang T, Zhu B *et al.* (2020):** Diagnostic role of postmortem CK-MB in cardiac death: a systematic review and meta-analysis. Forensic Science, Medicine, and Pathology, 16(2):287-294.
19. **Khan S, Ning H, Wilkins J *et al*. (2018):** Association of body mass index with lifetime risk of cardiovascular disease and compression of morbidity. JAMA cardiology, 3(4):280-287
20. **Csige I, Ujvárosy D, Szabó Z *et al.* (2018):** The impact of obesity on the cardiovascular system. Journal of diabetes research, 2018. https://doi.org/10.1155/2018/3407306.
21. **Kim D, Her S, Park H *et al.* (2019):** Association between body mass index and 1-year outcome after acute myocardial infarction. PloS one, 14(6): e0217525. https://doi.org/10.1371/journal.pone.0217525.