Pharmacology

Research Article

Pharmacology 2024; Volume 109, Issue 1: 94-105

Received: July 01, 2023
Accepted: Dec 12, 2023
Published online: Feb 15, 2024

Type 1 Myocardial Infarction in the Troponin-Elevated Diseases by Potential Biomarkers

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Keywords: Troponin-Elevated Diseases, Elevated Cardiac Troponin, Type 1 Myocardial Infarction, Mass Spectrometry, Proteomics.

Abstract

Background: Though the troponin is now most widely utilized biomarker, it cannot differentiate between subtypes of myocardial infarction and is not a specific marker for this condition. Earlier research has demonstrated that proteins referred to as myocardial infarction markers may be used to categorise different types of myocardial infarction. It is essential to categorize the myocardial infarction by the sub-type for proper care of patient.

Aim: This study's objective is to find the markers that can differentiate the type-1 myocardial infarction from other conditions when troponin is increased.

Method: We found additional potential disease categorization markers by comparing type-1 MI to other illnesses defined by the raised troponin levels using the mass spectrometry. We then confirmed these indicators, in addition to those previously linked to plaque rupture and cardiovascular disease. We found serotransferrin, corticosteroid-binding globulin, and α-1 acid glycoprotein 2 as possible diagnostic indicators.

Conclusion: We have found three distinct markers in our study that could successfully differentiate the type-1 myocardial infarction from other troponin-rising illnesses. To diagnose specifically the type 1 MI, it may be helpful to have these markers present along with additional data from complementary diagnostic procedures such chest discomfort, ECG, and troponin levels.

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INTRODUCTION

Annually, almost eight million people with acute myocardial infarction (MI) symptoms go to hospitals.[1] These patients receive a lot of attention in emergency rooms due to the importance of earlier diagnosis and the treatment for its prognoses.[2] Currently, the troponin is most consistent bio-marker to identify MI. With great sensitivity and minimal imprecision, troponin test able the patients to be examined with the myocardial injury quickly.[3] The high-sensitivity cardiac troponin (hs-cTn), which has higher detection limits than the prior approach, may now be able to diagnose people who would otherwise go undiagnosed.[4] Troponin increase has been found in a number of other disorders, though, as it signals injury to cardiac cells [8]. Various diseases have different troponin levels. As a result, it can be difficult to discern between various diagnoses.[5]

The pathophysiology classification of myocardial infarction into categories 1 and 2 is ubiquitous.[6] Type 1 myocardial infarction denotes the necrotic condition that results in the imbalanced supply of oxygen to heart muscle as a result of blood vessel erosion or atherothrombotic plaque disintegration. These can make the blood vessels smaller or completely occlude them. Instead of thrombotic plaque disruption, type 2 myocardial infarction is brought on by the imbalance in the demand or supply of oxygen.

Previous research has attempted to identify novel unique markers on the basis of type of the myocardial infarction because treatment and management approaches vary depending on the MI type (Excluding troponin).[7] The 29 biomarker panels were utilised by a study to find the markers that could differentiate between the type 1 myocardial infarction, the type 2 myocardial infarction, and the myocardial damage. The cardiac biomarker troponin is used to define myocardial injury in current study when it occurs in the absence of the ischemia's acute myocardial.[8] A study used hs-cTn I, hs-cTn T, and the seventeen cardiovascular bio-markers for identifying the markers that could differentiate type 2 myocardial infarction from myocardial infarction. To differentiate between type 1 MI and type 2 MI, a study evaluated diagnosis effectiveness of the 6-biomarkers, either individually or in the combination.[9] By contrasting the type 1 myocardial infarction with type 2 myocardial infarction or MI with myocardial damage, earlier research has tried to find markers for MI subtype classification. However, in order to differentiate between one specific kind of myocardial infarction and further diseases that could be suspected during the process of diagnosis, we should do the comparisons of the one type of the myocardial infarction with the other diseases into the account.[10]

This study's objective, to find the markers that could differentiate the type 1 myocardial infarction from other conditions when troponin is increased. Utilizing the mass spectrometry-based method, we discovered a novel marker by examining both the type 1 myocardial infarction and the other disorders with high levels of troponin as shown Figure 1. Type 1 MI has well-established causes such as thrombus and plaque development. So, in this work, we put out the hypothesis that elements associated to make-up or production of the thrombus and plaque might be used as markers for the type 1 myocardial infarction.

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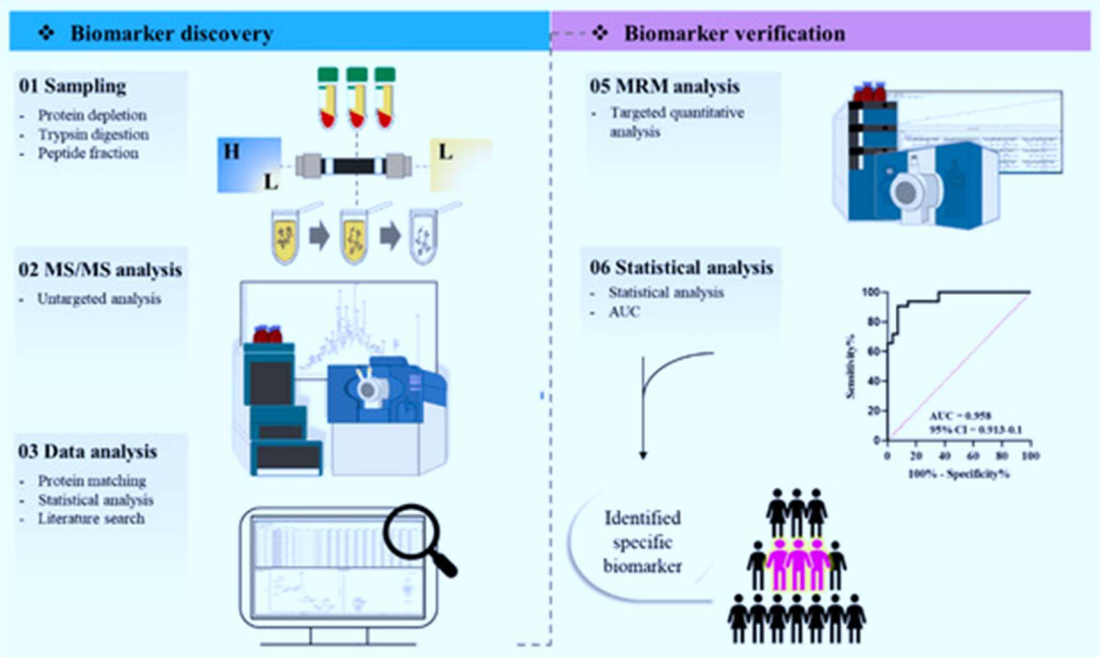

Figure 1. flowchart for experiment. Discovering and validating potential biomarker candidates made up the trial. In phase of discovery, samples of serum were pre-treated, and the untargeted mass spectrometry method was used to screen the whole protein. A literature search and statistical analysis were used to choose the final candidates. Proteins were chosen through quantitative analysis of multiple reaction monitoring (MRM) and statistical validation as the final indicators in the verification process.

MATERIALS AND METHODS

Study Population

Samples were taken between May 2021 and January 2022 at the University Hospital, Pakistan. The individuals in this study either underwent a cardiac troponin assay because they were hospitalised with suspected MI or went to the hospital for chest pain. Using the combined findings of the cardiac troponin test, coronary angiography, and the ECG, all the patients were categorised. The patients were labelled as with the type 1 myocardial infarction in coronary angiography when the lesions brought on by the coronary thrombosis or plaque rupture were found. However, the cardiologist's interventional used the fourth universal criteria to categorise patients having the stenosis of partial vascular as the "Others". All patient blood samples were taken 48 hours after admission during coronary angiography; however, for patients diagnosed with the type-2 myocardial infarction (the mechanism linked to the imbalance of oxygen), the samples of blood were taken 48 hours after the assay of cardiac troponin while hospitalised. Additionally included were healthy controls free of cardiovascular disease. Using a vacutainer without anticoagulant, all blood samples were drawn, held at the 25 °C for two hours, and then centrifugation at the 4000 g for five minutes for extracting serum.

Digestion of the Low-Abundance Protein

Low-abundance proteins were collected and concentrated at 12,000 g in the Nano-sep filter. Samples are then dried under the speed vacuum. The buffer lysis (0.1 Molar and 8 Molar urea Tris-HCl, pH 8.5) is used to dissolve the samples. The assay of BCA (Bicinchoninic acid) was used to measure the concentration of protein, and the concentrations of pooled and the individual sample are set to the 1mg

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and the 100g, respectively. The TCEP (Tris (2-carboxyethyl) phosphine) is added to the mixture at the final concentration of the 5mM to break down disulfide bonds. Mixture is then incubated at the 400-rpm speed at 37 °C for the 30 mins. Iodoacetamide and the 50mM of the Tris-HCl are added towards final concentration at the 15-mM for the alkylation and are incubated at the 25 °C with the 400 rpm for the 1 hour in order to raise the pH by 8.3. The 50mM of the Tris-HCl was used to seven-fold dilute samples. For digestion tryptic, trypsin was given to sample, which was then incubated for 15 hours at the temperature of 37 °C and the 800 rpm. A 10% FA addition quenched the activity, bringing pH down to the 3. Sample is then desalted, and subsequent process was carried out utilizing the cartridges C18. Cartridge was cleaned using 0.1 percent FA, 80 percent acetonitrile, and 100 percent methanol. The entire volume of digest is added, rinsed 7-times having 0.1 percent FA, then eluted using 50percent acetonitrile and 0.1 percent FA. Prior to the liquid chromatography the tandem mass spectrometry, the elutes were dried in the 40 Scan Speed combined with the Teflon and then resuspended in the 0.1 percent FA.

Peptide Fractionation

According to the isoelectric points, peptides from combined sample are divided into the fractionator of 12-well OFFGEL.

Data Processing

Using Protein Pilot programme and the database of Uniprot-human-SwissProt, peptide was identified. Homo sapiens, Triple TOF 5600 species, Cys alkylation, and the digestion were specified as search criteria (trypsin; allowing for the 2 missed cleavages). The Peakview 2.2 received ion library produced by the Protein Pilot and then imported it (SCIEX). The 5 peptides/protein, a false-discovery rate (FDR) threshold, and five transitions/peptide, of 1%, and the peptide confidence threshold of 95% were processing parameters used for the DIA data. MarkerView v.1.3.1 then imported the exported protein area value. The entire area sum was utilized to standardise data. With a p-value of < 0.05 and a fold change of ≥ 1.3, Welch's t-test was used to determine which proteins were expressed differentially between control and test groups. Furthermore, in the reference trial, final candidates for type 1 MI were chosen. We used PubMed to combine keyword searches to conduct a literature search. The keywords centred on the connection to thrombosis, a feature that can set type 1 MI apart from other illnesses. In MetaboAnalyts 5.0, a heatmap was used to visualise the data.

Statistical Analysis

To describe the continuous variables along with normal distributions the Means and the standard deviations are utilized, while 25th and 75th percentiles and medians were utilized to describe the continuous variables along with non-normal distributions. For the continuous variables, the unpaired Mann-Whitney U test or t-test was applied. To compare all the characteristics between the type 1 myocardial infarction and the type 2 myocardial infarction, Fisher exact test was utilized. Categorical variables were further specified as n (percent) variables. According to the outcomes of the normality test and variance, the MRM quantitative data also employed unpaired Welch's t-test, Mann-Whitney test, and t-test. According to findings and the variance of the normalcy test, the Kruskal-Wallis test that followed by test of Dunnett's multiple comparisons and Brown-Forsythe test that is followed by the Dunnett's T3 test of multiple comparisons were carried out to compare control and the disease groups. To identify the best biomarker, the curve of ROC (receiver-operating characteristic) was utilized. As a result, we were able to determine the cutoff, sensitivity, and specificity values. GraphPad Prism 8.4.2 was used to analyse all of the data. Microsoft Excel 2302 only ran the F-test to assess variance (Redmond, WA, USA).

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RESULTS

Patient Characteristics

The parameters from 60 participants were assessed in this study. Among these, 28 were categorised as Others, and 32 patients had type 1 MI (T1MI). The set of discovery contrasted the 12 patients from T1MI group to ten patients from others group for statistical analysis. Patients are selected at random. Though for the verification set, all subjects were examined.

Thirty-seven men and age (median) out of 73 were included in the study (61.7 percent). The distributions of sex and age of the 2 analysis sets did not differ significantly. Peak troponin and CK-MB (creatine kinase MB) isoenzyme had higher levels in the T1MI group in discovery set (T1MI verses Others, p value is 0.014 and 0.009, respectively). In the set of verification, significant differences statistically were observed in current smoker (T1MI verses Others; p value is 0.043), comprising the ECG (electrocardiogram) the results for the ST-depression (T1MI verses Others; p value is 0.02), the non-specific changes (T1MI verses Others; p is less than 0.0001), and the ST-elevation (T1MI verses Others; p value is 0.007), and in both the biomarkers of the heart disease, the CK-MB (T1MI verses Others; p value is 0.002) the troponin (T1MI verses Others; p value is 0.002). The Higher values are seen in the all parameters amongst the individuals having the type 1 MI compared to those in others group, excluding nonspecific abnormalities in the ECG (Table 1). Patients in the Others group had four different disease diagnoses, and O2 imbalance was one of the conditions that contributed to type 2 MI (the supplementary Table S1).

Table 1. Characteristics of Baseline.

	All	Verification			Discovery		
		Others a	T1MI	*p*-Value	Others a	T1MI	*p*-Value
Male, n (%)	37 (61.7)	15 (53.6)	22 (68.8)	0.291	4 (40)	10 (83.3)	0.074
Systolic BP, mmHg	130.4 ± 19.9	134.4 ± 21.3	126.9 ± 18.1	0.149	133.5 ± 12.7	135.3 ± 14.4	0.768
Age (year)	73 (60.5, 80)	72.5 (59, 80.8)	74 (62.0, 79.8)	0.805	70.1 ± 17.5	69.6 ± 11.6	0.935
Diastolic BP, mmHg	78.5 (69, 89.8)	80 (70, 90)	74 (67.5, 88.5)	0.394	79.5 (70, 84.8)	78.5 (71.3, 88.8)	0.936
BMI, kg/m2	23.5 (21.5, 26)	24.2 (21.5, 26.5)	23.4 (21.6, 25.6)	0.8	25.4 ± 2.6	23.1 ± 2.9	0.044
Past Medical History							
Previous heart failure	19 (31.7)	9 (32.1)	10 (31.3)	>0.999	2 (20)	3 (25)	>0.999
Previous revascularization	9 (15)	3 (10.7)	6 (18.8)	0.192	1 (10)	2 (16.7)	>0.999
Previous myocardial infarction	5 (8.3)	2 (7.1)	3 (9.4)	>0.999	1 (10)	1 (8.3)	>0.999
Risk Factors							

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Current smoker	13 (21.7)	4 (14.3)	13 (40.6)	0.043	0 (0)	4 (33.3)	0.096
Hyperlipidemia	19 (31.7)	10 (35.7)	9 (28.1)	0.586	5 (50)	3 (25)	0.377
Diabetes	25 (41.7)	14 (50.0)	11 (34.4)	0.296	6 (60)	3 (25)	0.192
Past smoker	9 (15)	5 (17.9)	4 (12.5)	0.721	2 (20)	2 (16.7)	>0.999
CAD family history	6 (10)	2 (7.1)	4 (12.5)	0.675	1 (10)	1 (8.3)	>0.999
Hypertension	32 (53.3)	16 (57.1)	16 (50.0)	0.613	7 (70)	5 (41.7)	0.231
Laboratory Findings							
CK-MB, ng/mL	23 (7.7, 64.9)	13.5 (5.6, 36.1)	51.0 (10.7, 137.6)	0.002	7.3 (5.0, 18.8)	31.7 (10.8, 104.7)	0.009
Hemoglobin, g/dL	12.6 ± 2	12.1 ± 2	12.9 ± 2	0.115	11.9 ± 2.1	13.19 ± 2	0.186
Peak troponin, ng/mL	0.9 (0.3, 2.5)	1.90 ± 1.2	2.7 ± 2.6	0.002	0.3 (0.2, 0.5)	1.4 (0.5, 2.1)	0.014
ECG							
ST elevation	18 (30)	4 (14.3)	14 (43.8)	0.007	1 (10.0)	6 (50)	0.074
ST-depression	15 (25)	3 (10.7)	12 (37.5)	0.02	0 (0)	5 (41.7)	0.04
Nonspecific change	27 (45)	21 (75.0)	6 (18.8)	<0.0001	9 (90.0)	1 (8.3)	0.0003

Identification of Protein Markers Specific for Type 1 Myocardial Infarction

With 208 proteins in group of T1MI and the 205 in others group, 216 of the proteins from DIA (data-independent-acquisition) data are matched to spectrum library as shown Figure 2. 208 proteins that are members of subset of the A-group and intersection of the B-group were used in the marker selection process among them. Additionally, according to the outcomes of the t-test, twenty-nine of them meet the requirements (p is less than 0.77- and 0.05-fold change or the fold change 1.3). (Supplementary Table S2). Additionally, complement group of human immune system was not included in list of potential biomarkers. [16] The literature supported the relationship between chosen proteins and the type 1 myocardial infarction. As a result, 10 proteins were considered as potential indicators. The proteins that were found were subjected to a heatmap analysis. Visual confirmation is available for each illness group's altered protein expression.

Groups of Protein Differentiating the Myocardial Infarction of Type 1: A Quantitative Comparison

Peptides are created with at least 94 percent purity. With the exception of apolipoprotein B-100, all curves of calibration for each peptide had the coefficients of the determination (R2) > 0.99. The MRM final parameter method was used to determine the values of 13 of 14 proteins, comprising 4 proteins identified in literature (the CD 40 ligand, myeloperoxidase (MPO), the pregnancy-associated plasma protein A (PAP-PA), and the matrix metallopeptidase 9 (MMP9)) that are thought to be linked with the plaque rupture and cardiac artery disease and were used as markers of the myocardial infarction. The finalizing method's parameters and transition of peptide dependent ion were listed (Table 2). Although it was taken from the references,

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matrix metallopeptidase 9 was not found in the study samples. Six proteins were found to significantly differ between the two groups after the quantitative data from 2 groups were compared, by the exculsion of outliers: -1 acid glyco-protein 2 (p is less than 0.0001), -1 acid glycoprotein 1 (p less than 0.0001), the CD40 ligand (p less than 0.0001), the cathelicidin antimicrobial peptide (p is less than 0.0001), and the corticosteroid binding globulin (p less than 0.0001) (Figure 4).

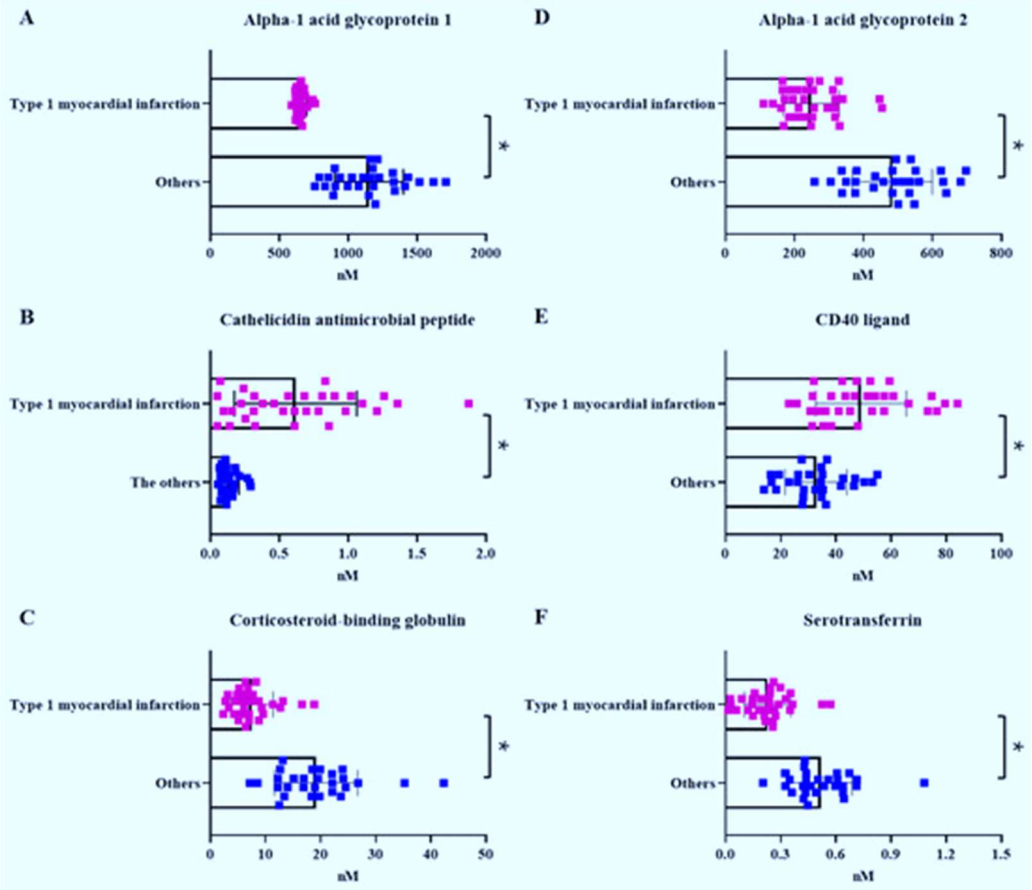

Figure 4. spread-out bar graph. the comparable groups' levels of quantitative protein. (A–F) Bar graph and quantitative scatter plot showing stark differences across groups. * $p < 0.0001$. Others: conditions other than the type 1 MI that have high cardiac troponin.

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Table 2. MRM settings for the peptide data collection technique.

Protein	Peptide	Transition	DP	CE	RT (min)
αα-1-acid glycoprotein 2	EHVAHLLFLR	617.9/267.1	35.1	76.2	6.3
ααα-1-acid glycoprotein 1	YVGGQEHFAHLLILR	877.0/235.3	74.6	45	7.3
Corticosteroid-binding globulin	GTWTQPFDLASTR	740.4/906.5	37.5	85.1	8.5
Cathelicidin antimicrobial peptide	FALLGDFFR	543.3/219.0	31.9	82.4	14.1
Apolipoprotein C-I	TPDVSSALDK	516.8/466.2	23.5	68.8	3.3
Apolipoprotein B-100	GFEPTLEALFGK	654.9/205.1	29.4	78.9	12.8
Prenylcysteine oxidase 1	LFLSYDYAVK	609.8/261.1	25.8	75.6	8.3
Serotransferrin	EGYYGYTGAFR	642.3/551.4	47.4	79.5	5
Matrix metallopeptidase 9	AVIDDAFAR	978.2/465.2	63	216	4.9
Pregnancy-associated plasma protein A	EQVDFQHHQLAEAFK	458.2/449.2	17	90.6	4.2
Fibronectin	WLPSSSPVTGYR	675.4/525.7	30.2	80.3	5.7
Serum amyloid oxidase 1	EALQGVGDMGR	566.7/691.3	30.3	72.4	3.5
CD40 ligand	SQFEGFVK	471.2/249.2	41.3	77.8	4.6
Myeloperoxidase	IANVFTNAFR	576.8/755.4	30.6	73.2	8.4

Marker selection and comparison with control group

If AUC (area under the curve) value of each biomarker in ROC curve was greater than 0.9, it indicated strong diagnostic ability for illness diagnosis.[11] Consequently, the 3 proteins with the AUC value of greater than 0.9 were chosen as the final indicators for the type 1 myocardial infarction diagnosis amongst illnesses with high levels of troponin (Figure 5). High specificity and sensitivity were displayed by each marker. The specificity and sensitivity for AGP2 were 90.63 (75.8-96.8) and 89.3 (72.8-96.3), respectively. The sensitivity and specificity of corticosteroid-binding globulin were 86.7 (70.3-94.7) and 85.7 (68.5-94.3), respectively. The sensitivity and specificity of serotransferrin were 84.4 (68.3-93.1) and 85.7 (68.5-94.3), respectively. AGP2, CBG, and TREA all had cutoff values that were, respectively, 337.3, 12.4, and 0.4 nM. Table 3 includes values of cutoff, specificity and sensitivity of each marker.

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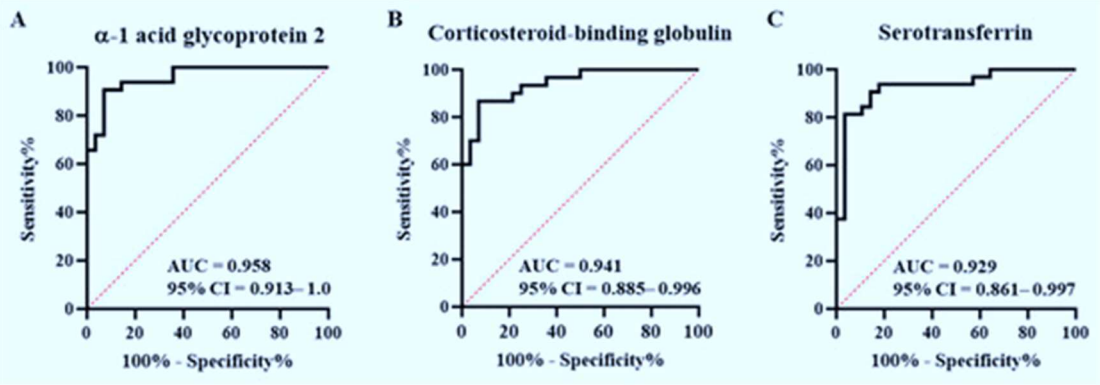

Figure 5. curve for receiver-operating characteristics (ROC). proof of the marker's diagnostic abilities. AUC of -1 acid glycoprotein two is the 0.958 in (A) and (B). AUC of the protein that binds corticosteroids is 0.941. (CI, confidence interval) Serotransferrin's AUC is 0.927.

Table 3. A comparison of markers' diagnostic capacities in brief.

Protein	Specificity	Sensitivity	Cut-off Value (nM a)
Serotransferrin	85.7 (68.5–94.3)	84.4 (68.3–93.1)	<0.4
Corticosteroid-binding globulin	85.7 (68.5–94.3)	86.7 (70.3–94.7)	<12.4
αα-1 acid glycoprotein 2	89.3 (72.8–96.3)	90.63 (75.8–96.8)	<337.3

Protein marker expression patterns were shown to be different between the disease group and healthy control group (free of the cardiovascular disease group) (Supplementary Table S3). The majority of the time, the proteins that had previously been identified as being associated with each disease showed statistically significant differences between the type 1 MI and healthy groups (Supplementary Figure S1).

DISCUSSION

To differentiate T1MI as from the other disorders with the high cardiac troponin, we looked into biomarkers other than troponin in the current study. Since it may screen proteins in sample without knowing anything about them beforehand, the nontargeted MS (mass spectrometry) method is useful for identifying the potential of the novel bio-markers.[11,12] Additionally, multiple reaction monitoring can be employed inexpensively and without the use of antibodies to confirm changes quantitatively in internal proteins linked to the pathophysiology.[13] Using mass spectrometry, we identified unique indicators between T1MI and the other illnesses with the increased troponin, such as T2MI, and subsequently confirmed their viability as the biomarkers. We have discovered 3 biomarkers that were highly successful at detecting type 1 MI: the CBG (corticosteroid-binding globulin), the -1 acid glycoprotein 2 (AGP), and the TRFE (serotransferrin).

Plaque and thrombus cause type 1 MI. Therefore, it was anticipated in this investigation that elements involved in the making of thrombus and plaque can serve as the particular T1MI markers. Plaque disintegration is caused by inflammatory, oxidative, and hypoxic conditions, and plaque-thrombus causal link encourages the thrombus development. Fibrin

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makes up the majority of the thrombus composition, with the remaining components being erythrocytes, platelets, leukocytes, and cholesterol crystals.[14]

Acute-phase protein α-1 acid glycoprotein 2 has the potential to serve as a clinical condition marker. Although it can be found in the myocardium, AGP is mostly synthesised in the liver.[15] The content of this protein, which inhibits inflammation, is modest in normal state then dramatically elevated in the conditions of acute-phase brought on by the inflammatory cytokines like the glucocorticoids, interleukin (IL)-1, CCAAT/enhancer binding proteins, tumour necrosis factor-, and the IL-6.[16] A significant inflammatory response & the elevated AGP2 levels are produced in MI as a result of immune system being stimulated to repair necrotic lesion after MI.[17] In this study, the others group's AGP2 levels were noticeably greater. This conclusion could be explained by a number of pathophysiological diseases and syndromes, but additional research is needed to confirm this.[17] Additionally, the comparison of the illness groups revealed protein levels that were differentially expressed. AGP2 may therefore serve as the biomarker to differentiate the T1MI. Both TRFE and CBG showed a clear decline in comparison to their levels in controls group, making them useful indicators for differentiating type 1 MI from other illnesses.

Although it can be detected in the heart as well as the liver, corticosteroid-binding globulin's tissue origin is unknown. Glucocorticoids are carried and bound by CBG, which also modulates the free hormones.[18] The majority of the cortisol's binding occurs with CBG, with the remainder binding with the free cortisol or albumin. The ability of the CBG to regulate free cortisol levels has lately gained attention.[19] The free hormone theory states that only the free corticosteroids, which don't bind to the CBG, have the biological activity in the clinical settings.[20] The affinity of the cleaved CBG for cortisol decreases during inflammation, while the release of free hormones increases. Because of its lower affinity for cortisol, CBG has the low expression in inflammation, which might be brought on by the synthesis degradation. Our findings are consistent with other research and attest to the elevated the free cortisol and the levels lower CBG in the MI.[17] Here, the severe form of the T1MI may be indicated by the clear decline in the T1MI compared to that in controls.

The potential for the inflammation, malignancy, hepatic dysfunction, and tissue necrosis is lowered in serotransferrin, the protein negative acute-phase.[21] The frequent transferrin deficit implies that the saturation of excessive iron and organisation in binding site of transferrin can result in the iron deposition and the iron overload. We can infer that the level of iron will rise in the acute-MI since ferritin levels were greater in the acute MI (premature) than in the healthy controls.[22] Heart function depends heavily on iron homeostasis, and iron overload and shortage have both been linked to the development of disorders related to heart.[23] An earlier study found that iron excess accelerated the generation of reactive oxygen species, which in turn enhanced blood arteries oxidative stress and hastened thrombus in the rats. Additionally, development of atherosclerotic plaques, the tighter fibrin networks, and the activated and reactive condition of the circulating platelets in the MI can all be caused by increased oxidative stress brought on by higher blood iron levels.[24] Since thrombogenesis had affected the development of the T1MI, decrease in the TRFE denotes the excess of the iron and that the elicited reactions might have an impact on thrombogenesis.

Biomarkers can successfully differentiate between MI subtypes, according to earlier research.[25] However, taking this circumstance into account is necessary as other illnesses besides the myocardial infarction might be suspected throughout diagnostic process.[22] Aside from factors connected to the cardiovascular system, type 2 MI and mortality can also be brought on by other complicated factors, which is also observed in patients in current investigation (Supplementary Table S1). Although many individuals who have type 1 MI have cardiac

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angiography do so because of the significant mortality rate associated with cardiovascular illness.[26] In other way, because T1MI patients have the high chance of the dying from the MI, it is critical that they are properly identified and treated. Based on their high specificity, the markers identified in this investigation can assist in differentiation of the type 1 MI. They are being investigated as markers in other disorders, such as cancer, although they are not heart-derived indicators. They can therefore be utilised in conjunction with troponin rather than as specific MI indicators.

The scope of our investigation has a number of restrictions. The study's patient population is too tiny, to start with. For the findings of this study to be validated, additional research with bigger participant populations is required. For instance, a large-scale study might not have excluded the outliers that were eliminated in the study on small-scale. Second, the control group lacked the diversity necessary to adequately represent all the disorders with high troponin levels (excluding T1MI). For two phenomena, T2MI accounted for more than 80% (46 + 36 percent) of the sample as a whole. Future research will be needed for validateing and confirming the findings, including more trials with disorders with high troponin levels. Third, there is no proof of the marker's diagnostic value when combined with troponin. Future research should take this into account to demonstrate that markers found in current study could supplement troponin and act as the T1MI specific marker.

CONCLUSIONS

We have found three distinct markers in our study that can successfully differentiate the type-1 MI from the other diseases of troponin-rising. These markers—CBG, TREA, and AGP2—can aid in the diagnosis of type 1 MI and advance our knowledge of the illness pathophysiology; validation will, however, require more studies. The indicators discovered in this study might also be found in other thrombotic and plaque-related illnesses. However, combining these indicators with additional symptoms such chest discomfort, an abnormal ECG, and high troponin levels might improve the accuracy of advanced MI diagnosis.

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