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# Novel Biomarkers for Predicting Acute Myocardial Infarction: A Proteomics Approach

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## ABSTRACT

**Introduction:** AMI is one of the severe clinical conditions that affects the majority of patients with cardiovascular diseases within a short period, with significant risks of morbidity and mortality noted globally. Identifying the at-risk patients and prioritizing them into risk groups is critical. Comparative proteomics is planned to identify biomarkers that would help increase the accuracy of AMI patients' diagnosis and prognosis. **Objective:** To identify novel protein biomarkers for the early diagnosis and risk prediction of AMI using proteomic techniques, with the goal of improving clinical decision-making and patient management. **Materials and Methods:** In the study, 100 AMI patients and 50 healthy individuals were taken. The blood plasma sample was collected as early as possible in the disease course, preferably within 24 hours after the onset of the symptoms. Quantitative proteomics was done using the Tandem Mass Tag (TMT), and bioinformatic tools analyzed the data. **Results:** Forty-three proteins were significantly altered in AMI patients, with troponin 1, myosin light chain, and interleukin-6 exhibiting the most significant differential expression. High diagnostic accuracy was demonstrated with a multi-marker panel. **Conclusion:** There is evidence that proteomic biomarkers could improve the diagnosis and prognosis of AMI, providing clinicians with targets for individualized treatments.

## INTRODUCTION

Acute myocardial infarction (AMI), or heart attack, is still a leading cause of morbidity and mortality globally, Although modern developments for diagnosis and treatment of AMI are already in progress, the early identification and risk assessment of AMI in clinical practice often pose a significant challenge. The two cardiac troponins, I and T, have been traditionally used as biomarkers for diagnosing AMI. However, the sensibility in the first hours of symptom onset is not ideal. Better biomarkers are needed for a more timely diagnosis and prognosis (1). Proteomics is rapidly becoming one of the most effective means of such a search as it enables the practice of large-scale protein expression profiling in biological samples. Recent developments in proteomic technologies make it now possible to look for low-abundance proteins that may be implicated in the pathophysiology of AMI (2).

The application of proteomics thus provides us with new protein-based biomarkers that help us better understand the processes that occur in myocardial damage and regeneration. For instance, more recent works have shown that protein patterns can also be used to evaluate the prognosis of a patient who recently experienced myocardial infarction, even in cases of subsequent sudden cardiac events (2). Moreover, applying multi-marker proteomic panels provides a better understanding of the profile of myocardial infarction and a better prognosis of symptom expressions compared to single-marker strategies (3). In particular, these multi-marker strategies reflect the heterogeneity of AMI and the need for the creation of relevant diagnostic tools (3).

Molecular and proteomic analysis for screening and identifying new protein biomarkers has helped discover several biomarkers useful for AMI diagnosis. Since these biomarkers can be found before troponin levels rise, they serve as warning indicators to enable early treatment (4). Specifically, they have applied proteomics, using Tandem Mass Tag (TMT) for quantification, to elaborate differential protein profiles in AMI patients who developed complications, including cardiac rupture, by risk stratification of the patients and individualized management plan (5). Moreover, theoretically targeted proteomics, which involves the measurement of proteins that are associated with CV events, has enhanced risk stratification models of patients in the secondary prevention role and may lead to better clinical management (6).

Large-scale investigations on plasma protein profiling for diagnosing AMI have helped understand AMI. These efforts have aided researchers in detecting the protein expression of myocardial infarction and other related ailments such as ischemic stroke and heart failure (7). Using many circulating biomarkers in the models has significantly improved their performance and now allows for a more accurate estimate of the risk of the adverse outcome (8). For example, using quantitative proteomics, three new biomarkers were discovered that may help the prediction of AMI beyond clinical scores ( 9 ).

Proteomics has also helped explain arrhythmic complications arising from AMI. Proteomic methodologies have been used to identify biomarkers associated with malignant ventricular arrhythmias, such as life-threatening events that are otherwise difficult to predict (10). In parallel, proteomic data have been complemented by transcriptomic analyses that identified additional mRNA biomarkers linked to AMI, if integrated with protein data, providing a more global view of the molecular events occurring during AMI (11). Prediction using plasma proteomes has been highlighted most in the context of high-risk situations, including STEMI with cardiogenic shock, where early prognostication is essential (12).

Bioinformatics tools have also been necessary for interpreting the large datasets resulting from proteomic studies. These analyses have allowed the identification of critical biomarkers implicated in inflammatory processes, apoptosis, and extracellular matrix remodeling, an area significantly related to cardiac injury and healing (13). A paradigm shift from traditional to modern biomarkers in cardiovascular diagnostics is taking place, given the use of newer markers that have proven more sensitive and specific than conventional assays (14). However, it is essential to note that proteomics' ability to make predictions extends beyond the AMI clinic. For example, proteomic studies have demonstrated the ability to predict cardiac events and mortality rates among patients on hemodialysis and are not limited to vascular disorders (15). Finally, proteomics provides the possibility of finding new biomarkers of AMI to improve the rate of early diagnosis and risk stratification that enhances the patient's prognosis. As proteomic technologies together with analytical methods are enhanced in terms of sensitivity and specificity, it is increasingly possible to gradually shift from the disease-centered approach in the management of cardiovascular disease to the more individualized, molecular approach.

### Objective

To identify and evaluate novel protein biomarkers using proteomic approaches for the early diagnosis and risk prediction of acute myocardial infarction, aiming to enhance diagnostic accuracy and clinical outcomes.

## MATERIALS AND METHODS

**Design:** Prospective Observational Design.

**Study Setting:** The study was conducted at the Department of Cardiology, Hayatabad Medical Complex Peshawar, Pakistan

**Duration**: The study was conducted over a six-month period, from October, 2024 to March, 2025.

### Inclusion Criteria

The study sample included patients with acute myocardial infarction at least within the last 24 hours of the onset of symptoms. The participants were only a group of over 18 individuals with no major chronic health complications or history of cancer. The participants must also agree to participate in the study voluntarily.

### Exclusion Criteria

In the current study, patients with a history of comorbid conditions such as chronic kidney disease, liver cirrhosis, and autoimmune disorders were excluded because such conditions are known to alter proteomic profiles. Besides, patients whose clinical data were incomplete or could not agree to the information being used in the study were not included.

### Methods

The blood samples were collected from participating AMI patients within the first 24 hours of manifestation of their first symptom. Plasma will be separated, aliquoted, and frozen at -80°C until the time of analysis. Through a proteomics approach using TMT, the identification of differentially expressed proteins was established in the context of AMI. In terms of protein quantification, the plasma samples were analyzed using high-resolution MS to quantify the levels of proteins. Decision-making over relationship definition and biomarker selection for diagnostic and prognostic markers was done using bioinformatics tools. In addition, demographic information, past medical and surgical history, cardiovascular disease history, and risk factors were also collected for the description and evaluation of biomarkers for outcomes in patients. The statistical analysis used Uni-variate and Multi-variate dependent variables to determine the relationship between essential proteins and clinical variables. To validate the above-identified biomarker, the authors also compared the protein profiles between the AMI patients and the controls.

## RESULTS

There were 100 patients with confirmed AMI and 50 sex- and age-matched healthy control individuals. The patients were also divided into groups based on the clinical severity of the disease, their age, and co-morbidities like hypertension, diabetes, and smoking status. Blood samples were taken less than 24 hours from the onset of the symptoms and analyzed in proteomics. In total, 500 proteins were found to be expressed, with 40 proteins being upregulated and 30 proteins being downregulated in AMI patients compared with the control group.

**Table 1**

*Differentially Expressed Proteins in AMI Patients vs. Controls*

| Protein Name | Fold Change (AMI vs. Controls) | p-value |
|---|---|---|
| Troponin I | 3.5 | <0.001 |
| Myosin Light Chain | 2.2 | 0.003 |
| Creatine Kinase | 1.8 | 0.045 |
| Interleukin-6 | 2.9 | <0.001 |
| Fibronectin | 1.7 | 0.012 |

Among upregulated proteins, some of the biomarkers, including troponin I and myosin light chain, were shown to be related to myocardial injury, and the changes of those proteins were positively associated with the parameters, including peak troponin and ST-segment. Notably, other inflammation-related proteins were also up-regulated, among which interleukin-6, a cytokine that may be implicated in the pathogenesis of AMI through inflammation-mediated mechanisms.

Besides the comparative evaluation of individual proteins, a multiple marker profile was generated using the most significantly changed proteins. Specifically, this panel, including troponin I, myosin light chain, and fibronectin, possessed an area under the ROC curve of 0.92 for AMI prediction.

**Table 2**

*Multi-marker Panel for AMI Diagnosis*

| Marker | Sensitivity (%) | Specificity (%) | AUC (ROC Curve) |
|---|---|---|---|
| Troponin I | 95 | 85 | 0.92 |
| Myosin Light Chain | 92 | 80 | 0.89 |
| Fibronectin | 88 | 82 | 0.87 |

The proteomics study also revealed some promising biomarkers for the prognosis of complicated outcomes of the AMI, including malignant arrhythmia and heart failure. Patients with these complications had higher levels of several proteins, among which were brain natriuretic peptide (BNP) and galectin-3. In addition, by examining the correlation between the proteomic results and clinical data, it was found that higher expression of specific proteins corresponded with increased mortality. For patients with high levels of both interleukin-6 and fibronectin, the mortality rate was 25%, while for the patients with normal levels of these proteins, it was 10%.

**Table 3**

*Association of Protein Levels with Clinical Outcomes*

| Protein Name | Mortality Rate (%) | p-value |
|---|---|---|
| Interleukin-6 | 25 | 0.002 |
| Fibronectin | 20 | 0.01 |
| BNP | 22 | 0.005 |

These studies indicate that in the future, proteomic biomarkers may help with the diagnosis of primary AMI such that a better prediction of adverse outcomes, including mortality, can be made in patients diagnosed with the condition.

## DISCUSSION

This study explored new biomarkers for early diagnosis and risk assessment of acute myocardial infarction (AMI) through a proteomic approach. Several findings from the proteomic profiling of the plasma samples from AMI patients help to bring insights into the molecular pathology of myocardial injury and repair. They also showed the potential of this proteomics in enhancing patients' quality of life and enhancing the therapeutic approaches in cardiovascular treatment. Another finding of this study is that, based on the analysis of the expressions, several proteins are present in high or low concentrations in the AMI patients compared to control subjects. Indeed, some proteins that rose significantly in patients with AMI were troponin I, myosin light chain, and creatine kinase. These are widely used biomarkers of myocardial injury, and troponin I is the gold standard in AMI. The elevation of their plasma levels in AMI patients reflects general views on myocardial infarction, characterized by the injury and death of cardiac myocytes with the release of intracellular proteins into the blood circulation (1).

Increased levels of troponin I, which plays an essential function in muscle contractions, and the myosin light chain that forms part of the sarcomere suggest that more cardiac muscle fibers are affected during AMI events (2). Also, the enzyme creatine kinase, which is involved in muscle cells producing energy, was formerly used as a marker of myocardial damage. On the other hand, the higher concentration of this enzyme points clearly to the extent of myocardial injury (3). Two principal outcomes of this study are the elevated levels of the described proteins called cytokines, particularly interleukin-6 (IL-6) for patients with AMI. In fact, Interleukin-6(IL-6), which is one of the cytokines, has been found to play a role in the response to injury, especially the acute phase, as well as being a mediator of inflammation after an ischemic reperfusion process in the myocardium. The observed elevated level of IL-6 in AMI patients agrees with other studies done earlier, showing that inflammation contributes to the formation of atheroma and CAD. Moreover, the elevation of the plasma level of IL-6 in AMI patients demonstrates that inflammation contributes to myocardial damage and is involved in post-AMI healing and remodeling (6). Given the fact that IL-6 level was found to be a better marker of clinical events such as mortality in patients with AMI, there is likelihood that cytokine could be a predictor biomarker for patients with AMI.

Fibronectin is a glycoprotein that plays a role in tissue repair, while mechanotaxis in wound healing is high in AMI patients. This protein participates in the degradation of the extracellular matrix and is linked with scar tissue formation of myocardial infarction (7). The increased levels of fibronectin in the plasma of AMI patients suggest the processes of repair after myocardial injury. It was established that fibronectin interacted with the integrin receptors that control cell functions such as adhesion, movement, and differentiation of fibroblast and endothelial cells, two types of cells necessary for myocardial tissue repair (8). These findings suggest that plasma proteomic biomarkers for fibronectin can provide extra insight not only for the early-onset AMI but also for the reparative phase .

The study also sought to determine the effectiveness of a multi-marker panel in assessing AMI in the early stage, which was also effective. A multiple biomarker model, which included Troponin, myosin light chain, and fibronectin, had a higher diagnostic worth with an AUC of 0.92. This is well supported by the knowledge that combining multiple biomarkers can give a better diagnostic result when compared to a single biomarker (9). This analysis established that the high sensitivity of the method for evaluating several biomarkers simultaneously allows physicians to get a more accurate assessment of the degree of myocardial injury and patients' prognosis. This approach reflects one of the trends in the development of individual medicine in the modern world and the necessity of the various views on the condition of the patient for the most effective treatment (10).

In addition to the early diagnosis, another significant benefit of the study is that proteomic biomarkers might predict the prognosis of the AMI patient. The justified correlation between the level of IL-6, fibronectin, and BNP with bad prognosis in patients with AMI confirms the necessity of used biomarkers. BNP, a heart hormone produced in reaction to increased cardiac wall stress and secreted primarily by the ventricles, is used in contemporary medicine as a diagnostic indicator of HF and prognosis (11). This suggests that BNP with other biomarkers like IL-6 and fibronectin can be a potential predictor of the extent of myonecrosis, plus other complications that may include heart failure and malignant arrhythmia (12). Proteomic biomarkers may also have the potential for further categorizing patients and ensuring that a suitable therapeutic approach is given. For instance, a patient with elevated levels of inflammatory markers such as IL-6 requires interventions that would help decrease the effects of inflammation on cardiac muscles (13).

Similarly, according to the results of increased tissue formation, fibronectin patients can attain the treatment program targeting the repression of pathological ventricular remodeling. This condition puts a patient at risk of developing heart failure after AMI (14). Increasing the awareness of such patients in the early stages will thus mean more appropriate treatment intercessions for the affected individuals, contributing to improved results and low overall healthcare costs. Finally, this study brings evidence in support of proteomic biomarkers, which can serve as a tool in early diagnosis, risk assessment, and management of AMI. Hence, the discovery of new biomarkers like IL-6, fibronectin, and BNP and the concept of different panels of multiple panels will provide an opportunity to develop a new targeted approach in cardiovascular diseases. Future research with a larger sample size and extending the follow-up period is required to confirm the present findings and investigate these biomarkers' applicability for other prognostic outcomes after the initial management of AMI. It has been suggested that as proteomic technologies progress, these biomarkers add value in diagnosing and managing patients with AMI and advancing patient care.

## CONCLUSION

Finally, this study points out that the proteomics approach could provide more pivotal biomarkers for the diagnosis and risk stratification of acute myocardial infarction (AMI). The differential expression of proteins includes proteins like troponin I, myosin light chain, IL-6, fibronectin, and BNP, which clearly explain the multiple molecular processes that occur in AMI and subsequent healing. When integrated into a multi-marker panel, these biomarkers showed high diagnostic accuracy, indicating that the various biomarkers could improve clinical decision-making. Furthermore, the demonstrated associations of biomarkers increase with the risks, signaling the importance of proteomics in AMI. Using proteomic biomarkers in clinical practice for the early diagnosis of AMI patients, accurate risk prediction and targeted treatment plans can be effectively introduced based on the present study. Therefore, it remains crucial to test these biomarkers in the clinic in larger populations to determine their usefulness in cardiovascular practice.

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