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RESEARCH ARTICLE

DIFFERENTIAL EXPRESSION OF CAVEOLIN-3, ST2, AND GDF-15 GENES IN ACUTE MYOCARDIAL INFARCTION: A CROSS-SECTIONAL STUDY

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Abstract

Introduction: Acute myocardial infarction (AMI) is a prominent cause of death and morbidity worldwide, requiring biomarkers for early diagnosis and prognosis. CAV-3, ST2, and GDF-15 are implicated in cardiovascular pathogenesis, but their differential expression and functional functions in AMI are unknown. This study aimed to evaluate the differential expression of CAV-3, ST2, and GDF-15 genes in AMI patients compared to controls and to investigate their potential as biomarkers and functional roles through bioinformatics analysis.

Methods: This study included 80 individuals (40 AMI patients and 40 non-AMI controls). Blood samples were analyzed using real-time PCR to measure the relative expression of the target genes. Bioinformatics tools were employed to identify gene enrichment pathways and protein-protein interactions. Statistical analysis was performed to assess the association of gene expression with clinical parameters.

Results: CAV-3, ST2, and GDF-15 were significantly upregulated in AMI patients compared to controls (fold changes: 3.45, 1.87, and 2.55, respectively; $p < 0.05$). Bioinformatics analysis identified CAV-3 as a critical player in cardiac conduction and calcium-mediated transport, interacting with proteins such as dysferlin and annexins. Correlation analysis revealed a strong association between CAV-3 expression and cardiac conduction abnormalities ($r = 0.65$, $p < 0.01$). ST2 and GDF-15 correlated with inflammatory and stress markers ($r = 0.58$, $r = 0.62$, $p < 0.05$).

Conclusion: The upregulation of CAV-3, ST2, and GDF-15 in AMI patients underscores their potential as biomarkers and highlights their roles in critical pathways such as cardiac conduction, inflammation, and

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stress response. These findings provide a foundation for future studies on diagnostic and therapeutic interventions in AMI.

Keywords: Acute Myocardial Infarction, Caveolin-3, ST2, GDF-15, Biomarkers, Cardiac Conduction

BACKGROUND/INTRODUCTION

Acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality worldwide, necessitating novel approaches to improve early diagnosis and prognosis. Despite advancements in clinical diagnostics, there is a growing need to identify robust biomarkers that provide insights into the molecular underpinnings of AMI and offer prognostic value [1]. Biomarkers such as Caveolin-3 (CAV-3), Suppression of Tumorigenicity 2 (ST2), and Growth Differentiation Factor-15 (GDF-15) have shown potential in elucidating the pathophysiology of cardiovascular diseases, particularly AMI [2,3].

CAV-3, a muscle-specific protein, plays a critical role in maintaining cardiac muscle integrity and function. It has been implicated in pathways such as smooth muscle contraction and calcium-mediated transport, which are vital in cardiac conduction and electrophysiology [4]. On the other hand, ST2, a member of the interleukin-1 receptor family, serves as a marker of cardiac stress and inflammation, with established links to adverse cardiac remodeling and heart failure [5,6]. GDF-15, a stress-responsive cytokine, is emerging as a critical biomarker associated with cardiovascular events, offering predictive value for mortality and major adverse cardiac events (MACE) in AMI patients [7].

Recent studies suggest that the upregulation of these genes is associated with myocardial damage, inflammation, and stress responses, underscoring their potential as diagnostic and prognostic tools. For instance, GDF-15 has been identified as a predictor of long-term mortality in AMI patients and has been shown to regulate cellular responses to ischemia/reperfusion injury [4]. Similarly, CAV-3 has been highlighted for its functional interactions with proteins such as dysferlin and annexins, which are crucial for muscle repair and calcium regulation [1].

This study aims to evaluate the differential expression of CAV-3, ST2, and GDF-15 genes in AMI patients compared to controls and to investigate their potential as biomarkers and functional roles through bioinformatics analysis.

MATERIALS AND METHODS

Study Design

A cross-sectional study.

Study Setting

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The study was carried out in a multi-specialty hospital with state-of-the-art laboratory facilities for molecular and bioinformatics analyses.

Participants

A total of 160 participants were recruited for the study. Blood samples were collected from:

- **AMI Patients (n=40):** Diagnosed with AMI based on clinical history, ECG findings, and elevated cardiac biomarkers.
- **Non-AMI Controls (n=40):** Individuals without any history of AMI or cardiovascular disease.

Inclusion Criteria

- AMI Group: Patients aged 30–70 years with confirmed AMI diagnosis.
- Control Group: Age- and sex-matched healthy individuals with no history of cardiovascular diseases or systemic illnesses.
- Participants who provided written informed consent.

Exclusion Criteria

- Individuals with chronic inflammatory conditions, autoimmune diseases, cancer, or ongoing infections.
- Pregnant or lactating women.
- Participants on immunosuppressive or steroid therapy.

Bias

To minimize selection bias recruitment was randomized within the eligible population. Age and sex matching were performed between AMI and control groups. To reduce measurement bias laboratory procedures followed standardized protocols, and all analyses were conducted in a blinded manner.

Variables

Variables included presence or absence of AMI, relative expression levels of CAV-3, ST2, and GDF-15 genes, age, sex, comorbid conditions, and lifestyle factors.

Data Collection

- **Sample Collection:** Blood samples were collected under sterile conditions, and RNA was isolated for gene expression analysis.
- **Gene Expression Analysis:** Real-time PCR (qPCR) was used to quantify the relative expression levels of CAV-3, ST2, and GDF-15. GAPDH was used as the reference gene for normalization.
- **In Silico Analysis:** Functional gene enrichment and protein-protein interaction networks were analyzed using bioinformatics tools such as STRING and DAVID.

Procedure

1. **Sample Preparation:** RNA was extracted from blood samples using a commercial RNA isolation kit. cDNA synthesis was performed for qPCR analysis.

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2. **Gene Expression Analysis:** Relative quantification of the target genes was performed using the ΔΔCt method. Fold change in gene expression was calculated for AMI patients compared to controls.
3. **Bioinformatics Analysis:** In silico tools identified key pathways and protein interactions involving CAV-3, emphasizing its role in smooth muscle contraction, cardiac conduction, and calcium-mediated transport.

Statistical Analysis

Baseline characteristics were summarised using descriptive statistics. The Mann-Whitney U test or the Student's t-test, as applicable, were used to analyse the gene expression data. To evaluate the relationships between gene expression levels and clinical factors, correlation analysis was done. P-values less than 0.05 were regarded as statistically significant. Version 25.0 of SPSS software was used for all statistical analyses.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

RESULTS

Baseline characteristics of the study participants were compared between AMI patients and controls.

Table no.1: Baseline Characteristics

Characteristic	AMI Patients (n=40)	Controls (n=40)	p-value
Age (years, Mean ± SD)	58.2 ± 8.1	57.5 ± 7.9	0.82
Male (%)	70	72	0.88
Smoking History (%)	60	35	0.03
Hypertension (%)	55	25	0.02
Diabetes Mellitus (%)	45	20	0.04

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Gene Expression Analysis

The expression levels of CAV-3, ST2, and GDF-15 were significantly higher in AMI patients compared to controls. CAV-3 showed the most substantial increase, indicating its critical role in AMI pathology.

Table no.2: Differential Expression of Target Genes

Gene	AMI Patients (Mean ± SD)	Controls (Mean ± SD)	Fold Change	p-value
CAV-3	3.45 ± 0.42	1.00 ± 0.12	3.45	0.001
ST2	1.87 ± 0.25	1.00 ± 0.15	1.87	0.015
GDF-15	2.55 ± 0.38	1.00 ± 0.11	2.55	0.005

Bioinformatics Analysis

In silico functional enrichment identified pathways and protein interactions relevant to each gene, as summarized below.

Table no.3: Functional Enrichment Analysis

Gene	Enriched Pathways	Biological Functions
CAV-3	Smooth muscle contraction, cardiac conduction, calcium transport	Regulation of cardiac electrophysiology and muscle function
ST2	Inflammation, cardiac remodeling	Modulation of inflammatory response and myocardial stress
GDF-15	Stress response, apoptosis regulation	Cell survival and apoptosis regulation under stress conditions

Table no.4: Protein-Protein Interaction (PPI) Network Analysis

Gene	Key Protein Interactions	Functional Relevance
CAV-3	Dysferlin, Annexins	Muscle repair, calcium regulation

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ST2	IL-33, Myocardial Proteins	Inflammatory signaling, cardiac tissue repair
GDF-15	TGF-beta, BMP Proteins	Cellular stress response, apoptosis regulation

Correlation Analysis

The correlation of gene expression with clinical parameters was explored. Significant correlations were observed, particularly for CAV-3 with cardiac conduction abnormalities.

Table no. 5: Correlation Analysis

Gene	Parameter	Correlation Coefficient (r)	p-value
CAV-3	Cardiac conduction abnormalities	0.65	< 0.01
ST2	Inflammatory markers	0.58	< 0.05
GDF-15	Stress biomarkers	0.62	< 0.05

These results highlight the significant upregulation of CAV-3, ST2, and GDF-15 in AMI patients and their correlation with key clinical parameters, suggesting their potential as biomarkers for AMI.

DISCUSSION

The study revealed a significant upregulation of CAV-3, ST2, and GDF-15 in AMI patients compared to controls, with fold changes of 3.45, 1.87, and 2.55, respectively. Among these, CAV-3 exhibited the highest level of upregulation (p = 0.001), underscoring its prominent role in AMI pathophysiology. ST2 and GDF-15, while moderately upregulated, were still significantly associated with AMI (p = 0.015 and p = 0.005, respectively). These findings suggest that these genes may serve as potential biomarkers for the early detection and progression of AMI.

In silico analyses demonstrated that CAV-3 plays a critical role in pathways related to smooth muscle contraction, cardiac conduction, and calcium-mediated transport. Protein-protein interaction (PPI) analysis highlighted key interactions of CAV-3 with dysferlin and annexins, emphasizing its involvement in muscle repair and calcium regulation. Similarly, ST2 was implicated in inflammation and cardiac remodeling through interactions with IL-33 and myocardial proteins, while GDF-15 was linked to stress response and apoptosis regulation via its association with TGF-beta and BMP proteins. These

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pathways reinforce the biological relevance of these genes in AMI.

Expression levels of CAV-3 were positively correlated with cardiac conduction abnormalities ($r = 0.65$, $p < 0.01$), highlighting its potential role in arrhythmogenesis and impaired cardiac electrophysiology in AMI patients. Additionally, ST2 and GDF-15 were moderately correlated with inflammatory and stress biomarkers ($r = 0.58$ and 0.62, $p < 0.05$, respectively), suggesting their contribution to systemic inflammation and cellular stress in AMI. These correlations indicate that the dysregulation of these genes is closely tied to key clinical manifestations of AMI.

Baseline comparisons revealed that AMI patients had higher incidences of smoking, hypertension, and diabetes mellitus compared to controls, with significant differences ($p < 0.05$). These factors may act as confounders influencing gene expression; however, their effects were mitigated through statistical adjustments.

The findings suggest that the upregulation of CAV-3, ST2, and GDF-15 is not only associated with the pathophysiology of AMI but also reflects their potential utility as diagnostic and prognostic biomarkers. The significant involvement of these genes in critical cardiac pathways and their correlations with clinical parameters provide insights into their functional roles. Specifically, CAV-3 emerges as a key player in cardiac conduction and muscle function, while ST2 and GDF-15 highlight the interplay between inflammation, stress, and myocardial damage in AMI. These results pave the way for future research into targeted therapies and biomarker development.

Even after controlling for conventional risk variables and other biomarkers, GDF-15 and TRAIL-R2 were found to be strong predictors of long-term all-cause death in patients with AMI. With an area under the curve (AUC) of 0.88, GDF-15 greatly enhanced the capacity to differentiate survivors from non-survivors during a 5-year period [8]. Certain genetic variations were linked to improved collateral circulation in non-ST-segment elevation MI patients, according to a study examining the GDF-15 +157 A/T polymorphism. These results imply that vascular adaptation during ischaemic episodes is influenced by genetics [9].

GDF-15 is one of the best indicators of death and heart failure hospitalisation in MI patients, according to an examination of 175 biomarkers. GDF-15 provided additional predictive value beyond clinical variables, but TRAIL-R2 was linked to recurrent heart failure [10]. Even after controlling for traditional risk variables, elevated GDF-15 levels were associated with a considerably increased risk of sudden cardiac death within 24 hours of the event MI. The usefulness of GDF-15 in early high-risk individual identification is highlighted by this study [11]. In stable post-MI patients, GDF-15 was the only inflammatory biomarker consistently linked to cardiovascular events. It outperformed ST2 and high-sensitivity CRP

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(hs-CRP) in terms of incremental predictive value for cardiovascular events [12].

Increased GDF-15 levels in MI patients were linked to DNA methylation alterations at particular loci, according to a genome-wide methylation analysis. This emphasises how GDF-15 is epigenetically regulated in cardiovascular disease [13]. Within three months of an AMI, major adverse cardiac events (MACE) were substantially correlated with high GDF-15 levels at admission. Patients with elevated GDF-15 levels had a considerably higher event rate for MACE, according to Kaplan-Meier survival analysis [14].

Although soluble ST2, galectin-3, and GDF-15 were elevated in heart failure and other inflammatory diseases, GDF-15 was the most consistently associated with cardiovascular risk. However, its lack of specificity limits its diagnostic utility [15]. In STEMI patients, citicoline treatment modulated the expression of microRNAs involved in cardioprotection, without altering Cav-3 levels. This suggests that citicoline may indirectly support myocardial recovery via exosomal pathways [16]. Buyang Huanwu Decoction demonstrated cardioprotective effects in an AMI mouse model by upregulating Cav-1 and VEGF expression. This resulted in improved angiogenesis and myocardial recovery [17].

CONCLUSION

This study highlights the significant upregulation of CAV-3, ST2, and GDF-15 in AMI patients, with CAV-3 showing the strongest association with cardiac conduction and muscle repair pathways. The bioinformatics analysis further elucidates their critical roles in inflammation, stress response, and myocardial remodeling. These findings underscore the potential of these genes as biomarkers for the diagnosis and prognosis of AMI, offering valuable insights into its molecular mechanisms and paving the way for targeted therapeutic strategies.

LIMITATION

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of comparison group also poses a limitation for this study's findings.

RECOMMENDATION

Further longitudinal studies with larger cohorts are recommended to validate these findings and explore the prognostic potential of these genes in AMI. Investigations into targeted therapies modulating these pathways may also offer novel treatment approaches.

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CONFLICT OF INTEREST

The authors have no conflicting interests to declare.

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LIST OF ABBREVIATION

AMI - Acute Myocardial Infarction

CAV-3 - Caveolin-3

ST2 - Suppression of Tumorigenicity 2

GDF-15 - Growth Differentiation Factor-15

MACE - Major Adverse Cardiac Events

ECG - Electrocardiogram

qPCR - Quantitative Polymerase Chain Reaction

cDNA - Complementary DNA

ΔΔCt - Delta Delta Cycle Threshold (method for quantifying gene expression)

PPI - Protein-Protein Interaction

IL - Interleukin

TGF-beta - Transforming Growth Factor-beta

BMP - Bone Morphogenetic Protein

VEGF - Vascular Endothelial Growth Factor

CRP - C-Reactive Protein

hs-CRP - High-Sensitivity C-Reactive Protein

AUC - Area Under the Curve

STEMI - ST-Elevation Myocardial Infarction

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