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A Multivariate Approach to The Relationships of Cardiac Biomarkers with Oxidative and Inflammatory Status in Rats

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ABSTRACT

Cardiac biomarkers are considered one of the fundamental elements in the evaluation of cardiac function, while oxidative stress and inflammatory processes can also be regarded as important factors in the assessment of cardiovascular health. This study examined the relationships between cardiac biomarkers, oxidative stress, and inflammation, aiming to uncover the multidimensional interactions among these parameters. Cardiac parameters, inflammatory cytokines, and oxidative stress markers in heart tissue were analyzed in twenty-four male rats. Pearson and canonical correlation analyses were employed to assess the complex relationships between these datasets. Creatine kinase (CK) and creatine kinase–myocardial band (CK-MB) were positively correlated with malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and negatively correlated with glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase ($p<0.05$). Furthermore, cTnI showed a positive correlation with TNF-α and a negative correlation with GSH-Px. The canonical correlation coefficients for the cardiac-oxidative stress and cardiac-inflammation datasets were significant ($r_c=0.852$, $p<0.05$; $r_c=0.821$, $p<0.05$). The proportion of oxidative stress and inflammation parameters explaining the variance in cardiac biomarkers was 52.20% and 49.40%, respectively. Canonical correlation analysis, which incorporates multiple relationships, revealed the intriguing multidimensionality of the interactions among the parameters. The results suggest that the association between oxidant-antioxidant and inflammatory status is notably more intricate with CK and CK-MB than with cTnI. These significant findings offer valuable insights that could contribute to advancing diagnostic and therapeutic strategies in the field of cardiology.

INTRODUCTION

Cardiovascular diseases are a major cause of mortality and a significant public health concern in modern society (Balakumar et al., 2016; Haybar et al., 2023). According to the World Health Organization (WHO), an estimated 17.3 million people died from cardiovascular diseases in 2008, accounting for approximately 30% of all global deaths. It is projected that by 2030, cardiovascular disease-related deaths will increase to approximately 23.6 million, mainly driven by heart disease and stroke (Norton et al., 2011). Additionally, 5.8 million heart patients in the USA have healthcare costs exceeding 39 billion dollars (Norton et al., 2011). After discovering cardiac biochemical markers, clinicians can obtain invaluable information from serum cardiac biomarkers, patient history, and electrocardiographic analysis. This information makes it easier for clinicians to determine the diagnosis, select treatment, and assess prognosis. However, current cardiac biomarkers, while highly sensitive and specific to myocardial damage, have the disadvantage of being unable to precisely identify the extent of the damage (Howie-Esquivel and White, 2008).

According to clinical studies, inflammatory disorders are seen as a risk factor for cardiovascular diseases. It has also been reported that various inflammatory cells cause

vascular oxidative stress (Steven et al., 2019). It is reported that cardiovascular diseases are closely related to increased oxidative stress and inflammation, and antioxidant and anti-inflammatory therapies could be necessary to treat cardiovascular diseases (Daiber et al., 2021). Inflammatory cytokines modulate the phenotype and function of all myocardial cells and may cause impairments in cardiac function by suppressing contractile function in cardiomyocytes (Hanna and Frangogiannis, 2020). In addition to cardiac biomarkers, immune components play an essential role in diagnosing, treating, and prognosing cardiovascular diseases. For instance, plasma levels of IL-1α, IL-18, IL-33, IL-6, and IL-8 positively correlate with atherosclerosis, while plasma levels of other interleukins, such as IL-35, negatively correlate with acute myocardial infarction or cardiac angina. Additionally, the IL-1 superfamily plays a critical role in various cardiovascular diseases, including atherosclerosis, with IL-20 having a pro-atherogenic role, while others, such as IL-10 and IL-19, serve an anti-atherogenic function (Haybar et al., 2023). Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's antioxidant defense capacity, as small amounts of ROS are typically produced inside cells to play a role in cell signaling, and the body's antioxidant defense system can easily handle these small amounts, but in certain conditions, such as illness, the production of ROS can overwhelm the body's antioxidant system, leading to damage and cell death (Van der Pol et al., 2019). Increased oxidative stress in heart diseases like heart failure is responsible for damaging heart tissue (Ahmed and Tang, 2012; Aimo et al., 2020). Some studies suggest that antioxidant treatments can be used for heart conditions (Ahmed and Tang, 2012).

Given the ongoing challenge of predicting cardiovascular diseases despite the wide range of cardiovascular biomarkers available, the study aims to analyse the relationship between cardiac parameters, inflammatory cytokines, and oxidative stress using the Pearson and canonical correlation method. The goal is to uncover the detailed relationships between commonly used clinical parameters cardiac biomarkers (CK, CK-MB, cTnI), inflammatory cytokines (TNF-α, IL-6, IL-1β), and oxidative stress markers (MDA, GSH, GSH-Px, and catalase) in the study. By doing so, we hope to contribute to improving diagnosis and treatment processes for cardiovascular diseases.

MATERIALS AND METHODS

Ethics and animals

The Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (HADYEK) approved the study (Decision No: 2023/05-10). Twenty-four male wistar rats (220-250 g) were provided with food and water ad libitum and exposed to a 12-hour light and 12-hour dark cycle. The animals were anaesthetised with xylazine (10 mg/kg) and ketamine (80 mg/kg) and then euthanised. Blood samples were collected from the heart chambers for biochemical analyses, and heart tissue samples were collected after euthanasia.

Biochemical analyses

The collected blood samples were centrifuged, and cardiac parameters were analyzed using an electrochemiluminescence immunoassay. Oxidative stress markers (MDA, GSH, GSH-Px, and catalase) were assessed via spectrophotometric methods, and inflammatory cytokines (TNF-α, IL-6, and IL-1β) in heart tissue were quantified using commercial ELISA kits.

Statistical analyses

The study rigorously controlled the variables by applying the D'Agostino-Pearson test to assess normality and Levene's test to evaluate the homogeneity of variances. The Pearson correlation coefficient was used to assess the relationships between cardiac, oxidative stress, and inflammation parameters. Prior to conducting canonical correlation analysis, the variables were evaluated for multicollinearity with the scores for the variance inflation factor (VIF). Canonical correlation analysis was then used to examine the associations between the independent set (X), consisting of cardiac biomarkers (CK, CK-MB, and cTnI), and the dependent set (Y), which included MDA, GSH, GSH-Px, catalase, and inflammation markers (TNF-α, IL-6, and IL-1β). This analysis explored the interrelationships between variables in the cardiac parameter set and those in the oxidative stress and inflammation parameter sets. The canonical correlation coefficients' F values were analyzed using Wilks' Lambda values, a significant test. The redundancy index was used to calculate the ratio of explained variance. The significance level was set at $p<0.05$.

RESULTS

Pearson correlation coefficient between cardiac biomarkers, oxidative stress biomarkers and inflammatory cytokine levels

A moderate positive and significant correlation was observed between CK and MDA ($p<0.01$). In contrast, a negative and significant correlation was found between GSH, GSH-Px, and catalase ($p<0.05$, $p<0.05$, and $p<0.01$, respectively). A moderate positive and significant correlation was also identified between CK and TNF-α, IL-6, and IL-1β ($p<0.01$). Similar relationships were found between CK-MB and oxidative stress and inflammation parameters, as shown in Table 1. Furthermore, a moderate negative and significant relationship was discovered between cTnl and GSH-Px ($p<0.05$), and a moderate positive and significant relationship was found with TNF-α ($p<0.01$). Notably, no statistically significant relationship was found between cTnl and other oxidative stress and inflammation parameters ($p>0.05$). The correlation coefficients for the analysed parameters are presented in Table 1.

Canonical correlation between cardiac biomarkers and oxidative stress

The canonical correlation coefficient between the cardiac and oxidative stress biomarkers was found to be 0.852, indicating a statistically significant relationship ($p<0.05$). The oxidative stress parameters accounted for 52.20% of the variance in the cardiac parameters. Notably, CK, CK-MB, and cTnI demonstrated the most substantial impact on the cardiac parameters, while catalase, MDA, GSH, and GSH-Px significantly influenced the oxidative stress parameters. Further details, including the canonical correlation results, can be found in Table 2.

Table 1. Pearson correlation coefficient between cardiac biomarkers, oxidative stress biomarkers and inflammatory cytokine levels.

	MDA	GSH	GSH-Px	Catalase	TNF-α	IL-6	IL-1β
CK	0.639**	-0.523*	-0.477*	-0.675**	0.677**	0.578**	0.574**
CK-MB	0.514*	-0.548*	-0.553*	-0.575**	0.700**	0.559*	0.563**
cTnl	0.377	-0.331	-0.518*	-0.375	0.590**	0.314	0.262

*: p<0.05; **: p<0.01

Table 2. Canonical correlation between cardiac and oxidative stress biomarker levels

Variables		Canonical Loadings
Cardiac Parameters	CK	0.979
	CKMB	-0.892
	cTnl	-0.636
Oxidative Stress Parameters	MDA	0.741
	GSH	0.648
	GSH-Px	0.635
	Catalase	0.789
Canonical Correlation Coefficient		0.852 (p<0.05)
R^2		0.726
Explained Variance Ratio (%)		52.20

Canonical correlation between cardiac biomarkers and inflammatory cytokines

The canonical correlation coefficient between cardiac biomarkers and inflammatory cytokines was 0.821 (p<0.05). The rate of inflammatory cytokines explaining cardiac parameters was 49.40%. CK, CK-MB, and cTnI exhibited the most significant associations with cardiac parameters, while TNF-α, IL-6, and IL-1β demonstrated the most significant impact on inflammatory cytokines, respectively. Detailed results are available in Table 3.

Table 3. Canonical correlation between cardiac biomarkers and inflammatory cytokine levels.

Variables		Canonical Loadings
Cardiac Parameters	CK	0.945
	CKMB	0.947
	cTnl	0.637
Inflammatory Cytokines	TNF-α	0.900
	IL-6	0.725
	IL-1β	0.719
Canonical Correlation Coefficient		0.821 (p<0.05)
R^2		0.675
Explained Variance Ratio (%)		49.40

DISCUSSION AND CONCLUSION

Cardiac parameters are fundamental biomarkers that have been widely utilized in clinical practice for many years (Collinson, 1998; Penttila et al., 2000). In patients with cardiac pathology, assessing these parameters during standard diagnostic procedures is essential for evaluating cardiac function and identifying underlying pathophysiological conditions (Nawaytou and Bernstein, 2014). Despite advancements in diagnostic techniques, differentiating the potential causes of chest pain remains challenging, with approximately one in ten patients experiencing acute myocardial infarction being mistakenly discharged from the emergency department (Collinson, 1998). Previous studies have emphasized that the evaluation of multiple cardiac parameters is essential for the early diagnosis and management of acute myocardial infarction, ultimately contributing to reduced mortality rates (Kim et al., 2022). Creatine kinase (CK), creatine kinase-MB (CK-MB), and cardiac troponin I (cTnI) are commonly measured biomarkers for assessing cardiac status in cardiovascular diseases (Mladenka et al., 2009; Mladenka et al., 2014). A study conducted in rats demonstrated a strong correlation between cardiac troponin T (cTnT), stroke volume index, ventricular weight, and myocardial calcium levels (Mladenka et al., 2009). Similarly, another study reported that serum cTnT concentrations exhibit a significant correlation with parameters reflecting the extent of myocardial damage, such as calcium overload and stroke volume (Mladenka et al., 2013). However, in a separate study on rats, cTnT did not show a strong correlation with myocardial calcium levels, ventricular weight, or electrocardiographic (ECG) parameters, including T-wave, R-wave, and J-junction amplitudes (Mladenka et al., 2014). Given these findings, further investigation into the potential relationships between cardiac biomarkers, oxidative stress parameters, and inflammatory status may provide valuable insights for improving the diagnosis and treatment of cardiac diseases in clinical settings.

According to a recent study, associations between cardiac parameters, oxidative stress, and inflammatory biomarkers have been demonstrated (Gökçek, 2024). In

particular, recent research has shown that groups with elevated cardiac biomarkers, such as CK-MB, CK, and cTnI, also exhibit higher levels of inflammatory cytokines and oxidative stress markers, along with decreased antioxidant capacity (Çömez et al., 2020). In the study, Pearson correlation analyses further revealed significant relationships between CK and CK-MB and multiple oxidative stress and inflammatory markers, including MDA, GSH, GSH-Px, catalase, TNF-α, IL-6, and IL-1β. In contrast, cTnI demonstrated significant correlations only with GSH-Px and TNF-α.

In preclinical research, multivariate analyses are crucial for examining complex datasets, with canonical correlation analysis (CCA) being one of the most widely utilized statistical methods, as it constructs linear combinations that maximize the correlation between two datasets, thereby identifying the most relevant relationships (Figueroa et al., 2020; Vargas, 2022). For instance, a recent study employed similar multivariate analysis techniques to investigate the associations between sperm parameters, oxidative stress markers, and inflammatory biomarkers in detail (Kaya and Olğaç, 2024). Given its ability to elucidate intricate relationships, CCA may prefer as an appropriate method for exploring the connections among cardiac biomarkers, oxidative stress, and inflammatory markers.

Oxidative stress is a well-established contributor to cardiovascular pathophysiology, while antioxidant defense systems play a crucial role in maintaining cardiac health (Tsutsui et al., 2011). A strong association between oxidative stress and cardiac biomarkers has been reported (Çömez et al., 2020; Gökçek, 2024). For instance, a study demonstrated a significant relationship between oxidative stress markers and cardiac parameters (Çömez et al., 2020). In an experimental study on rats, cTnT exhibited a weak negative correlation with total blood glutathione, whereas no significant association was observed with plasma TBARS (Mladenka et al., 2014). Similarly, another study reported weak or no correlation between cTnT and certain oxidative stress parameters, suggesting that cTnT may not be a reliable indicator of cardiovascular function in this context (Mladenka et al., 2013). In the present study, a correlation was identified between oxidative stress markers and cardiac biomarkers, with catalase, MDA, GSH, and GSH-Px contributing most significantly to this association. Inflammatory cytokines are also known to play a pivotal role in modulating cardiac function under both physiological and pathological conditions. Their involvement in various cardiac pathologies, such as myocardial infarction and heart failure, depends on the nature of the disease, as well as the extent and duration of tissue damage (Bartekova et al., 2018). Cardiac inflammation has the potential to impair cardiac function and induce lasting damage (Lafuse et al., 2020), to the extent that systemic inflammation is considered a contributing factor in heart failure (Carrillo-Salinas et al., 2019). A study further demonstrated that inflammatory conditions were strongly associated with heart failure in patients with uremia (Zhang et al., 2021). There is growing evidence supporting the correlation between cardiac biomarkers and inflammatory cytokines (Bartekova et al., 2018; Çömez et al., 2020). In a study investigating myocardial injury, increased CK-MB levels, along with impaired ECG findings, confirmed heart damage, while a positive correlation between CK-MB and inflammatory cytokines was observed (Durdagi et al., 2021). Furthermore, a recent study in rats reported a significant positive correlation between cardiac biomarkers (CK, CK-MB, and cTnI) and the gene expressions and protein levels of inflammatory cytokines, including TNF-α, IL-6, NF-κB, COX-2, and Nrf-2, in cardiac tissue following ketamine-induced cardiotoxicity (Çömez et al., 2020). Notably, TNF-α, IL-6, and IL-1β were identified as the primary contributors to this correlation, further highlighting the intricate interplay between inflammatory status and cardiac function.

In conclusion, cardiac biomarkers serve as essential tools in the clinical assessment of cardiac function; however, their diagnostic utility may be enhanced when considered alongside additional physiological markers. This study utilized various correlation analyses to explore the relationships between cardiac biomarkers, oxidative stress, and inflammatory status. Multivariate methods such as canonical correlation provide an advantage to researchers in the evaluation of health biomarkers that vary under the influence of multiple factors. The findings demonstrated that CK played a significant role in influencing cardiac parameters, while catalase and TNF-α were the most prominent contributors to oxidative stress and inflammatory responses, respectively. The complex interplay between cardiac biomarkers, particularly CK and CK-MB and oxidative stress and inflammatory parameters underscores the potential for a more comprehensive approach to diagnosing and managing cardiac diseases. These findings, rooted in the complex biochemical and physiological interactions governing cardiovascular health, highlight the importance of further research. Future studies may build upon this foundation to develop targeted therapeutic strategies and precision medicine approaches aimed at improving cardiovascular outcomes.

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Ethical Declaration

The Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (HADYEK) approved the study (Decision No: 2023/05-10).

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: I.G., U.K., Design: U.K., Data Collection or Processing: I.G., U.K., Analysis or Interpretation: I.G., U.K., Literature I.G., Writing: I.G., U.K.

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