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Evaluative Value of Apelin-12 in Acute Myocardial Infarction

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ABSTRACT

Background: In acute myocardial infarction (MI), hypoxia, through the hypoxia-inducible factor pathway, increases the level of apelin, protecting the myocardium from ischemia-reperfusion injury.
Objectives: We aimed to evaluate the level of apelin-12 in the early and late phases of MI.
Methods: In this prospective observational study, 98 patients were randomly included with the following criteria: chest pain lasting for more than 30 minutes, a 12-lead ECG with ST-segment elevation, a rise of cardiac troponin I, and a primary percutaneous coronary intervention (PCI) within 12 hours. Blood samples were collected on the first day (early phase) and the seventh day (late phase) after reperfusion therapy. Continuous variables are expressed as mean ± standard deviation or median (range), while categorical variables are expressed as percentages. We compared the variables using the Wilcoxon test and evaluated the variability of apelin values with the Kruskal-Wallis test. We checked the degree of association and correlation between two variables with the Mann-Whitney test and Pearson correlation, respectively.
Results: On the first day of the early phase after MI, the median apelin-12 level was 2.73 (0.46 –15.24), which was significantly higher than the seventh-day value of 2.32 (0.25 – 10.89) (P = 0.003 on Wilcoxon test). With the Kruskal-Wallis test, variability in apelin-12 values was noted on the first day relative to segmental wall motion abnormalities (P = 0.043) and on the seventh day relative to the number of coronary stenoses (P < 0.001). The Mann-Whitney test of the post-PCI final thrombolysis in MI (TIMI) flow grade (patients without reperfusion injury: TIMI 3; patients with reperfusion injury: TIMI ≤ 2) and the apelin-12 levels during the early phase of MI revealed a statistically significant difference (P = 0.002).
Conclusions: Differences in apelin-12 between the early and late phases may reflect the activity of the apelin-angiotensin receptor-like axis in patients with MI.

1. Introduction

Acute myocardial infarction (AMI) remains one of the main causes of worldwide cardiovascular mortality and morbidity, especially in those with ST-segment elevation myocardial infarction (STEMI) (1). Apelin and angiotensin receptor-like-1 (APJ) are widely expressed in various tissues, including the myocardium and vascular endothelial cells (2). Apelin is an adipocytokine, a peptide encoded by the apelin gene located on the human X chromosome, with positive effects on the cardiovascular system (3-6). Apelin increases intracellular Ca^{2+} concentration and elevates the pH level, thereby increasing the sensitivity of cardiac myofilaments to intracellular Ca^{2+} and regulating cardiac contractility (7-10).

Acute myocardial infarction due to occlusion of the coronary artery results in myocardial oxygen supply-demand imbalance, necessitating urgent reperfusion to save the infarcted myocardium (11). Myocardial reperfusion is the main treatment for myocardial infarction, salvaging ischemic myocardium from an expected death. However, besides restoring forward blood flow to the infarct-related artery, reperfusion therapy may lead to myocardial ischemic reperfusion injury (MIRI). The major mechanism behind

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this phenomenon is the generation of excessive reactive oxygen species (ROS) due to the diminished concentration of antioxidative agents in ischemic cells. ROS promotes endothelial dysfunction, DNA damage, and local inflammation, causing structural cellular damage and cell death (12-17).

In rats with acute myocardial infarction, hypoxia through the hypoxia-inducible factor pathway increases the apelin level, which might improve cardiac function (18). Apelin is involved in myocardial ischemia-reperfusion injury (MIRI), activating the reperfusion injury salvage kinase (RISK) pathway components, such as phosphatidylinositol-3-OH kinase (PI3K), Akt/protein kinase B, and p44/42 mitogen-activated protein kinase (MAPK) [Figure 1] (19, 20). By increasing cardiac contractility and reducing cardiac load, apelin may also be used to treat patients with ischemic heart failure (21-25). Apelin mediates the vasodilator function of endothelial nitric-oxide synthase (eNOS) and is inversely correlated with LDL cholesterol, suggesting its role in hypertension and atherosclerosis (26-29). Apelin also has vascular effects and plays a role in fluid balance (30, 31).

In patients with acute myocardial infarction, the apelin-APJ axis may be up-regulated or down-regulated, potentially limiting infarct size and improving myocardial function (2, 6).

2. Objectives

This study aimed to evaluate the level of apelin-12 in the early and late phases of myocardial infarction.

3. Methods

3.1. Study Design and Patients

In this dual-center prospective observational study, a total of 98 patients were included. These patients either presented themselves or were referred to the Coronary Care Unit of Dubrava University Hospital, Zagreb, and the University Clinical Center of Kosova, Pristina, during the year spanning from March 2022 to March 2023. The inclusion criteria were: chest pain lasting for more than 30 minutes; a 12-lead ECG characterized with ST-segment elevation (measured at the J-point) in at least two contiguous leads ≥ 2.5 mm in men < 40 years, ≥ 2mm in men ≥ 40 years, or ≥ 1.5 mm in women in leads V_2-V_3 and/or ≥ 1 mm in other leads; rise of cardiac troponin I; and having undergone primary percutaneous coronary intervention (PCI) within 12 h from initiation of chest pain.

All patients underwent an echocardiographic examination while draped and prepared for reperfusion therapy. The left ventricular (LV) ejection fraction was calculated using the biplane Simpson method. The wall motion alterations were defined with the 17-segment model (6 basal, 6 midventricular, 5 apical). All segments of the left ventricle were scored with the usual method: 1-normokinesis, 2-hypokinesis, 3-akinesis, 4-dyskinesis, and 5-aneurysm, through the short axis projections and apical 2-, 3- and 4-chamber visualization. Left ventricular segments were assigned to five different territories supplied by coronary arteries (32). The territory supplied by the left descending coronary artery (LAD) includes segments 1, 6, 7, 12, 13, 16, and 17. The territory supplied by the right coronary artery (RCA) includes segments 2, 3, and 9. The territory supplied by the LAD or left circumflex coronary artery (LCx) includes segments 5, 11, and 15. The territory supplied by the RCA or LAD includes segments 8 and 14. The territory supplied by the RCA or LCx includes segments 4 and 10.

Based on coronary angiography findings, we determined the number of diseased vessels, the culprit lesion (luminal diameter stenosis ≥ 70% in epicardial vessels or ≥ 50% in left main stem (LMS) lesions), coronary perfusion according to the Thrombolysis in Myocardial Infarction (TIMI) flow grade, endoluminal thrombi, and signs of plaque rupture. Reperfusion injury was defined as TIMI flow grade ≤ 2.

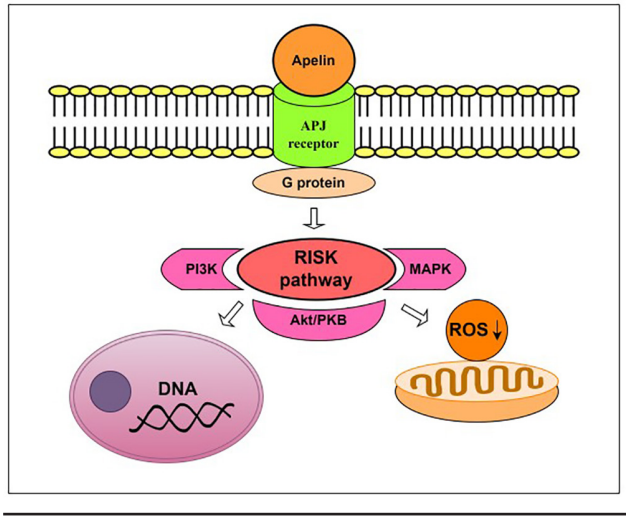
Data regarding patient characteristics, medical history, and current medications were obtained from the hospital ward and emergency department admission using a standardized questionnaire. Blood samples were collected on the first day (early phase) and the seventh day (late phase) after reperfusion therapy; the serum was obtained by allowing the blood to solidify in a serum tube for 30 min. After centrifugation (2,000 rpm, 15 minutes), serum samples were stored at -80 °C to prevent degradation. Apelin was measured using the apelin-12 microplate ELISA assay kit (Phoenix Pharmaceuticals Inc.) according to the manufacturer's instructions. Blood samples for routine laboratory parameters were also collected at admission. Patients who underwent analysis on both day one and day seven were the ones taken into consideration.

The institutional ethics committee of Dubrava University Hospital, Zagreb, and the University Clinical Center of Kosova, Pristina, approved the study. Informed consent was obtained from all individuals participating in the study.

3.2. Statistical Analysis

The clinical endpoint was the apelin value in the early (98 patients) and late phase (98 patients) of myocardial infarction, confirming the effect of apelin/APJ in patients

Figure 1. Protective Effect of Apelin in Myocardial Ischemia-Reperfusion Injury



APJ receptor: angiotensin receptor like-1 receptor, PI3K: phosphatidylinositol-3-OH kinase, PKB: protein kinase B, MAPK: mitogen-activated protein kinase, RISK: reperfusion injury salvage kinase, ROS: reactive oxygen species, DNA: deoxyribonucleic acid

with myocardial infarction. Continuous variables are expressed as mean ± standard deviation or median (range), while categorical variables are expressed as percentages. Continuous variables were compared with the Wilcoxon test (paired samples) with a prespecified statistical significance of $P < 0.001$. The Kruskal-Wallis test evaluated the variability of apelin values on the first day based on segmental wall motion abnormalities (SWMAs) and apelin values on the seventh day based on different numbers of stenotic coronary arteries. The degree of association and correlation between variables were analyzed using the Mann-Whitney and Pearson correlation tests, respectively. All statistical analyses were performed using SPSS statistics version 22.

4. Results

This study included 98 patients. The mean age was 74.53 ± 4.86 in the age group ≥ 65 years and 54.51 ± 7.62 in the age group < 65 years. Fifty-nine patients (60.2%) were males. Coronary risk factors included hypertension (59.18%), diabetes mellitus (18.36%), dyslipidemia (30.80%), smoking (31.83%), and a family history of cardiovascular disease (CVD) (19.38%).

4.1. Early and Late Phase after Myocardial Infarction and Apelin Level

On the first day of the early phase after myocardial infarction, the median apelin-12 level was 2.73 (0.46 - 15.24), and on the seventh day of the late phase, it was 2.32 (0.25 - 10.89). The Wilcoxon test revealed a statistically significant difference ($P = 0.003$) in apelin values between the first and seventh days. Variability was observed in the apelin values on the first day (Kruskal-Wallis test) based on segmental wall motion abnormalities (SWMAs) ($P = 0.043$), and on the seventh day based on the number of coronary lesions and stenosis ($P < 0.001$) (Table 1). Also, there was a significant association between apelin-12 and ejection fraction (EF), with a P-value < 0.04. Figure 2 shows the high values of apelin-12 on the 1st day based on segmental wall motion abnormalities (SWMAs), while Figure 3 shows the low value of apelin-12 on the 7th day based on the number of occluded coronary arteries.

Table 1. Main Characteristics and Apelin-12 Levels of Study Participants

	Apelin-12; first day (n = 98)	P	Apelin-12; seventh day (n = 98)	P
No of SWMAs, median (range)				
5, 6	5.47 (1.80-5.90)	0.043	2.33 (2.30-4.14)	0.43
7, 8	5.60 (0.46-7.08)		2.97 (1.87-5.28)	
9, 10	2.40 (1.30-13.15)		2.10 (0.49-6.98)	
11, 12	4.00 (0.56-15.25)		1.95 (0.26-3.53)	
No of stenoses, median (range)		0.059		<0.001
1	3.17 (0.45-11.05)		3.43(0.83-10.9)	
2	4.85 (0.60-13.15)		2.74 (0.47-6.98)	
3	1.75 (0.46-12.00)		1.70 (0.26-9.25)	
4	1.93 (1.00-6.96)		2.04 (0.49-2.4)	
5	2.80 (1.14-8.39)		1.8 (1.18-2.68)	
Final TIMI grade flow, median (range)				
≤2	1.80 (0.46-9.25)	0.002	1.94 (0.26-8.88)	0.01
3	3.93 (0.45-15.25)		2.42 (0.49-10.90)	
Age, median (range)				
≥65	2.60 (0.45-15.25)	0.78	2.20 (1.44-10.90)	0.96
<65	2.92 (0.51-13.50)		2.36 (0.26-8.88)	
Gender, median (range)				
M	2.30 (0.45-13.50	0.34	2.33 (0.26-10.90)	0.91
F	3.72 (0.50-15.25)		2.42 (0.55-9.75)	
Hypertension, median (range)				
Yes	2.73 (0.45-15.25)	0.55	2.10 (0.26-10.90)	0.058
No	2.71 (0.50-12.00)		2.58 (0.83-9.75)	
Diabetes mellitus, median (range)				
Yes	2.73 (0.56-9.33)	0.94	1.90 (0.55-4.63)	0.029
No	2.73 (0.45-15.25)		2.41 (0.26-10.90)	
Dyslipidemia, median (range)				
Yes	3.51 (0.45-12.00)	0.79	2.40 (0.55-10.90)	0.44
No	2.67 (0.46-15.25)		2.33 (0.26-9.75)	
BMI, median (range)				
>30	3.72 (0.45-11.05)	0.46	2.40 (0.47-10.90)	0.81
≤30	2.40 (0.46-15.25)		2.31 (0.26-9.75)	
Smoking, median (range)				
Yes	3.82 (0.45-13.15)	0.3	2.33 (0.47-10.90)	0.76
No	2.1o (0.46-15.25)		2.40 (0.26-9.75)	

*Kruskal-Wallis test; #Mann-Whitney test. Abbreviations: SWMAs: segmental wall motion abnormalities; TIMI: thrombolysis in myocardial infarction; BMI: body mass index.

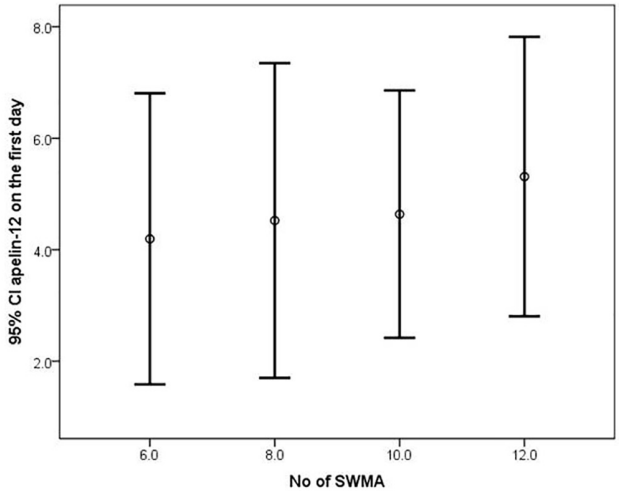


Figure 2. Multiple Comparisons Graph Showing the Proportional Value of Apelin-12 on the 1st Day based on the Number of Segmental Wall Motion Abnormalities (SWMAs).

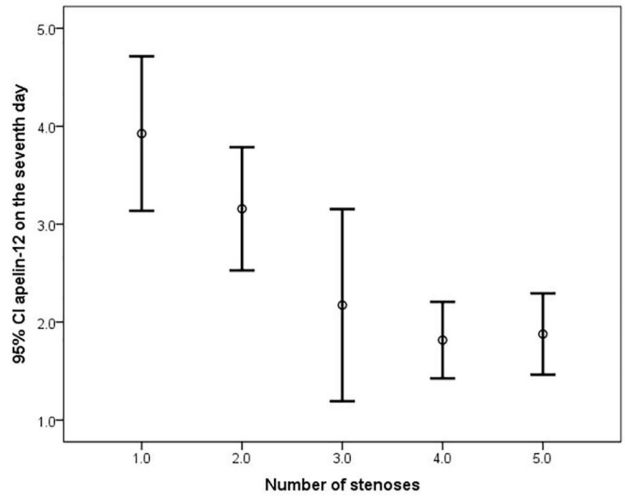


Figure 3. Multiple Comparisons Graph Showing the Proportional Value of Apelin-12 on the 7th Day based on the Number of Stenoses.

4.2. Reperfusion Injury and Apelin

Myocardial ischemia-reperfusion injury after reperfusion therapy was present in 25.51% of patients according to the angiographic criteria. Based on the Mann-Whitney test, the relationship between apelin-12 and the final TIMI grade flow (in patients without reperfusion injury with TIMI flow grade 3, and in patients with reperfusion injury with TIMI flow grade ≤ 2) in the early phase after myocardial infarction was significant (P = 0.002) (Table 1). In the adjusted logistic regression analysis, apelin-12 was correlated with TIMI flow independent of other risk factors (age, gender, hypertension, dyslipidemia, and diabetes mellitus) with a relative risk of 1.34 (95% confidence interval 1.05 – 1.70; P = 0.016.

5. Discussion

In this observational study, we investigated the difference in apelin-12 between myocardial infarction's early and late phases. In rats with acute myocardial infarction, hypoxia-inducible factor 1 alpha (HIF-1a) increases due to ischemia and hypoxia of the myocardium, augmenting the level of apelin, which induces angiogenesis, limits infarct size, and improves myocardial function (18, 33-35). In the late phase, with the rise in oxygen levels, HIF-1a is rapidly degraded, thereby decreasing the apelin level. In our patients with myocardial infarction, the median apelin-12 level was 2.73 (0.45 – 15.25) on the first day, and it was 2.33 (0.26 – 10.90) on the seventh day, revealing a statistically significant difference (P = 0.003) in apelin values between the early and late phases of myocardial infarction. The increase in apelin on the first day due to hypoxia is reflected by the variability in these values (Kruskal-Wallis test) relative to the segmental wall motion abnormalities (SWMAs) (P = 0.043), highlighting the protective effect of apelin during the early phase of myocardial infarction (36). We recorded a significant association between apelin-12 and ejection fraction (EF), with a P-value < 0.04. Apelin/APJ expression patterns were found to be inversely associated with human aortic and coronary atherosclerosis. A low level of apelin on the seventh day was inversely associated with the number of coronary stenoses (37, 38).

Hypoxia enhances the expression of HIF-1α, which upregulates apelin/APJ signaling and activates downstream PI3K/Akt signaling, an important promoter of endothelial progenitor cell (EPC) proliferation. Therefore, apelin/APJ may serve as a potential target for preventing hypoxic/ischemic injury in the cardiovascular system (39).

A structural analog of apelin-12, chemically modified apelin-12 (MA), reduces irreversible cardiomyocyte damage, improves cardiac dysfunction, and enhances metabolic restoration and membrane integrity according to experimental models of myocardial I/R injury (40). This cardioprotection is mediated by signaling via phospholipase C (PLC) and survival kinases, such as protein kinase C (PKC), phosphoinositide-3-OH kinase (PI3K), and mitogen-activated protein kinase (MEK1/2) by activating downstream targets, such as NO synthase, mito-KATP channels, and sarcolemmal Na(+)/H(+) and Na(+)/Ca(2+) exchangers (41). A lack of apelin enhances susceptibility to ischemia-reperfusion (IR) injury (42).

In our study, myocardial ischemia-reperfusion injury after reperfusion therapy was present in 25.51% of patients according to the angiographic criteria. In the early phase of myocardial infarction, the apelin level in patients without reperfusion injury with TIMI flow grade 3 differed significantly from patients with reperfusion injury with TIMI flow grade ≤ 2 (P = 0.002). In the adjusted logistic regression analysis, apelin-12 was correlated with TIMI flow independent of other risk factors (age, gender, hypertension, dyslipidemia, and diabetes mellitus) with a relative risk of 1.36 (95% confidence interval 1.07 – 1.72; P = 0.016).

5.1. Study Limitations

Certain limitations need to be taken into account. This observational study was conducted with a relatively restricted sample size, encompassing only patients diagnosed with ST-segment elevation myocardial infarction.

5.2. Conclusion

Differences in apelin-12 between the early and late phases may confirm the effect of the apelin-APJ axis in patients

with myocardial infarction. The increased apelin level during the early phase of myocardial infarction indicates a protective effect from reperfusion injury. A low level of apelin during the late phase of myocardial infarction was inversely associated with the number of coronary stenoses.

5.3. Ethical Approval

Ethical Statement Case no: 380-59-10106-19-2524/2 This research was approved by the Ethical Committee of the Faculty of Medicine, University of Zagreb, and the Ethical Committee of the University Clinical Centre of Kosova. The research was conducted according to the Basics of Good Clinical Practice and Helsinki Declaration.

5.4. Informed Consent

Informed consent was obtained from all participants.

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Authors' Contribution

X.K. conceived and designed the evaluation, performed the statistical analysis, and drafted the manuscript. J.V. re-evaluated the clinical data and revised the manuscript. D.K. collected the clinical data and interpreted them. A.B. reanalyzed the clinical and statistical data and revised the manuscript.

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