Thymosin beta4 levels after successful primary percutaneous coronary intervention for acute myocardial infarction

Akut miyokart enfarktüsü için başarılı primer perkütan koroner girişim sonrasında timozin beta4 düzeyleri

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ABSTRACT

Objectives: Thymosin beta4 (Tβ4) has been shown to have an important role in healing of damaged tissues and promoting cardiomyocyte survival in acute coronary syndromes. We evaluated endogenous Tβ4 levels in patients presenting with ST-elevation acute myocardial infarction (STEMI) before and after successful primary percutaneous coronary intervention (PCI).

Study design: The study included 24 consecutive patients (7 females, 17 males; mean age 55.0±10.9 years) who underwent successful primary PCI for STEMI and 24 age- and sex-matched healthy controls (13 females, 11 males; mean age 57.5±11.7 years) with angiographically normal coronary arteries. To determine Tβ4 levels, blood samples were obtained from STEMI patients on admission and 48 hours after successful PCI, and from controls immediately after coronary angiography.

Results: Compared to controls, baseline levels of high-density lipoprotein cholesterol (46.2±8.9 *vs.* 34.2±7.2 mg/dl, p<0.001) and Tβ4 (2.9±1.5 *vs.* 1.5±1.0 μg/ml, p<0.001) were significantly lower, and white blood cell counts (7.6±2.2 *vs.* 11.4±3.0 10^3/μl, p<0.001) were significantly higher in the STEMI group. After 48 hours of PCI, the mean Tβ4 level increased significantly to 2.3±0.8 μg/ml (p<0.001) and became similar to that of the control group (p=0.068). There was a significant negative correlation between serum Tβ4 and white blood cell count (r=-0.347, p=0.016).

Conclusion: Considering the significant increase in serum Tβ4 levels following successful primary PCI in patients with STEMI, Tβ4 may prove to be a new marker in the assessment of reperfusion success in addition to those used currently.

ÖZET

Amaç: Timozin beta4'ün (Tβ4) hasarlı dokuların iyileşmesinde ve akut koroner sendromlarda kardiyomiyositlerin canlı kalmasında önemli rol oynadığı gösterilmiştir. Bu çalışmada, ST yükselmeli akut kalp krizi (STYKK) ile başvuran hastalarda başarılı primer perkütan koroner girişim (PKG) öncesi ve sonrasındaki endojen Tβ4 düzeyleri değerlendirildi.

Çalışma planı: Çalışmaya STYKK nedeniyle başarılı primer PKG uygulanan 24 ardışık hasta (7 kadın, 17 erkek; ort. yaş 55.0±10.9) ve kontrol grubu olarak, anjiyografide koroner arterleri normal bulunan, yaş ve cinsiyet uyumlu 24 sağlıklı kişi (13 kadın, 11 erkek; ort. yaş 57.5±11.7) alındı. Timozin β4 düzeylerinin belirlenmesi için, STYKK grubunda başarılı PKG öncesinde ve 48 saat sonrasında, kontrol grubunda ise koroner anjiyografiden hemen sonra kan örnekleri alındı.

Bulgular: Kontrol grubuyla karşılaştırıldığında, STYKK grubunda başvurudaki yüksek yoğunluklu lipoprotein kolesterol (46.2±8.9 ve 34.2±7.2 mgr/dl, p<0.001) ve Tβ4 (2.9±1.5 ve 1.5±1.0 μgr/ml, p<0.001) düzeyleri anlamlı derecede düşük, beyaz kan hücresi sayısı anlamlı derecede yüksek (7.6±2.2 ve 11.4±3.0 10^3/μl, p<0.001) idi. Başarılı PKG'den 48 saat sonra ortalama Tβ4 düzeyi anlamlı artış (2.3±0.8 μgr/ml, p<0.001) göstererek kontrol grubuyla benzer düzeye yükseldi (p=0.068). Serum Tβ4 düzeyi ile beyaz kan hücresi sayısı arasında anlamlı negatif ilişki gözlendi (r=-0.347, p=0.016).

Sonuç: Başarılı primer PKG sonrasında STYKK'li hastalarda serum Tβ4 düzeylerinde görülen anlamlı artış göz önüne alındığında, halen kullanılmakta olan birçok belirtece ek olarak, Tβ4 reperfüzyon başarısının değerlendirilmesinde yeni bir gösterge olabilir.

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Thymosin beta4, originally isolated from the thymus gland, plays an important role in the regeneration, remodeling, and healing of injured tissues. It is involved in a wide variety of biological activities, including regulation of actin, endothelial cell migration, epithelialization, angiogenesis, prevention of apoptosis, stimulation of adult epicardial stem cell differentiation, and anti-inflammation.[1-4] It is found in high concentrations in platelets, white blood cells, wound fluids and in other tissues of the body. Some experimental studies have shown that Tβ4 is an important factor in the setting of myocardial infarction. Recently, it has been shown that Tβ4 is expressed in the developing heart and stimulates migration of cardiomyocytes and endothelial cells,[5] and finally plays an essential role in cardiac vessel development.[6] Thus, Tß4 is currently being investigated as a therapeutic agent for treatment of ischemic heart disease, in hopes that it may have a significant therapeutic potential to protect the myocardium and to promote cardiomyocyte survival in the acute stages of ischemic heart disease.[5-9] Furthermore, Tβ4 has been shown to have anti-inflammatory properties, which could be so important since inflammation plays a central role in acute coronary syndromes.[10-12] Although Tβ4 has been found to have an essential role in myocardial healing after acute coronary syndromes, endogenous levels of Tβ4 in different populations and the role of endogenous Tβ4 in the physiopathology of ST-elevation myocardial infarction remain to be elucidated.

The aim of the current study was to evaluate serum Tβ4 levels in patients presenting with STEMI in comparison with subjects having normal coronary arteries, and to assess changes in Tβ4 levels after successful primary percutaneous coronary intervention.

PATIENTS AND METHODS

Patients

The study included 24 consecutive patients (7 females, 17 males; mean age 55.0±10.9 years) with a diagnosis of STEMI and 24 age- and sex-matched controls (13 females, 11 males; mean age 57.5±11.7 years) with NCA. Inclusion criteria for the STEMI group were the presence of the following: symptoms of ischemia-like typical chest pain, ST-segment elevation of ≥1 mm in at least two contiguous electrocardiographic leads with reciprocal ST depression in the contralateral leads or new onset left bundle branch block, elevated creatine kinase-myocardial band isoenzyme activity at least 1 value above the 99th percentile of the upper reference limit, or elevated troponin I level. A troponin I value of ≥0.1 ng/ml was considered to be positive. The control group included healthy subjects with normal findings on coronary angiogram performed due to a skeptical positive treadmill test or angina equivalent symptoms. Patients with known coronary artery disease (a previous history of myocardial infarction, coronary artery bypass surgery or PCI), heart failure (left ventricular ejection fraction <45% or symptoms and/or findings consistent with congestive heart failure), thyroid disorders, connective tissue or other inflammatory disorders, hepatic dysfunction, renal failure (serum creatinine >1.5 mg/dl in males, >1.0 mg/dl in females), and those with moderate or severe valvular insufficiency or stenosis were excluded from the study.

Abbreviations:

CAG	*Coronary angiogram/angiography*
HDL	*High-density lipoprotein*
NCA	*Normal coronary arteries*
PCI	*Percutaneous coronary intervention*
STEMI	*ST-elevation myocardial infarction*
Tβ4	*Thymosin beta4*
WBC	*White blood cells*

The study was approved by the institutional ethics committee and written informed consent was obtained from all participants. All procedures were conducted in conformity with the principles stated in the Declaration of Helsinki.

A detailed medical history was obtained from each subject. All patients were evaluated via physical examination, 12-lead electrocardiogram, and echocardiography for the evaluation of valvular and left ventricular function. The decision for thrombolytic or direct primary PCI therapy was made quickly and the patient was transferred to the cardiac catheterization laboratory as rapidly as possible for coronary angiography. Following identification of the culprit lesion, reperfusion with PCI was achieved. All STEMI patients underwent primary PCI within eight hours following the onset of chest pain.

Successful reperfusion after primary PCI was defined as follows: improved chest pain, rapid normalization of the ST segments (resolution of ST elevation by >70%), reperfusion arrhythmias (accelerated idioventricular rhythm) or an early but short-lasting elevation in serum biomarkers (8-12 hours), grade 3 postprocedural Thrombolysis In Myocardial Infarction flow, and residual stenosis of less than 50%. All patients with STEMI received aspirin (300 mg), clopidogrel (300 mg initially and 75 mg as a maintenance dose), metoprolol (50 mg), angiotensin-converting enzyme inhibitor (perindopril 5 mg/day, the dose was higher in hypertensives), and

Table 1. Baseline clinical and laboratory characteristics of the patients with ST-elevation myocardial infarction (STEMI) in comparison with controls having angiographically normal coronary arteries

	STEMI group (n=24)			Control group (n=24)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			55.0±10.9			57.5±11.7	0.448
Gender							0.079
Male	17	70.8		11	45.8		
Female	7	29.2		13	54.2		
Body mass index (kg/m^2)			25.7±2.5			27.1±2.9	0.081
Smoking	16	66.7		13	54.2		0.376
Diabetes mellitus	9	37.5		6	25.0		0.350
Hypertension	8	33.3		14	58.3		0.082
Blood glucose (mg/dl)			124.8±42.74			118.6±36.3	0.592
Serum creatinine (mg/dl)			0.9±0.2			0.9±0.2	0.073
Hemoglobin (g/dl)			14.1±1.5			13.6±1.3	0.207
White blood cell count (10^3/µl)			11.4±3.0			7.6±2.2	**<0.001**
Platelet count (10^3/µl)			265.3±62.9			268.6±71.3	0.866
Total cholesterol (mg/dl)			182.2±24.3			199.1±39.5	0.080
HDL cholesterol (mg/dl)			34.2±7.2			46.2±8.9	**<0.001**
LDL cholesterol (mg/dl)			112±22			124±36	0.142
Triglyceride (mg/dl)			172.7±88.5			145.9±47.1	0.197
Thymosin beta 4 (µg/ml)			1.5±1.0			2.9±1.5	**<0.001**
Heart rate (beat/min)			75.7±13.2			70.9±10.4	0.196
Pulse pressure (mmHg)			49.2±13.2			48.8±14.7	0.918
Systolic blood pressure (mmHg)			129.6±22.9			128.5±21.1	0.870
Diastolic blood pressure (mmHg)			80.4±13.3			79.8±13.2	0.871
Left ventricular ejection fraction (%)			56.2±6.9			59.1±6.8	0.149

statin (rosuvastatin 20 mg/day). Unfractionated heparin (as recommended in the AHA/ACC guidelines) was administered as a bolus of 100 U/kg (maximum 10,000 units) before the procedure, followed by 12 U/kg per hour (maximum 1,000 U/hr). Glycoprotein IIb/IIIa inhibitors were not used.

Coronary angiography

In all patients and controls, coronary angiography was performed using the standard Judgkins technique on a Siemens Angioscop X-ray equipment (Axiom Artis, Siemens, Germany). Left and right selective CAGs were obtained in multiple projections.

Blood sampling and the measurement of plasma Tβ4 and cardiac enzyme levels

Blood samples were obtained from STEMI patients on admission and 48 hours after successful reperfusion therapy, and from controls immediately after CAG. Blood was collected without anticoagulant and spun at 3000 g for 10 min, and the serum obtained was immediately stored at -80°C until Tβ4 measurement. Thymosin β4 was measured with a newly developed commercial enzyme-linked immunosorbent assay (Immunodiagnostik AG, Bensheim, Germany). Biochemical variables (glucose, creatinine, and lipid profile) were determined by standard methods on a chemistry autoanalyzer using original reagents (P 800, Roche Diagnostics, Germany). Complete blood count was measured by an automated hematology analyzer (Beckman Coulter Gen-S, Coulter Corp, Miami, USA).

Statistical analysis

All statistical calculations were performed using the SPSS statistical software (SPSS for Windows 15.0). Continuous variables were given as mean±standard deviation, categorical variables were defined as per-

centage. Data were tested for normal distribution using the one-sample Kolmogorov-Smirnov test. Categorical variables were compared using the chi-square test. The Student's t-test was used for the univariate analysis of the continuous variables. A paired-sample t-test was used to assess the difference between the Tβ4 values before and 48 hours after primary PCI within the STEMI group. Comparison of the Tβ4 levels between the groups (pre-PCI, post-PCI and NCA) was performed using one-way analysis of variance (ANOVA). The point-biserial correlation coefficient was used to compare categorical variables and Tβ4, whereas the Pearson correlation coefficient was used to compare continuous variables and Tβ4. All tests of significance were two-tailed. Statistical significance was defined as $p<0.05$.

RESULTS

The baseline clinical and laboratory characteristics of the patients and controls are summarized in Table 1. There were no significant differences between the two groups with respect to age, gender, smoking, and the frequencies of hypertension, diabetes mellitus, and hyperlipidemia. The biochemical characteristics of the patients did not differ significantly from controls except for high-density lipoprotein cholesterol level, WBC count, and Tβ4 level, where HDL and Tβ4 levels were significantly lower, and WBC counts were significantly higher in the STEMI group (Table 1).

Correlations of serum Tβ4 levels with the baseline clinical characteristics and laboratory data are presented in Table 2. A negative correlation was found between serum Tβ4 level and WBC count ($r=-0.347$, $p=0.016$), whereas there were no correlations between Tβ4 and other demographic, clinical and laboratory variables including HDL cholesterol level ($r=0.172$, $p=0.242$).

Thymosin β4 levels before primary PCI were significantly lower compared with the control group (1.5±1.0 *vs*. 2.9±1.5 μg/ml, $p<0.001$) and post-PCI levels (1.5±1.0 vs. 2.3±0.8 μg/ml, $p<0.001$) (Fig. 1). After 48 hours of PCI, the Tβ4 levels increased significantly and became similar to those of the control group ($p=0.068$).

Table 2. Correlation of thymosin beta 4 levels with baseline demographic, clinical and laboratory variables*

	r	*p*
Age	0.214	0.143
Gender*	0.028	0.848
Body mass index	0.248	0.089
Diabetes mellitus*	-0.230	0.112
Hyperlipidemia*	-0.050	0.733
Hypertension*	0.076	0.604
Smoking*	-0.122	0.404
Blood glucose	-0.192	0.191
Serum creatinine	-0.054	0.716
Hemoglobin	-0.023	0.878
Platelet count	0.012	0.938
White blood cell count	-0.347	**0.016**
HDL cholesterol	0.172	0.242
LDL cholesterol	0.030	0.838
Triglyceride	-0.253	0.082
Pulse pressure	-0.110	0.455
Heart rate	-0.078	0.614
Systolic blood pressure	-0.133	0.368
Diastolic blood pressure	-0.104	0.480
Left ventricular ejection fraction	0.100	0.507

*The point-biserial correlation coefficient was used for comparison of categorical variables and the Pearson correlation coefficient was used for continuous variables.

DISCUSSION

Literature data suggest that Tβ4 is an important factor in the setting of myocardial ischemia to protect the myocardium and thus it should be studied further. In the present study, it was found that Tβ4 levels were initially lower in STEMI patients and increased after primary PCI. However, it is not clear whether lower Tβ4 levels led to the development of AMI or *vice versa*. Moreover, the underlying mechanism for increased Tβ4 levels following successful primary PCI or whether Tβ4 will also increase after thrombolytic therapy for AMI remain unknown.

In the current study, admission HDL cholesterol levels of STEMI patients were significantly lower than those of controls with NCA, which is compatible with the results of Goswami et al.[13] who showed a significant decline in HDL cholesterol in patients with AMI compared to healthy controls. The epidemiological data generally support an independent inverse association between HDL cholesterol level and coronary artery disease. Low HDL cholesterol level (<40 mg/dl in men and <45 mg/dl in women) is a known major cardiovascular risk factor[14] for the development of ischemic events as seen in our study. The present

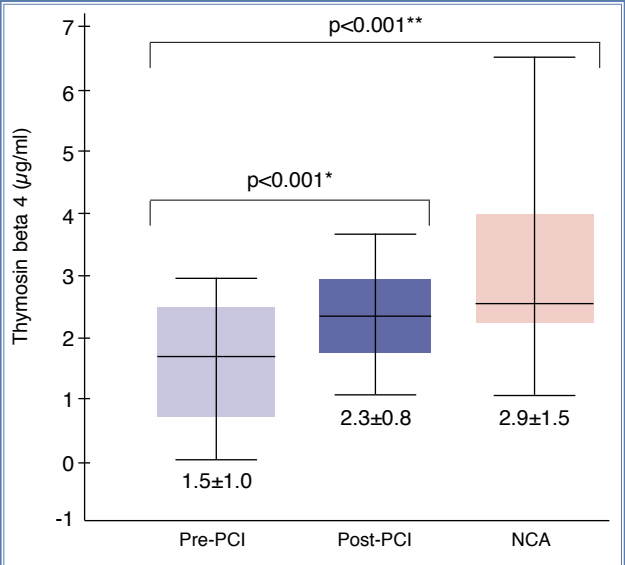

Figure 1. Significantly lower thymosin beta 4 levels in the pre-PCI group compared with the post-PCI and NCA groups (p<0.001). *paired-sample t-test, **ANOVA; PCI: Percutaneous coronary intervention; NCA: Normal coronary artery.

study also showed that HDL cholesterol level was not correlated with Tβ4 levels, a finding whose clinical importance remains to be determined. In addition, the WBC count was significantly higher in the STEMI group compared with the NCA group. Inflammation plays a key role in the development and progression of atherosclerosis and its complications.[15] Patients with coronary artery disease show both local inflammation within the coronary arteries and increased acute-phase proteins and WBC suggesting a systemic inflammatory response.[15,16] Elevation or activation of leukocytes represent an increased risk for cardiovascular events and mortality.[17,18] In the setting of STEMI, elevated baseline WBC counts have been found to be associated with worse angiographic findings and a higher 30-day mortality rate.[11] Furman et al.[18] examined the association between WBC count and short-term in-hospital mortality and found that there was an independent association between WBC count and in-hospital survival. Lower Tβ4 levels may also be associated with a poor prognosis since the present study showed a negative correlation between serum Tβ4 levels and WBC counts. Further studies are necessary to fully understand the complex interplay between Tβ4 and WBC or other prognostic factors and the role of endogenous Tβ4 in the pathogenesis of atherothrombotic vascular disease.

It has been shown that Tβ4 can influence all important characteristics of wound healing.[19-22] Activation of human platelets results in increased concentrations of Tβ4 nearby the clots and tissue damage, which in turn contributes to wound healing, angiogenesis, and inflammatory response.[19-25] It has been demonstrated that Tß4 has a number of important biological activities that appear to be useful for a wide range of medical conditions. It can reduce inflammation by down-regulating some inflammatory molecules, suggesting a potential role for diseases characterized by increased inflammation, such as AMI or other inflammatory diseases.[1,2,23,26] Even though inflammation represents a requisite for infarct healing, attempts to limit the inflammatory response may be of potential therapeutic use to limit unfavorable remodeling. Therefore, Tβ4 may be of great interest to pharmaceutical companies focusing on its therapeutic potential, particularly for dermal, ophthalmic, and cardiovascular wound healing.[3-5]

Srivastava et al.[7] reported that, after coronary artery ligation in mice, Tß4 treatment resulted in upregulation of integrin-linked kinase and protein kinase B activity in the heart, enhanced early myocyte survival, and improved cardiac function. These findings suggest that Tβ4 promotes cardiomyocyte and endothelial cell migration *in vitro* and *in vivo* by acting as a potent chemoattractant factor, enhances survival and repair, and thus may be a novel therapeutic target for acute myocardial damage.[7] Hinkel et al.[27] showed that myeloperoxidase activity, which is important in myocardial damage, was decreased in neonatal rat cardiomyocytes by retrograde application of Tβ4 into the anterior interventricular vein in the setting of anterior AMI.

Bock-Marquette et al.[5] reported that Tβ4 could prevent apoptosis after induction of myocardial infarction in rodents, indicating that both cardiac function and cardiac muscle were preserved most likely by prevention of apoptosis resulting from ischemia.

Acute myocardial infarction is one of the most important causes of morbidity and mortality in humans throughout the world. Following AMI, early and successful myocardial reperfusion is the most important strategy for reducing the size of a myocardial infarct and improving the clinical outcome. It has been demonstrated that restoration of epicardial flow alone does not guarantee adequate myocardial perfusion and several markers have been evaluated for the success of reperfusion.[28-32] In the present study, increases in Tβ4 levels in the STEMI group following successful reperfusion may be attributed to the onset of myocardial regeneration, where Tβ4 may be associated with

myocardial healing. Moreover, Tβ4 might be a new candidate in the assessment of success in reperfusion in addition to several markers currently used. Unfortunately, no data exist in the literature regarding the baseline levels of Tβ4 in healthy subjects, and alterations in Tβ4 levels in the presence of AMI. Further investigations with a longer follow-up and larger sample size are needed to elucidate the role of Tβ4 in the physiopathology of STEMI and to clarify the clinical importance of our results.

We report for the first time that the levels of Tβ4 are lower in patients with STEMI than in subjects having NCAs. However, it is unclear whether these findings are of clinical importance or what they explain in STEMI patients. Thymosin β4 might be a new candidate in the assessment of successful reperfusion in addition to several markers currently used. We still have much to learn about the pathophysiology of acute coronary syndromes.

Limitations of the study

Our study has several limitations. An important limitation is the relatively small sample size, which might have decreased the statistical power to detect significance between groups. There is a need for larger studies for increased statistical power. It may be useful to measure the Tβ4 levels in serial blood samples after the infarction to determine the rate of increments and the course of the levels with time. Since the prognostic value of Tβ4 levels was not investigated in the current study, and Tβ4 levels were not measured following failed reperfusion, we could not conclude that the Tβ4 level is a predictor of successful reperfusion. We should include a no-reperfusion group if the value of Tβ4 is assessed as a reperfusion indicator.

Conflict-of-interest issues regarding the authorship or article: None declared

REFERENCES

1. Shrivastava S, Srivastava D, Olson EN, DiMaio JM, Bock-Marquette I. Thymosin beta4 and cardiac repair. Ann N Y Acad Sci 2010;1194:87-96.
2. Sosne G, Szliter EA, Barrett R, Kernacki KA, Kleinman H, Hazlett LD. Thymosin beta-4 promotes corneal wound healing and decreases inflammation in vivo following alkali injury. Exp Eye Res 2002;74:293-9.
3. Goldstein AL, Hannappel E, Kleinman HK. Thymosin beta4: actin-sequestering protein moonlights to repair injured tissues. Trends Mol Med 2005;11:421-9.
4. Philp D, Badamchian M, Scheremeta B, Nguyen M, Goldstein AL, Kleinman HK. Thymosin beta-4 and a synthetic peptide containing its actin-binding domain promote dermal wound repair in db/db diabetic mice and in aged mice. Wound Repair Regen 2003;11:19-24.
5. Bock-Marquette I, Saxena A, White MD, Dimaio JM, Srivastava D. Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. Nature 2004;432:466-72.
6. Crockford D. Development of thymosin beta4 for treatment of patients with ischemic heart disease. Ann N Y Acad Sci 2007;1112:385-95.
7. Srivastava D, Saxena A, Michael Dimaio J, Bock-Marquette I. Thymosin beta4 is cardioprotective after myocardial infarction. Ann N Y Acad Sci 2007;1112:161-70.
8. Hinkel R, Bock-Marquette I, Hatzopoulos AK, Kupatt C. Thymosin beta4: a key factor for protective effects of eEPCs in acute and chronic ischemia. Ann N Y Acad Sci 2010;1194:105-11.
9. Cavasin MA. Therapeutic potential of thymosin-beta4 and its derivative N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) in cardiac healing after infarction. Am J Cardiovasc Drugs 2006;6:305-11.
10. Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995;91:2844-50.
11. Barron HV, Cannon CP, Murphy SA, Braunwald E, Gibson CM. Association between white blood cell count, epicardial blood flow, myocardial perfusion, and clinical outcomes in the setting of acute myocardial infarction: a Thrombolysis In Myocardial Infarction 10 substudy. Circulation 2000;102:2329-34.
12. Cannon CP, McCabe CH, Wilcox RG, Bentley JH, Braunwald E. Association of white blood cell count with increased mortality in acute myocardial infarction and unstable angina pectoris. OPUS-TIMI 16 Investigators. Am J Cardiol 2001;87:636-9.
13. Goswami B, Rajappa M, Singh B, Ray PC, Kumar S, Mallika V. Inflammation and dyslipidaemia: a possible interplay between established risk factors in North Indian males with coronary artery disease. Cardiovasc J Afr 2010; 21:103-8.
14. Viles-Gonzalez JF, Fuster V, Corti R, Badimon JJ. Emerging importance of HDL cholesterol in developing high-risk coronary plaques in acute coronary syndromes. Curr Opin Cardiol 2003;18:286-94.
15. Friedman GD, Klatsky AL, Siegelaub AB. Leukocyte count and myocardial infarction: correction [Letter]. N Engl J Med 1974;291:1361.
16. Ernst E, Hammerschmidt DE, Bagge U, Matrai A, Dormandy JA. Leukocytes and the risk of ischemic diseases. JAMA 1987;257:2318-24.
17. Yarnell JW, Baker IA, Sweetnam PM, Bainton D, O'Brien JR, Whitehead PJ, et al. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. Circulation 1991;83:836-44.
18. Furman MI, Becker RC, Yarzebski J, Savegeau J, Gore

JM, Goldberg RJ. Effect of elevated leukocyte count on in-hospital mortality following acute myocardial infarction. Am J Cardiol 1996;78:945-8.
19. Huff T, Otto AM, Müller CS, Meier M, Hannappel E. Thymosin beta4 is released from human blood platelets and attached by factor XIIIa (transglutaminase) to fibrin and collagen. FASEB J 2002;16:691-6.
20. Makogonenko E, Goldstein AL, Bishop PD, Medved L. Factor XIIIa incorporates thymosin beta4 preferentially into the fibrin(ogen) alpha C-domains. Biochemistry 2004;43:10748-56.
21. Crockford D, Turjman N, Allan C, Angel J. Thymosin beta4: structure, function, and biological properties supporting current and future clinical applications. Ann N Y Acad Sci 2010;1194:179-89.
22. Smart N, Risebro CA, Melville AA, Moses K, Schwartz RJ, Chien KR, et al. Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization. Nature 2007;445:177-82.
23. Malinda KM, Sidhu GS, Mani H, Banaudha K, Maheshwari RK, Goldstein AL, et al. Thymosin beta4 accelerates wound healing. J Invest Dermatol 1999;113:364-8.
24. Huff T, Ballweber E, Humeny A, Bonk T, Becker C, Müller CS, et al. Thymosin beta(4) serves as a glutaminyl substrate of transglutaminase. Labeling with fluorescent dansylcadaverine does not abolish interaction with G-actin. FEBS Lett 1999;464:14-20.
25. Sosne G, Qiu P, Goldstein AL, Wheater M. Biological activities of thymosin beta4 defined by active sites in short peptide sequences. FASEB J 2010;24:2144-51.
26. Sosne G, Xu L, Prach L, Mrock LK, Kleinman HK, Letterio JJ, et al. Thymosin beta 4 stimulates laminin-5 production independent of TGF-beta. Exp Cell Res 2004; 293:175-83.
27. Hinkel R, El-Aouni C, Olson T, Horstkotte J, Mayer S, Müller S, et al. Thymosin beta4 is an essential paracrine factor of embryonic endothelial progenitor cell-mediated cardioprotection.Circulation 2008;117:2232-40.
28. van't Hof AW, Liem A, de Boer MJ, Zijlstra F. Clinical value of 12-lead electrocardiogram after successful reperfusion therapy for acute myocardial infarction. Zwolle Myocardial infarction Study Group. Lancet 1997; 350:615-9.
29. Ito H, Tomooka T, Sakai N, Yu H, Higashino Y, Fujii K, et al. Lack of myocardial perfusion immediately after successful thrombolysis. A predictor of poor recovery of left ventricular function in anterior myocardial infarction. Circulation 1992;85:1699-705.
30. Vuotikka P, Uusimaa P, Niemelä M, Väänänen K, Vuori J, Peuhkurinen K. Serum myoglobin/carbonic anhydrase III ratio as a marker of reperfusion after myocardial infarction. Int J Cardiol 2003;91:137-44.
31. van't Hof AW, Liem A, Suryapranata H, Hoorntje JC, de Boer MJ, Zijlstra F. Angiographic assessment of myocardial reperfusion in patients treated with primary angioplasty for acute myocardial infarction: myocardial blush grade. Zwolle Myocardial Infarction Study Group. Circulation 1998;97:2302-6.
32. Schröder R, Dissmann R, Brüggemann T, Wegscheider K, Linderer T, Tebbe U, et al. Extent of early ST segment elevation resolution: a simple but strong predictor of outcome in patients with acute myocardial infarction. J Am Coll Cardiol 1994;24:384-91.

Key words: Angioplasty, balloon, coronary; coronary angiography; ischemia; myocardial infarction; reperfusion; thymosin.

Anahtar sözcükler: Anjiyoplasti, balon, koroner; koroner anjiyografi; iskemi; miyokart enfarktüsü; reperfüzyon; timozin.