Original Article

Association of Serum Pleiotrophin Levels with Acute Coronary Syndrome

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Abstract

Background and Aim: Pleiotrophin (PTN) is known as a multifunctional cytokine and a neurite outgrowth-promoting factor and appears as an angiogenic response to numerous processes such as ischemic injury affecting a region of the heart and the brain. We evaluated the serum concentration of PTN in clients with acute coronary syndrome (ACS), besides assessing the clients with no visible stenosis (NVS) in elective invasive coronary angiography (ICA). **Materials and Methods:** Through conducting a prospective research, we compared 110 consecutive patients with ACS referred for ICA with 47 stable patients with NVS. **Results:** We determined that ACS group had higher PTN levels than NVS group (244.94 ± 89.09 vs. 199.46 ± 70.71, $P < 0.001$). Based on the multivariate analysis, besides older age, smoking, and white blood cell, PTN ≥207.66 ng/ml remained independently associated with ACS. **Conclusion:** The serum concentration of PTN in clients was determined to have an association with ACS. The potential diagnostic and prognostic impact of PTN in ischemic heart disease may deserve to be further evaluated. PTN may also be studied as an angiogenic therapeutic factor in patients with ACS in additional studies.

Keywords: Acute coronary syndrome, angiogenesis, pleiotrophin

Introduction

Acute coronary syndrome (ACS) is considered a significant factor that leads to morbidity and mortality worldwide, affecting more than 1 million patients admitted annually to hospitals in the United States.[1] Despite recent advances in drug therapy and interventional techniques, the survivors of ACS can suffer from repetitive infarction and experience the risk of mortality at least five or six times higher than the population who are not affected by the coronary artery disease (CAD).[2] Alternative treatments are investigated for ACS besides resorting to traditional strategies. Angiogenesis occurs with the remodeling of new blood vessels growing out of the existing vasculature. Numerous cells secrete pro-angiogenic agents under the conditions of ischemia and hypoxia. Therapeutic vascular growth stimulated by proteins or genes is asserted to potentially enhance blood flow to ischemic tissue by inducing angiogenesis and vasculogenesis.[3] Supporting the infarcted myocardium by supplying blood through angiogenesis can strengthen the performance of heart muscles subsequent to myocardial infarction. Numerous preclinical and clinical researches have scrutinized the effect of angiogenic agents on therapeutic angiogenesis when adequate blood flow to the myocardium is obstructed. Despite the fact that vascular endothelial growth factor (VEGF) represents an essential growth factor for angiogenesis, as tested in lab analysis, VEGF may result in unpleasant effects such as atherosclerotic lesions and hemangiomas.[4]

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Being a cytokine which constitutes a group of heparin-binding proteins along with midkine (MK),[5] pleiotrophin (PTN) serves as a neurite outgrowth-promoting factor and emerges as an angiogenic response to numerous processes such as ischemic injury affecting a region of the heart and the brain.[4] It has been confirmed that PTN has a chemoattractant effect on endothelial progenitor cells, much the same as VEGF and stromal cell derived factor 1 alpha (SDF-1α).[6] Moreover, Sharifi discovered that PTN can stimulate monocytes to transdifferentiate into functional endothelial cells.[7] Christman *et al*.[8] reported that delivery of PTN plasmid increases neovasculature formation, unlike the control group. The capillary and arteriole density was observed to be much higher in the PTN plasmid-injected patients in comparison to that of the control group. Besides, the group supported with the PTN plasmid was observed to show no sign of developing macroscopic or histological risk of forming tumors.[8]

Türker Duyuler *et al*.[9] reported that serum PTN levels had a close correlation with maintaining coronary collateral circulation in clients who suffer from angina pectoris. However, serum PTN levels were not identified in clients with ACS. As an objective of our research, we assessed the serum levels of PTN in clients with ACS and patients with no visible stenosis (NVS) through applying elective invasive coronary angiography (ICA).

Materials and Methods

This single-center prospective study enrolled 110 consecutive clients having the symptoms of ACS referred for ICA within 24 h of symptom onset and 47 stable patients with NVS. NVS was defined as complete absence of stenosis or plaque formation in coronary arteries.[10]

Exclusion criteria were applied for the participants who experienced CAD, peripheral arterial disease, cerebrovascular disease, cancer, myocardial infarction with non-obstructive coronary arteries (MINOCA), severe congestive heart failure, atrial fibrillation, severe valvular disease, hypertrophic cardiomyopathy, or severe renal or liver failure.

Diabetes mellitus was determined as a fasting plasma glucose level >126 mg/dl or currently on antidiabetic treatment. Hyperlipidemia was detected as a fasting serum low-density lipoprotein (LDL), with the cholesterol level >130 mg/dl or currently being treated with lipid-lowering drugs. Patients with resting blood pressure ≥140/90 mmHg on at least two measurements or currently taking antihypertensive medication were deemed to have hypertension.

Data collection

Patient gender, age, medical history, comorbidities, 12-lead electrocardiography, and transthoracic echocardiography were collected from the medical records of our hospital.

Laboratory tests

We collected blood samples for biochemistry parameters and complete blood count after 12-h of fasting. Tests such as complete blood count, serum urea, creatinine, triglyceride, total cholesterol, high-density lipoprotein cholesterol, LDL cholesterol, and glucose levels were performed via routine laboratory techniques. We drew venous blood sample for PTN testing before ICA in the ACS group and after ICA in the NVS group. We centrifuged the venous blood samples in the biochemical tubes of 3000 g for 10 min to deliver serum samples, followed by storing them at the freezing temperature of –80°C. Levels of PTN were calculated by means of a commercial PTN Enzyme-linked immunosorbent assay Kit (*SunRed Biotechnology Company*, *Shanghai SunRed Biological Technology Co., Ltd. Hu Tai Road, Baoshan District, Sanghani, China*), adhering to the instructions recommended in the owner's manual.

Invasive coronary angiography

The indication for ICA in acute and elective conditions was decided according to the guidelines.[11,12] ICA was performed by means of the Judkins coronary catheter via radial or femoral access. Four experienced invasive cardiologists interpreted the ICA recordings, and the therapeutic approach was determined by taking into consideration guidelines of 2018 ESC/EACTS on myocardial revascularization.[13]

Statistical analysis

Sample size calculations were performed using G*Power 3.1. All data were analyzed via SPSS software (ver. 22.0; SPSS Inc., Chicago, IL, USA), whereas continuous data were demonstrated as the mean ± standard deviation and analyzed with the *t*-test. Pearson's Chi-square was preferred to analyze qualitative variables. The area under the receiver operating characteristic curve was used to measure the cutoff point of PTN for ACS. Univariate and multivariate logistic regression was used to predict the markers of ACS in clients without history of CAD. We applied two-tailed tests for both the groups, as the $P < 0.05$ was assessed to be statistically significant.

Ethical Statement

All patients were referred for ICA between February 2022 and June 2022. Prepared in line with the principles of the Declaration of Helsinki, version 1975, the research protocol was confirmed by the local ethics committee of Ankara City Hospital (E1-22-2375, dated February 9, 2022).

Results

We analyzed 110 patients with ACS (n = 110, 71.8% of males, mean age = 61.47 ± 11.00 years) and 47 patients with NVS (n = 47, 51.1% of males, mean age = 54.53 ± 9.06 years). Table 1 summarizes their demographic, clinical, and laboratory data. Smoking (59.1% vs. 34.0%, $P = 0.004$) was more prevalent in the group with ACS in comparison to the group with NVS.

We determined that ACS (+) group had higher PTN levels than NVS group (244.94 ± 89.09 vs. 199.46 ± 70.71, $P < 0.001$). The cutoff value of PTN for ACS was 207.66. PTN ≥207.66 ng/ml predicted ACS with a sensitivity of 79.1% and specificity of 59.6% (area under the curve: 0.697 [0.598–0.797], 95% confidence interval, $P < 0.001$]) [Figure 1].

According to the findings of univariate logistic regression analysis, older age, male gender, smoking, and PTN ≥207.66 ng/ml predicted ACS. In the multivariate analysis, besides older age, smoking and white blood cell (WBC), PTN ≥207.66 ng/ml remained independently associated with ACS [Table 2].

Discussion

In this research, we concluded that PTN levels were higher in ACS patients when compared to NVS patients, and the PTN cutoff point for ACS was 207.66 ng/dl. Presumably, our research is the first attempt applied on people in terms of revealing an association between high levels of PTN serum and ACS.

Table 1: Demographic and clinical data of study population

	NVS (*n*=47)	ACS (*n*=110)	*P*
Age (year)	54.53±9.06	61.47±11.00	<0.001
Gender (male), *n* (%)	24 (51.1)	79 (71.8)	0.012
Comorbidities, *n* (%)			
DM	12 (25.5)	41 (37.3)	0.154
HT	20 (42.6)	46 (41.8)	0.932
Smoking	16 (34.0)	65 (59.1)	0.004
Laboratory findings			
Creatinine, mg/dl	0.77±0.20	0.83±0.26	0.172
Uric acid, mg/dl	5.47±2.00	5.60±2.00	0.280
HDL-C, mg/L	47.09±17.22	43.04±10.30	0.07
LDL-C, mg/L	124.00±33.00	126.00±47.25	0.068
WBC, 10^3/uL	8.20±2.30	9.40±3.93	0.001
Neutrophil 10^3/uL	5.23±1.54	7.08±2.73	<0.001
Lymphocyte 10^3/uL	2.17±0.73	1.95±1.09	0.157
Hb, g/dl	14.00±2.40	13.90±2.20	0.802
Plt, 10^3/uL	252.43±58.13	248.83±66.95	0.749
Pleiotrophin, ng/ml	199.46±70.71	244.94±89.09	<0.001
Echocardiographic findings			
LVEF, %	59.64±4.57	43.98±9.03	<0.001

ACS=Acute coronary syndrome, DM=Diabetes mellitus, Hb=Hemoglobin, HDL-C=High-density lipoprotein cholesterol, HT=Hypertension, LDL-C=Low-density lipoprotein cholesterol, LVEF=Left ventricular ejection fraction, Plt=Platelet, NVS=No visible stenosis, WBC=White blood cell

ACS involves a wide range of clinical syndromes occurring in the form of instability or rupture of coronary atherosclerotic plaque and microvascular occlusion developing in the coronary artery, which is mostly experienced as coronary thrombosis. Despite the improvements in interventional and medical therapeutic approaches, ACS is still considered a major cause of morbidity and mortality all around the world. Sufficient supply of blood to the ischemic region can remarkably prevent the myocardial injury, which contributes to the improvement of heart function and maintenance of healthy of life.

Angiogenesis is the growth of new blood vessels from adjacent vasculature providing collateral blood supply of the tissues subject to chronic ischemia. By this way, the viability of the ischemic tissue may be maintained. An adequately coordinated angiogenic response is linked by promising results in animal models of acute MI as proved by tiny infarct scars, minor remodeling, and diastolic heart failure. Similarly, Fang *et al*.[4] demonstrated in a study with mice that delivery of myoblast mediated *PTN* gene could enhance blood distribution and protect tissues in the ischemic hindlimb muscle. We detected an increase in PTN level in acute ischemia of myocardium.

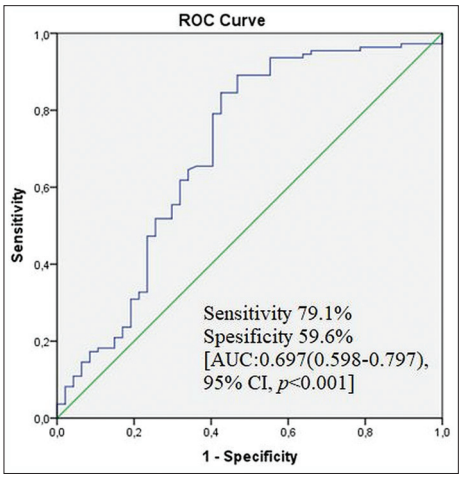

Figure 1: ROC curves for PTN associated with ACS. ROC = Receiver operating characteristic, PTN = Pleiotrophin, ACS = Acute coronary syndrome

Table 2: Univariate and multivariate logistic regression analysis showing the independent predictors of acute coronary syndrome

	Univariate		Multivariate	
	Odds ratio 95% CI	*P*	Odds ratio 95% CI	*P*
Age, year	1.064 (1.028-1.102)	<0.001	1.106 (1.052-1.163)	<0.001
Gender, male	2.442 (1.204-4.952)	0.013	1.457 (0.516-4.109)	0.477
Smoking	1.799 (1.372-5.710)	0.005	3.358 (1.138-9.913)	0.028
Hypertension	0.970 (0.486-1.937)	0.932		
Creatinine	2.956 (0.619-14.117)	0.174		
HDL-C	0.976 (0.949-1.004)	0.096		
WBC	1.306 (1.112-1.534)	0.001	1.390 (1.137-1.700)	0.001
PTN ≥207.66 ng/ml	5.574 (2.654-11.707)	0.014	3.994 (1.649-9.676)	0.002

HDL-C=High-density lipoprotein cholesterol, PTN=Pleiotrophin, WBC=White blood cell, CI=Confidence interval

This increase in PTN level in the acute phase of myocardial infarction may affect angiogenesis and by this way may reduce ischemia in the myocardium. Our study is the first to demonstrate the increase of PTN in the acute phase of myocardial infarction in humans. Promoting angiogenesis in the ischemic area may evolve as a novel approach to ACS treatment.[14] Early treatment with angiogenic factors in ACS may help rescue the jeopardized ischemic myocardium. Further studies are warranted for the effect of angiogenic factors like PTN in the treatment of ACS patients.

Obama *et al*.[15] studied the expression of MK in the mouse heart due to experimental myocardial infarction. As detected via IHC staining, myocytes and endothelial cells in the non-infarcted cardiac area produced dense immunoreactivity of MK 6 h after ligation of the left anterior descending coronary artery.[15] Similarly, our study demonstrated the increased level of PTN in the acute phase of myocardial infarction. We made a comparison between the clients with ACS and those with NVS. PTN has also been reported at increased levels in stable patients with good coronary collateral compared to poor collateral patients. No study has been designed comparing ACS with chronic coronary syndrome patients. The increased level of PTN in good collateral stable patients suspends PTN from being a diagnostic marker for ACS patients. Nonetheless, additional studies are required for evaluating PTN as a diagnostic marker in ACS.

Yeh *et al*.[16] designed a study to discover a proof that PTN can enhance tissue recovery. They examined PTN's expression pattern after designing focal cerebral ischemia model in rats and demonstrated that both the endothelial cells in neovasculature and the cells that are associated with the sites of angiogenesis exhibit intense PTN mRNA signals, initially at day 3 and continued up to day 14. Our study revealed that PTN level increased in the acute phase of myocardial ischemia. If we had scrutinized the patients' PTN levels included in our study on the 7th day, perhaps we would have seen even higher PTN values. In addition, there may be a difference in PTN levels between patients with ACS who are revascularized and those who are followed up medically.

Apoptosis is defined as cell death and removal without activating an inflammatory process based on DNA and cellular fragmentation. Abrupt reduction in coronary blood flow leads to hypoxia and hypoperfusion of the myocardium, generally resulting in cell death. Hence, therapeutic modulation on cardiac myocyte apoptosis may be a good target for the treatment of ACS patients. Several treatment strategies such as pharmacological and genetic interventions to modulate ion channels, nitric oxide, growth factors, and downstream signaling molecules have been studied to reduce apoptotic cell damage.[17] None of them have been shown to be useful clinically until now. It has been suggested that PTN signaling provides a strong anti-apoptotic effect. Bowden *et al*.[18] demonstrated that PTN through its receptor anaplastic lymphoma kinase could provide a survival signal for epithelial and fibroblast cells. However, Li *et al*.[19] demonstrated that PTN was released from cardiomyocytes in response to cell stress and can potentiate cardiomyocyte apoptosis through inhibition of protein kinase B (AKT) signaling. Du *et al*.[20] showed that suppression of PTN activity promoted apoptosis of cell lines in leukemia patients. PTN may have different effects on apoptosis in various cell lines through diverse signaling pathways. The effects of increase in PTN levels in ACS patients may be a subject of further studies.

Study limitations

While study population is limited in number, patients were included according to their statements relating subjective symptoms. Actual time of initiation of symptoms may be different. Our study population consisted of unstable angina, non-ST-elevation myocardial infarction, and ST-elevation myocardial infarction patients. This might have led to a heterogeneous study group in terms of biomarker kinetics. Amount of PTN in the tissue levels may vary with respect to the serum level, since we only measured serum PTN levels.

Conclusion

We determined that the serum PTN level had an association with ACS. Furthermore, older age, smoking, WBC, and PTN ≥207.66 ng/ml were independent predictors of ACS. The potential diagnostic and prognostic impact of PTN in ischemic heart disease may deserve to be further evaluated. PTN may also be studied as an angiogenic therapeutic factor in patients with ACS in additional studies.

Declaration of patient consent

We obtained written informed consent from each client before enrolling them in the study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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