THE EXPRESSION OF HGF, PDGF, VEGF AND ANGI IN PERIPHERAL ARTERIAL BLOOD AND CORONARY ARTERY BLOOD OF PATIENTS WITH ACUTE CORONARY SYNDROMES AND THE RELATIONSHIP WITH DISEASE SEVERITY

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ABSTRACT

Objective: *To examine the expression levels of hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), angiopoietin I (AngI)in peripheral arterial blood and coronary artery blood of patients with acute coronary syndromes (ACS) and the relationship with disease severity.*

Methods: *We analysed 148 ACS patients treated by coronary angiography (CAG) in our hospital from July 2016 to June 2017. In addition, there were 50 volunteers with normal CAG results in a control group. The expression levels of HGF, PDGF, VEGF and AngI in the peripheral arterial blood of all volunteers and coronary artery blood of ACS patients were detected and compared.*

Results: *The expression levels of HGF and PDGF in coronary artery blood of ACS patients were higher than the levels in peripheral arterial blood ($p<.05$); but the expression levels of VEGF and AngI in coronary artery blood of ACS patients were lower than the levels in peripheral arterial blood ($p<.05$). The expression levels of HGF, PDGF and VEGF in the peripheral arterial blood of ACS patients were higher than in the control group ($p<.05$). However, there was no statistical significance of AngI between the two groups ($p>.05$). The Gensini score of ACS patients was (36.93±1.47), which was higher than the control group ($p<.05$). The Gensini score had a positive correlation with HGF, PDGF and VEGF ($p<.05$); but there was no relationship between the Gensini score and AngI ($p>.05$).*

Conclusion: *There were significant differences in the levels of HGF, PDGF, VEGF and AngI in peripheral arterial blood compared to coronary artery blood of patients with acute coronary syndromes. (HGF and PDGF were higher; VEGF and AngI were lower.) In addition, the levels of HGF, PDGF and VEGF in the peripheral arterial blood of patients with acute coronary syndromes could be higher than in the control group and they were correlated with disease severity.*

Keywords: *Acute coronary syndromes, coronary artery blood, Gensini score, hepatocyte growth factor, platelet-derived growth factor.*

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Introduction

Acute coronary syndromes (ACS) are severe coronary heart diseases (CHD) involving complete or partial vessel occlusion caused by the secondary rupture of coronary artery atherosclerotic plaque. The specific pathogenesis is unknown and the mortality rate is high, including instability angina pectoris (UA), ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI)[1, 2]. At present, common clinical methods to treat CHD include conservative drug treatment, percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG) [3]. However, due to the severity and complications of ACS, common methods to treat CHD clinically are not ideal in the case of ACS. Therefore, it is imperative to explore the pathogenesis of ACS and new treatment strategies; the vascular factors related to endothelial injury especially have received serious attention in recent years. Therapeutic angiogenesis provides new treatment directions and hope for ACS

patients. This study examines the expression of four pleiotropic growth factors-hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and angiopoietin I (Ang I-in peripheral arterial blood and coronary artery blood of patients with acute coronary syndromes (ACS). It also examines the relationship with disease severity. The results provide preliminary theoretical support for individualized treatment of ACS related to angiogenesis factor.

Data and method

Clinic data

This study randomly analysed 148 ACS patients treated by coronary angiography (CAG) in our hospital from July 2016 to June 2017. There were 81 male patients and 67 female patients 44-81 years old, with an average of 62.86±7.35 years old. This group included 52 UA patients, 51 STEMI patients and 45 NSTEMI patients.

After examination for CAG in our hospital, 50 patients whose examinations of myocardial enzyme and cardiac troponin were negative were included as a control group in this study. The Gensini score was used to evaluate the disease levels of the ACS patients. This study has been approved by the local Ethics Committee.

There was no statistical difference (p>.05) between the groups in terms of age, gender, body mass index (BMI), diabetes history, hypertension history and smoking history. See Table 1.

Group	Cases (case)	BMI (kg/m^2)	Average age (years)	Gender (case)		Diabetes history (n, %)	Hypertension history (n, %)	Smoking history (n, %)
				Male	Female			
Control group	50	21.67 ±4.17	62.86 ±7.35	28	22	9 (18.0)	13 (26.0)	13 (37.14)
ACS group	148	22.45 ±2.39	61.99 ±8.02	81	67	31 (20.95)	49 (33.11)	61 (41.22)

Table 1: General clinical data analysis of patients.

Inclusion criteria

• Clinical diagnosis of all patients was ACS through CAG, clinic symptoms and auxiliary examination;

• The patients and their family members signed informed consent.

Exclusion criteria

• Patients did on meet the inclusion criteria;

• Patients had severe disturbance of consciousness, hypertensive crisis, and acute heart failure before treatment;

• Severe combined liver and kidney function impairment;

• Severe combined blood system diseases or haemorrhagic disease; (5) malignant tumour.

Main instruments and reagent

GTR16-2 high-speed desktop refrigerated centrifuge (purchased from Beijing Times Beili Centrifuge Co., Ltd.); US ELX-808 fully automatic quantitative drawing microplate reader (purchased from Shanghai Maisha Biotechnology Co., Ltd.).

HGF, PDGF, VEGF and AngI ELISA kits (purchased from US R&D Biotechnology Co., Ltd.).

Coronary angiography (CAG)

Before the examination, the patient relaxed the wrist properly. After puncture at 2 cm of processus styloideus radii, 3000 U-6000 U heparin was injected through sheathing canal. An all-round X-ray examination of the left and right coronary arteries was conducted and a DSA image processing system was used for quantitative analysis of the degree of coronary artery stenosis.

The Gensini scoring system was adopted for evaluation:

• 1 score, stenosis <25%;
• 2 scores, 25%-50% stenosis;
• 4 scores: 51%-75% stenosis;
• 8 scores: 76%-90% stenosis;
• 16 scores: 91%-99% stenosis;
• 32 scores, 100% stenosis.

Coefficient of each section of the coronary artery:

• Left main coronary artery × 5;

• Proximal segment, middle segment and distal segment of anterior descending branch ×2.5, ×1.5, ×1 respectively;

• The first and second diagonal branches × 1, ×0.5 respectively;

• Proximal segment, middle segment and distal segment of circumflex branch × 2.5, ×1.5, ×1 respectively;

• Proximal segment, middle segment and distal segment of the right coronary artery, the posterior descending branch, and the posterior branch of the left ventricle ×1. The sum of disease scores was the total score.

Detection of indicators of angiogenesis-related factors

For each ACS patient, 5 ml of coronary artery

blood was extracted with a driver. When all subjects were processing CAG, 5 ml blood of radial artery or peripheral blood of femoral artery was collected.

Blood samples were centrifuged at room temperature and the supernatant was taken and placed at -80oC for storage. According to the experimental procedure specified by the ELISA reagent, expression levels of HGF, PDGF, VEGF and AngI serums were detected. A fully automatic quantitative drawing microplate reader was adopted to measure OD values at 450 nm of wavelength.

Statistical treatment

SPSS 21.0 software was used for statistical analysis. Measurement data were expressed in ($\bar{x}\pm s$), t-test was used to for comparison of data of both groups; count data were expressed in percent, and variance analysis was adopted. A Pearson examination or a Spearman examination was used for correlation analysis. $p<.05$ meant the difference was statistically significant.

Results

Comparison of preoperative examination results of patients in both groups

Blood biochemical indexes of the subjects of both groups were examined by the laboratory. The results showed that the platelet count (PLT) and the fasting blood-glucose content of ACS patients were higher than those of the control group, and the difference was statistically significant at $p<.05$. Differences in other indexes of the two groups-glycosylated haemoglobin (HbAlc), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL)-were without a statistical difference, $p>.05$. See Table 2.

Group	PLT ($\times10^9$/L)	PFG (mmol/l)	HbAlc (%)	TG (mmol/l)	TC (mmol/l)	HDL (mmol/l)	LDL (mmol/l)
Control group	176.95 ±69.37	5.41 ±1.48	6.33 ±1.56	1.23 ±1.24	4.11 ±1.65	1.26 ±0.17	2.52 ±0.21
ACS group	217.43 ±81.62[a]	6.58 ±1.28[a]	6.24 ±0.97	1.05 ±1.33	3.98 ±1.44	1.26 ±0.34	2.57 ±0.16

Table 2: Comparison of examination results of subjects of both groups at admission ($\bar{x}\pm s$).
Note: [a]Compared with control group, $p<.05$.

HGF, PDGF, VEGF and AngI levels of peripheral arterial blood and coronary artery blood of ACS patients

HGF, PDGF, VEGF and AngI contents of peripheral arterial blood and coronary artery blood of ACS patients were examined by the laboratory. The results showed that the content values of HGF and PDGF in the coronary artery blood of ACS patients were higher than those in peripheral arterial blood. However, levels of VEGF and AngI in the coronary artery blood were lower than in peripheral arterial blood. The differences were statistically significant, $p<.05$. See Table 3.

ACS	HGF (ng/ml)	PDGF (ng/ml)	VEGF (pg/ml)	AngI(ng/ml)
Coronary artery blood	39.05±8.33	3.87±1.04	1.36±0.38	7.57±2.16
Peripheral arterial blood	7.42±1.08[b]	1.02±0.36[b]	91.24±26.42[b]	32.69±10.22[b]

Table 3: Comparison of HGF, PDGF, VEGF and AngI levels of peripheral arterial blood and coronary artery blood of ACS patients ($\bar{x}\pm s$).
Note: [b]Compared with the blood content of the coronary arteries of ACS patients, $p<.05$.

Comparison of HGF, PDGF, VEGF and AngI levels in peripheral arterial blood and Gensini scores of subjects in both groups

HGF, PDGF, VEGF and AngI contents in the peripheral arterial blood of ACS patients and subjects in the control group were examined by the laboratory. The results showed that the content values of HGF, PDGF and VEGF of peripheral arterial blood of ACS patients were higher than those of patients in the control group with a statistical difference of $p<.05$. The AngI levels in the peripheral arterial blood of subjects in both groups had no statistical difference, $p>.05$.

In addition, the Gensini score of ACS patients was (36.93±1.47), and it was higher than for the control group, with a statistical difference, $p<.05$. See Table 4.

Group	HGF (ng/ml)	PDGF (ng/ml)	VEGF (pg/ml)	AngI (ng/ml)	Gensini score
Control group	1.27±0.18	0.26±0.02	19.83±7.49	29.76±9.15	5.48±0.62
ACS group	7.42±1.08[a]	1.02±0.36[a]	91.24±26.42[a]	32.69±10.22	36.93±1.47[a]

Table 4: Comparison of HGF, PDGF, VEGF and AngI levels of peripheral arterial blood and gensini scores of subjects in both groups ($\bar{x}\pm s$).
Note: [a]Compared with control group, $p<.05$.

Correlation analysis of HGF, PDGF, VEGF, AngI and Gensini score

Pearson examination was used for correlation analysis.

The Gensini score and expression of HGF, PDGF and VEGF of ACS patients showed a positive correlation, $p<.05$; but they were not related to the expression of AngI, $p>.05$. See Table 5 and Figure 1.

Variable	*r*	*p*
HGF	0.419	.012
PDGF	0.731	.003
VEGF	0.486	.010
AngI	0.119	.078

Table 5: Correlation analysis of Gensini score and expression of HGF, PDGF, VEGF and AngI

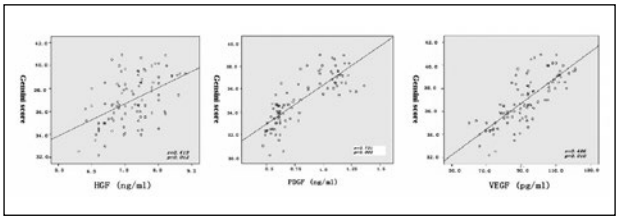

Figure 1: Correlation analysis of Gensini scores and expression of HGF, PDGF and VEGF.

Discussion

Coronary heart disease, one of the cardiovascular and cerebrovascular diseases that severely impair human health, has a high mortality rate and disability rate. In recent years, there has been a trend of youthfulness. The pathogenesis is complicated and it has added a great economic burden to the family of patients[4, 5]. ACS is the most severe coronary heart disease[6]. In recent years, several studies have confirmed that coronary endothelial dysfunction, plaque rupture, platelet aggregation, inflammation and other aspects are closely related to generation and development of ACS[3, 7]. In particular, endothelial injury inflammation theory is increasingly recognized by relevant scholars. Various disease mechanisms eventually lead to arterial intimal injury[8]. Therefore, this study focuses on the expression of endothelial-repair-related factors of HGF, PDGF, VEGF and AngI of peripheral arterial blood and coronary artery blood of ACS patients and the relationship with disease severity.

HGF, PDGF, VEGF and AngI are multifunction cell factors, and they are closely related to the generation of an inflammatory reaction, angiogenesis, apoptosis and other physiological and pathological processes[9]. HGF is derived from Leydig cells; its specificity combines with C-met receptor to activate multiple signal pathways, participate in angiogenesis, inhibit apoptosis and so on. Meanwhile, it is also involved in the repair and proliferation of endothelial cells to protect ischemic myocardial cells[10, 11]. Like HGF, PDGF also has various physiological effects that promote vasoconstriction by inhibiting the synthesis of endothelium-derived relaxing factor. After endothelial injury of ACS patients, platelets are activated, promoting the release of PDGF and AngI and inducing migration of smooth muscle cells into the intima to form a thrombus[12]. In recent years, studies have used PDGF as a candidate gene to target ACS treatment or for coronary artery bypass grafting[13]. VEGF secreted from vascular endothelial cells is a necessary cell factor for growth and differentiation of endothelial cells. It shows an expression of high specificity in vascular endothelial cells of CHD patients, and it promotes angiogenesis by promoting migration and proliferation of endothelial cells and degrading the release of protein kinases related to the basement membrane[14]. AngI also can promote the angiogenesis of VEGF, induce endothelial cell aggregation to promote vascular damage, and then adopt endothelialisation[15]. Therefore, theoretically, HGF, PDGF, VEGF and AngI are involved in the generation and development of ACS and other pathological processes.

This study found that content values of HGF and PDGF in coronary artery blood of ACS patients were higher than those in peripheral arterial blood, but VEGF and AngI levels were lower in coronary artery blood than in peripheral arterial blood. The differences were statistically significant, $p<.05$. This suggests that, although HGF and PDGF are expressed in various organs through endocrine pathway, there is physiological activity only in local impaired vascular endothelia tissues that are hypoxic and ischemic in the coronary artery. So, expression levels of HGF and PDGF in coronary artery blood of ACS patients are higher than in peripheral arterial blood. Generally, the expression level of VEGF in the body is lower. Under the condition of ischemia and anoxia or stimulation of an inflammatory response, the body secretes large VEGF, repair of ischemic tissues requires the consumption of large local VEGF, and the body will further secrete VEGF for compensation. So, the content of VEGF of peripheral arterial blood is higher than that of coronary artery blood.

In addition, results of this study also show that content values of HGF, PDGF and VEGF of peripheral arterial blood of ACS patients are higher than those of the patients in the control group with a statistical difference, $p<.05$; AngI levels of peripheral arterial blood of subjects of both groups have no statistical difference, $p>.05$. Presumably, AngI mainly plays a role in vascular remodelling and maturation during late angiogenesis, and it is closely related to VEGF. AngI and VEGF have effects on new vessels, promote the connection of endothelial cells with the

extracellular matrix and maintain the stability of new vessels. Therefore, the AngI content in the peripheral arterial blood of ACS patients was higher than in coronary artery blood; there is no statistical difference for subjects of the control group. A Pearson examination, used for correlation analysis, showed a positive correlation between the disease severity of ACS patients and expression of HGF, PDGF and VEGF at $p<.05$. These results and the study of the mechanisms of HGF, PDGF and VEGF are basically the same. Higher expression levels of HGF, PDGF and VEGF indicate that the condition of an ACS patient is more severe. However, no such correlation was found for the expression level of AngI, $p>.05$. This result may be related to the small sample size of this study or the mechanism of AngI. This needs further study.

Above all, HGF, PDGF, VEGF and AngI are closely related to the generation and development of ACS. They can be molecular markers for ACS diagnosis and prediction of the severity of disease severity. They also provide a potential target and direction for individualized gene treatment for angiogenesis.

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