Therapeutic and Diagnostic Value of Caspase-12 and Study of Growth Differentiation Factor-15 in Patients with Acute Myocardial Infarction

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Abstract

Background: To investigate the therapeutic and diagnostic value of caspase-12 and study of growth differentiation factor-15 (GDF-15) in patients with acute myocardial infarction (AMI).

Methods: Overall, 78 patients with AMI admitted to Weifang People's Hospital Brain Hospital, Weifang, China from Apr 2016 to Apr 2019 were enrolled as AMI group. Seventy-five non-AMI people undergoing physical examination during the same period were enrolled as non-AMI group. The expression levels of caspase-12 and GDF-15 were detected and compared. The correlation between the expressions of GDF-15, caspase-12 and clinical characteristics and efficacy was detected. Single and combined detection of GDF-15 and caspase-12 were performed analyze their role in the early diagnosis, the prediction of efficacy, and the guidance of clinical therapy.

Results: After treatment, the levels of GDF-15 and caspase-12 in AMI group were significantly lower than those before treatment ($P<0.001$). The expression levels of GDF-15 and caspase-12 were significantly correlated with blood pressure ($P<0.05$). The expression levels of GDF-15 and caspase-12 were significantly negatively correlated with clinical efficacy in AMI group. The diagnostic value of combined detection of GDF-15 and caspase-12 was higher than that of single diagnosis. The levels of serum caspase-12 and GDF-15 proteins were significantly up regulated in AMI patients. With the better therapeutic effect, the levels of serum caspase-12 and GDF-15 proteins decreased significantly.

Conclusion: The levels of serum caspase-12 and GDF-15 proteins may be a key indicator in the clinical diagnosis of acute myocardial infarction and may be used to guide the treatment of AMI patients and predict the therapeutic efficacy.

Keywords: Acute myocardial infarction (AMI); Therapeutic value; Diagnostic value

Introduction

Acute myocardial infarction (AMI) is a myocardial necrosis caused by acute, persistent ischemia and hypoxia in the coronary arteries. In recent years, the number of coronary heart disease in China has been increasing. Because the epidemic trend of risk factors for coronary heart disease has been increasing, it leads to a rising mortality rate of coronary heart disease (1, 2). In the past 30 years, developed countries were in the opposite direction of the rising trend of China, and

mortality of coronary heart disease was significantly reduced (3). The primary cause of death in coronary heart disease is AMI. It reflects the health education level of residents and the quality of medical and health services and shows a steady decline trend in developed countries (4, 5).

The growth differentiation factor-15 (GDF-15), a member of the transforming growth factor-β (TGF-β) superfamily (6), belongs to the GDFs family. GDF-15 was associated with cardiovascular disease (7). GDF-15 increases in tissue damage and inflammation and is associated with cardiac metabolism risk (8). Expression levels of GDF-15 were altered in acute heart failure (9), and chronic inflammation of the prostate (10). It also involved in tumor formation, deterioration, angiogenesis and metastasis, and stress response, bone formation, hematopoietic development, adipose tissue function and regulation of cardiovascular disease (11). Caspase-12 colonized on the outer membrane of the endoplasmic reticulum belongs to the family of cysteinyl aspartate specific proteinase (caspase). One of the important parts of the apoptotic link is the endoplasmic reticulum (12). Endoplasmic reticulum stress is a new way to initiate apoptosis. It can be triggered by persistent myocardial ischemia caused by AMI to induce apoptosis (13-15).

In this study, we analyzed whether the expression levels of serum GDF-15 and caspase-12 were related to AMI and analyzed whether it had therapeutic and diagnostic value for AMI patients.

Materials and Methods

Clinical baseline data

Seventy-eight patients with AMI admitted to Weifang People's Hospital Brain Hospital, Weifang, China from Apr 2016 to Apr 2019 were enrolled as AMI group, including 45 males and 33 females with an average age of (62.41±7.62) yr old. Seventy-five non-AMI people undergoing physical examination during the same period were enrolled as non-AMI group, including 43 males and 32 females with an average age of (61.62±8.24) yr old.

Inclusion criteria: the patient was accompanied by family members and diagnosed as AMI at admission. Diagnostic criteria for AMI: at least 2 of the following 3 criteria must be met: 1) clinical history of ischemic chest pain; 2) dynamic evolution of electrocardiogram; 3) Dynamic changes in serum myocardial marker concentrations of myocardial necrosis.

Exclusion criteria: congenital heart disease, history of cardiac surgery, liver and kidney dysfunction, history of malignant tumors, acute pulmonary embolism, cerebral hemorrhage or cerebral infarction, acute phase of various infections; history of mental disease and family history of mental disease; autoimmune system defect; history of severe organ diseases; history of craniocerebral trauma; history of drug dependence; unable to cooperate with the examination due to communication barrier such as aphasia, dysphoria, unconsciousness and lipothymia.

The study was approved by the Ethics Committee of our hospital. The experimental content in detail were described to the subjects and their families in advance. The subjects and their families agreed and signed a complete informed consent form.

Methods of treatment

Patients with AMI were immediately given an electrocardiogram examination, oxygen inhalation and electrocardiogram monitoring after admission, and venous access was quickly established to prepare for defibrillation. The patients first orally took 300 mg of aspirin, then received 1,500,000 U of urokinase intravenous infusion for thrombolysis and 100 mg of heparin intravenous infusion. Thrombin time was measured every 6 h, and demerol or nitroglycerin was used to relieve patient's pain.

Collection of blood

Before admission and after treatment for 14 d, 4 mL fasting venous blood was extracted from AMI patients. 3-4 ml peripheral venous blood was collected, put into anticoagulative tube and sent to the laboratory department. In the morning of the physical examination of the non-AMI

group, 4 ml peripheral blood was collected on an empty stomach, put into anticoagulant tube and sent to the laboratory department. After coagulation for 60 min (20-25 ℃), it (Beijing Bohaoxing Instrument Co., Ltd., Sigma 3-18 ks) was centrifuged at 3500 r/min for 15 min with a centrifuge radius of 10 cm and centrifuge temperature of 4 ℃, and then separated the upper serum and stored it for use. After obtaining the upper serum, the concentration of GDF-15 (Wuhan Hualianke Biotechnology Co., Ltd., HM10819) and caspase-12 (Nanjing Laifusai Biotechnology Co., Ltd., EK2113) in serum was detected by enzyme-linked immunosorbent assay (ELISA). The detection process was carried out in strict accordance with the kit instructions. The specific steps of the ELISA were as follows: 100 μL of the sample and standard sample were added to the reagent diluent, sealed the plate and incubated for 2 h at room temperature. The plate was washed. 100 μL of the detection antibody was added to each hole, sealed the plate and incubated for 2 h at room temperature. The plate was washed. One hundred μL of streptavidin-biotin-HRP working dilution was added to each hole, sealed the plate and incubated for 20 min at room temperature in the dark. The plate was washed. One hundred μL of substrate solution was added to each hole, and then incubated at 37 °C for 20 min in the dark. Fifty μL of terminal liquid was added. The reading at 450 nm of the ELXS00 enzyme-labelling measuring instrument was used within 15 min.

Evaluation of efficacy

Efficacy evaluation (16, 17) was divided into markedly effective, effective and ineffective. Markedly effective: the patient's clinical symptoms and signs improved significantly after treatment. Effective: the patient's clinical symptoms and signs improved after treatment, but the improvement effect was not significant. Ineffective: the patient's clinical symptoms and signs were not significant change or deterioration after treatment.

Observation indexes

Before treatment, the expression levels of serum GDF-15 and caspase-12 in the AMI group and the non-AMI group were compared. The correlation between expression levels of GDF-15 and caspase-12 in serum and efficacy was compared after treatment in the AMI group. The expression levels of GDF-15 and caspase-12 in serum before and after treatment in the AMI group were compared.

Statistical methods

SPSS20.0 (IBM Corp, Armonk, NY, USA) was used for all statistical analyses of the experimental results. All graphical results were plotted by using GraphPad Prism 7 (San Diego Graphpad Software, Inc.). The count data were represented by [n(%)]. The chi-square test was used for comparison between groups. Measurement data were expressed by (x ± s). The two groups were compared by t test. The diagnostic and predictive values were analyzed by using the receiver-operating curve (ROC). The correlation was analyzed by using the Spearman correlation coefficient. The difference was statistically significant with $P<0.05$.

Results

Comparison of baseline data

There was no statistical difference between the AMI group and the non-AMI in terms of gender, age, body mass index, presence or absence of smoking, complications, Glu, TC, Cr, HDL-C, hs-CRP (all $P>0.05$) and there were statistical differences in LDL-C, TG and UA between the two groups (all $P < 0.05$) (Table 1).

Comparison of expression levels of GDF-15 and caspase-12 between the AMI group and the non-AMI group before treatment

The serum expression levels of GDF-15 and caspase-12 in the AMI group were significantly higher than those in the non-AMI group (all $P<0.0001$) (Fig. 1).

Table 1: Comparison of baseline clinical data between the two groups ($\bar{x}\pm s$)/[n(%)]

| Variable	AMI group (n=78)	Non-AMI group (n=75)	T/X²	P
Gender			0.002	0.96
Male	45 (57.69)	43 (57.33)		
Female	33 (42.31)	32 (42.67)		
Age/yr old	62.41±7.62	61.62±8.24	0.62	0.54
Body mass index	27.32±2.68	26.82±2.51	1.19	0.24
Smoking			2.31	0.13
Yes	52(66.67)	41(54.67)		
No	26(33.33)	34(45.33)		
Complications				
Diabetes mellitus	22 (28.21)	19 (25.33)	0.16	0.69
Hypertension	42 (71.29)	30 (74.67)	2.94	0.09
Glu (mmol/L)	7.62±2.43	7.14±2.32	1.25	0.21
TC (mmol/L)	4.36±1.21	4.42±1.46	0.28	0.78
TG (mmol/L)	1.59±0.47	1.82±0.51	2.90	0.004
UA (µmol/L)	358.19±118.30	316.32±95.24	2.41	0.02
Cr (mmol/L)	73.12±12.23	68.82±13.14	1.61	0.11
LDL-C (mmol/L)	3.12±0.65	2.91±0.52	2.2.	0.03
HDL-C (mmol/L)	1.21±0.36	1.12±0.32	1.63	0.10
hs-CRP (mg/L)	3.18±1.02	2.91±0.92	1.71	0.09
Medication history				
Nitroglycerin	32 (41.03)	34 (45.33)	0.29	0.59
Aspirin	16 (20.51)	14 (18.67)	0.08	0.77
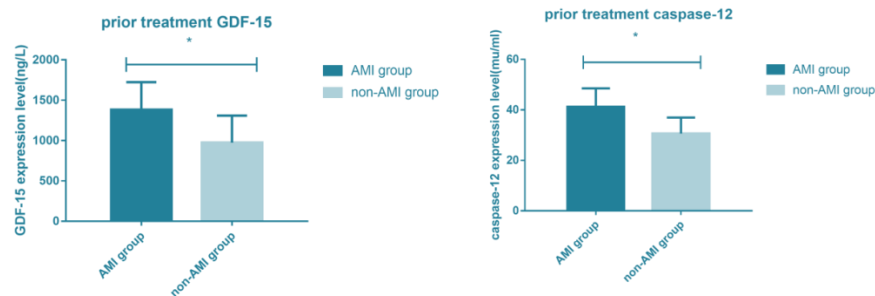
Fig. 1: Comparison of expression levels of serum GDF-15 and caspase-12 in the two groups before treatment
The expression levels of serum GDF-15 and caspase-12 in the AMI group were significantly higher than those in the non-AMI group. Note: * indicates the comparison between the two groups, *P*<0.05

Relationship between expression levels of serum GDF-15 and caspase-12 and clinical characteristics in patients with AMI
The expression levels of GDF-15 and caspase-12 were not significantly correlated with gender, age, smoking and presence or absence of diabetes (all *P*>0.05), while were significantly correlated with blood pressure (all *P*<0.05) (Table 2).

Comparison of serum GDF-15 and caspase-12 levels before and after treatment in the AMI group
After treatment, the levels of GDF-15 and caspase-12 in AMI patients in the AMI group were significantly lower than those before treatment (all *P*<0.001) (Fig. 2).

Correlation between the expression levels of GDF-15 and caspase-12 in the AMI group and clinical efficacy

GDF-15 and caspase-12 levels were significantly negatively correlated with efficacy (*P*<0.05). See Fig. 3 According to the therapeutic effect, the AMI group was divided into the effective group (n=64) and the ineffective group (n=14). The serum GDF-15 and caspase-12 levels in the ineffective group were significantly higher than the effective group (all *P*<0.05) (Table 3).

Table 2: Relationship between levels of serum GDF-15 and caspase-12 and clinical features in patients with AMI (x±sd)

| *Variable*	*n*	*GDF-15(ng/L)*	*t*	*P*	*caspase-12(mu/ml)*	*t*	*P*
Gender			0.37	0.71		1.78	0.08
Male	45	1391.62±364.61			43.26±6.24		
Female	33	1361.51±331.19			40.48±7.52		
Age			0.26	0.79		1.40	0.17
< 65 yr old	49	1362.54±321.47			39.96±5.98		
≥65 yr old	29	1382.71±332.86			42.12±7.51		
Smoking			0.48	0.63		1.46	0.15
Yes	52	1410.64±323.45			42.85±6.71		
No	26	1372.95±334.49			40.51±6.58		
Diabetes mellitus			0.03	0.97		11.64	0.11
Yes	22	1389.65±371.52			43.11±7.55		
No	56	1386.52±365.47			40.26±6.65		
Blood pressure			2.08	0.04		2.74	0.01
Hypertension	42	1489.58±323.98			44.31±6.12		
Normal	36	1335.91±326.78			40.30±6.81		
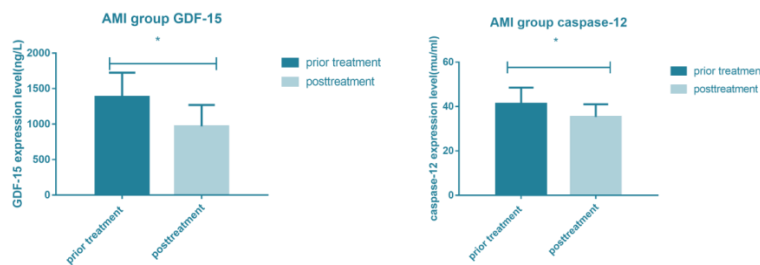
Fig. 2: Comparison of expression levels of serum GDF-15 and caspase-12 before and after treatment in the AMI group**.** The levels of GDF-15 and caspase-12 of AMI patients in the AMI group after treatment were significantly lower than those before treatment. Note: * indicates the comparison between the two groups, *P*<0.05
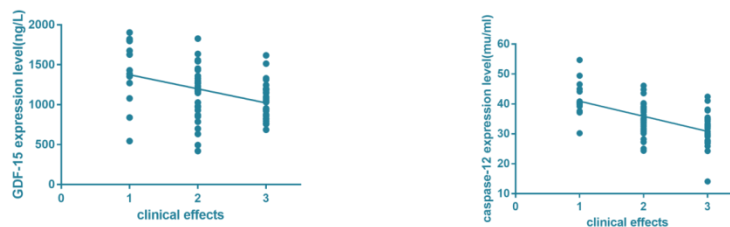

Fig. 3: Correlation between the expression levels of GDF-15 and caspase-12 and clinical efficacy
The expression levels of GDF-15 and caspase-12 were negatively correlated with the clinical efficacy. The worse the therapeutic effect, the higher the levels of GDF-15 and caspase-12
Note: Clinical efficacy 1: ineffective 2: effective 3: markedly effective

Table 3: Comparison of expression levels of GDF-15 and caspase-12 between the effective group and the ineffective group (x±s)

variable	Effective group (n=64)	Ineffective group (n=14)	t	P
GDF-15 (ng/L)	1075.04±271.74	1383.42±332.16	3.69	0.0004
caspase-12 (mu/ml)	33.83±5.53	42.14±6.46	4.94	<0.0001

Comparison of diagnostic value of serum GDF-15 and caspase-12 single detection and two combined detection for AMI in the two groups before treatment

The sensitivity from high to low was combined detection, caspase-12, GDF-15. The specificity from high to low was combined detection, GDF-15, caspase-12. The AUC combined detection was up to 0.93 (Fig. 4 and Table 4).

Comparison of predictive value of GDF-15 and caspase-12 single detection and two combined detections for AMI efficacy before treatment in the effective group and the ineffective group

The sensitivity from high to low was combined detection, caspase-12, GDF-15. The specificity from high to low was GDF-15, combined detection, caspase-12. The AUC combined detection was up to 0.91 (Table 5 and Fig. 5).

Table 4: Comparison of diagnostic value of GDF-15 and caspase-12 single detection and two combined detections for AMI

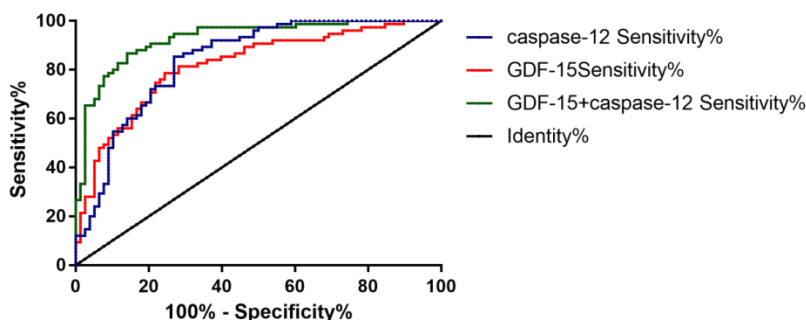| Items	Sensitivity	Specificity	You-den index	Best threshold	AUC	P	95% confidence interval	
							Upper limit	Lower limit
GDF-15	78.67%	75.64%	0.03	<1175	0.82	<0.0001	0.89	0.75
Caspase-12	85.33%	73.08%	0.03	<35.79	0.84	<0.0001	0.90	0.78
GDF-15+ Caspase-12	86.67%	85.90%	0.02	-	0.93	<0.0001	0.97	0.89

ROC curve: ROC of DF-15+caspase-12 ROC

Fig. 4: ROC curves of GDF-15 and caspase-12 single detection and two combined detection before treatment in the two groups**.** The order of sensitivity from high to low was combined detection, caspase-12 and GDF-15. The order of specificity from high to low was combined detection, GDF-15 and caspase-12. AUC combined detection was up to 0.93
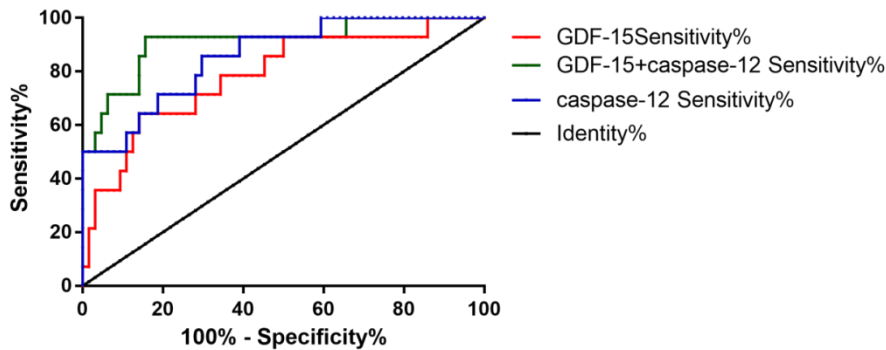
Fig. 5: ROC curves of GDF-15 and caspase-12 single detection and two combined detections before treatment in the effective group and the ineffective group. The order of sensitivity from high to low was combined detection, caspase-12 and GDF-15. The order of specificity from high to low was GDF-15, combined detection and caspase-12. AUC combined detection was up to 0.91

Table 5: Predictive value of GDF-15 and caspase-12 single detection and two combined detections for AMI efficacy

Items	Sensitivity	Specificity	You-den index	Best threshold	AUC	P	95% confidence interval	
							Upper limit	Lower limit
GDF-15	64.29%	85.94%	0.07	<1612	0.79	0.0009	0.92	0.65
Caspase-12	85.71%	70.31%	0.06	<40.61	0.86	<0.0001	0.96	0.75
GDF-15+ Caspase-12	92.86%	84.37%	0.05	-	0.91	<0.0001	1.00	0.82

Discussion

AMI is usually caused by erosion and rupture of coronary artery unstable plaques to form secondary thrombosis, which may lead to acute ischemic necrosis of myocardium (18). After the first AMI, the concentration of cTn will last for 10-14 days. During this period, it is impossible to judge whether the patient has the second AMI, which is not conducive to the timely treatment of the patient and doctor's judgment (19). At the same time, the concentration of cTn in myocardium damaged by myocarditis, septicemia, electrical cardioversion and radiofrequency ablation also increased (20,21). Therefore, it is of great clinical significance for early AMI diagnosis to search and find markers with high specificity and sensitivity.

GDF-15 may be a good indicator in the diagnosis and treatment of cardiovascular diseases (22). Studies have also shown that the increase of the expression levels of GDF-15 is associated with heart failure, acute coronary syndrome, primary pulmonary hypertension and other diseases (23, 24). Caspase-12 rats can alleviate apoptosis of ERS-induced, but they are more sensitive to other death stimuli (25). This indicated that caspase-12 activation could induce apoptosis independently and not rely on other pathways.

The expression levels of serum GDF-15 in the AMI group was higher than that in the non-AMI group, which was similar to the results of the studies (26, 27). The expression levels of serum caspase-12 was also higher in the AMI group than in the non-AMI group, which was consistent with the results of Jing et al (28) for the

expression of CaSR, p-p65 and caspase-12 as well as the secretion of th-1 and th-2 cytokines during the onset of acute myocardial infarction. GDF-15 may be a marker for recognizing ventricular hypertrophy or hypertensive heart disease (29). In the study of GDF-15 blocking norepinephrine-induced cardiac hypertrophy by inhibiting trans activation of epidermal growth factor receptor, GDF-15 inhibited norepinephrine-induced EGF receptor deactivate and related hypertrophy and was positively correlated with cardiac hypertrophy in hypertensive patients(30). Cardiomyocyte apoptosis can occur in cardiovascular diseases such as hypertension, and ERS is one of the intrinsic apoptotic pathways. CHOP and caspase-12 dependent pathways of SHR rats are activated in endoplasmic reticulum stress-induced apoptosis of hypertensive and ERS can cause apoptosis of myocardial cells in hypertensive patients (31). In this study, the levels of GDF-15 and caspase-12 in this AMI group after treatment were significantly lower than those before treatment (all $P < 0.001$). It is consistent with our results in a study (28), and in caspase-12.

Since the sample size in this study was small and both groups were given drugs, it may affect the detection results of the levels of serum caspase-12 and GDF-15 proteins in both groups. Furthermore, long-term follow-up was not conducted. For the above shortcomings, analysis that is more experimental will be carried out in the future to improve experimental results.

Conclusion

The levels of serum caspase-12 and GDF-15 proteins may be key indicators in the clinical diagnosis of acute myocardial infarction and may also be used to guide the treatment of AMI patients and predict the therapeutic efficiency.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Conflict of interest

The authors declare that there is no conflict of interest.

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