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Correlation between growth differentiation factor-15 and collagen metabolism indicators in patients with myocardial infarction and heart failure

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Abstract

Background Growth differentiation factor (GDF)-15, a divergent member of the transforming growth factor beta super-family does appear to be up-regulated in response to experimental pressure overload and progression of heart failure (HF). HF frequently develops after myocardial infarction (MI), contributing to worse outcome. The aim of this study is to assess the correlation between GDF-15 levels and markers related to collagen turnover in different stages of HF. **Methods** The study consists of a cohort of 179 patients, including stable angina pectoris patients (AP group, n = 50), old MI patients without HF (OMI group, n = 56), old MI patients with HF (OMI-HF group, n = 38) and normal Control group (n = 35). Both indicators reflecting the synthesis and degradation rates of collagen including precollagen I N-terminal peptide (PINP), type I collagen carboxy-terminal peptide (ICTP), precollagen III N-terminal peptide (PIIINP) and GDF-15 were measured using an enzyme-linked inmunosorbent assay. **Results** The plasma GDF-15 level was higher in OMI-HF group (1373.4 ± 275.4 ng/L) than OMI group (1036.1 ± 248.6 ng/L), AP group (784.6 ± 222.4 ng/L) and Control group (483.8 ± 186.4 ng/L) (P < 0.001). The indicators of collagen turnover (ICTP, PINP, PIIINP) all increased in the OMI-HF group compared with Control group (3.03 ± 1.02 μg/L *vs.* 2.08 ± 0.95 μg/L, 22.2 ± 6.6 μg/L *vs.* 16.7 ± 5.1 μg/L and 13.2 ± 7.9 μg/L *vs.* 6.4 ± 2.1 μg/L, respectively; P < 0.01). GDF-15 positively correlated with ICTP and PIIINP (r = 0.302, P < 0.001 and r = 0.206, P = 0.006, respectively). GDF-15 positively correlated to the echocardiographic diastolic indicators E/Em and left atrial pressure (r = 0.349 and r = 0.358, respectively; P < 0.01), and inversely correlated to the systolic indicators left ventricular ejection fraction and the average of peak systolic myocardial velocities (Sm) (r = –0.623 and r = –0.365, respectively; P < 0.01). **Conclusion** Plasma GDF-15 is associated with the indicators of type I and III collagen turnover.

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Keywords: Biomarkers; Collagen turnover; Growth differentiation factor-15; Heart failure; Myocardial infarction

1 Introduction

Heart injury often ends up with pathologic remodeling and fibrosis, which may contribute to heart failure (HF).[1] Changes of myocardial cells and extracellular matrix (ECM) such as collagen deposition and fibrosis in ECM are the structural bases for ventricular remodeling. Collagen is a structural protein abundantly expressed in myocardial tissue; the complex reticular structure composed of collagen fiber enclosures and cross-links with myocardial cells, muscle fibers and bundles from the cardiac collagen network. Our previous study showed that plasma growth differentiation factor (GDF)-15 rose following a staged progression of HF and blocked norepinephrine-induced myocardial hypertrophy via inhibiting the phosphorylation of epidermal growth factor (EGF) receptors and downstream kinases involving inhibition of epidermal growth factor receptor transactivation.[2,3] GDF-15 is a latest marker in the heart. It was originally identified as macrophage inhibitory cytokine 1 (MIC-1),[4] and participates in a number of overlapping pathways in the heart, which makes it a good choice for evaluation.[5] GDF-15 is not expressed in the normal adult heart but is induced in response to conditions that promote hypertrophy and dilated cardiomyopathy.[6] In view of the fact that GDF-15 might be a protective marker of multiple stress pathways in the heart, Kempf, *et al*.[7] reported that the concentration of GDF-15 was increased in chronic HF patients and was closely related to disease severity and

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prognosis. Transforming growth factor (TGF)-β plays an important role in ECM metabolism.[8] Some peptide fragments in the process of precollagen metabolism may be used as a non-invasive method for *in vivo* evaluation of myocardial fibrosis. In this study, we investigated the correlation between GDF-15, a member of the TGF-β cytokine super-family, and the following biomarkers of collagen metabolism: indicators reflecting *in vivo* synthesis and degradation rates of type I collagen, precollagen I N-terminal peptide (PINP) and type I collagen carboxy-terminal peptide (ICTP), and the indicator reflecting transformation (synthesis and degradation) rates of type III collagen, precollagen III N-terminal peptide (PIIINP).[9]

2 Methods

2.1 Study population

A total of 179 participants were recruited from the Department of Cardiology at the Peking University Third Hospital (Beijing, China) between July 2010 to June 2011, including stable angina pectoris patients (AP group, n = 50): patients with coronary atherosclerotic heart disease confirmed by coronary angiography in the absence of myocardial infarction (MI) or HF, old MI patients (OMI group, n = 56); patients with a definite history of MI but no clinical manifestations of HF; patients with a definite history of MI and symptoms of past or present HF (OMI-HF group, n = 38); and healthy volunteers without HF risk factors, cardiac structural lesions or HF symptoms (Control group, n = 35).

Exclusion criteria included acute MI during the preceding 12 weeks, impairment of renal function or liver function, systemic inflammatory diseases, infectious diseases, cancers, acute cerebral infarction and pregnancy; we also excluded users of steroids that might affect the levels of biomarkers.

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval of the Ethics Committee of Peking University. Written informed consent was obtained from all participants.

2.2 Biochemical analysis

Five milliliter blood was drawn from an antecubital vein and transferred to an ethylenediamine tetraacetic acid (EDTA) treated tube in the morning after overnight fasting. Plasma samples were processed within 30 min after collection by centrifugation at 3000 g for 15 min at 4°C. To avoid repeated freeze-and-thaw cycles, each plasma sample was divided into 0.5 mL aliquots and frozen immediately at –80°C. Plasma GDF-15 levels were determined using a commercially available sandwich ELISA kit (R&D Company, USA). Plasma ICTP, PINP and PIIINP levels were determined using a commercially available human sandwich ELISA kit (MyBioSource Company, USA). The samples were analyzed blindly at the Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education using the procedures which totally followed the instructions.

Levels of creatinine, uric acid, white blood cell count and percentage of neutrophils were evaluated at the central chemistry laboratory of Peking University Third Hospital.

2.3 Echocardiography

Echocardiography was performed with the patient lying in the left decubitus position using a GE-VingMedVecho-cardiographic machine (Vivid 7) with a 3.3 MHz multistage array probe. The echocardiography and calculation of different cardiac dimensions and volumes were performed according to the guidelines of the American Society of Echocardiography.

2.4 Statistical analysis

The Kolmogorov-Smirnov test was used to test for normal distribution of continuous variables. Data were expressed as mean ± SD in continuous variables of normal distribution, median ± quartile ranges (QR) in abnormal distribution and percentage, or proportion in categorical variables as appropriate. The Student's t test was used to compare means between two groups, and one-way ANOVA for muti-group comparisons. Proportions were compared by chi-square statistics. Parametric correlation was calculated using Pearson correlation coefficient and nonparametric correlation using the Spearman test. Multivariate linear regression analysis was used to identify factors that were independently associated with GDF-15 concentration. All analyses were conducted using SPSS 17.0 (SPSS Inc, Chicago, IL, USA) with two tailed alpha = 0.05.

3 Results

3.1 Patients' clinical and biochemical characteristics parameters

Compared with the other three groups, the OMI-HF group was substantially older, and had higher plasma uric acid, N-terminal B-type pronatriuretic peptide (NT-proBNP), glycosylated hemoglobin (HbA1C) levels and lower creatinine clearance. The OMI and OMI-HF groups had more smokers (Table 1).

3.2 The correlation between clinical Groups and GDF-15 and collagen metabolism indicators level

The plasma GDF-15 levels were higher in the OMI-HF group (1373.4 ± 275.4 ng/L) than OMI group, AP group and Control group (1036.1 ± 248.6 ng/L, 784.6 ± 222.4

ng/L, and 483.8 ± 186.4 ng/L, respectively; $P < 0.001$) (Figure 1A). Plasma concentrations of ICTP were higher in the OMI-HF and OMI groups than the AP and Control groups (3.03 ± 1.02 μg/L & 2.75 ± 1.47 μg/L *vs*. 2.02 ± 0.69 μg/L & 2.08 ± 0.95 μg/L, $P < 0.01$) (Figure 1B). PINP and PIIINP were elevated in the OMI-HF group compared with Control group (22.2 ± 6.6 μg/L *vs*. 16.7 ± 5.1 μg/L and 13.2 ± 7.9 μg/L *vs*. 6.4 ± 2.1 μg/L, $P < 0.01$) (Figure 1 C, D); No significant differences were found among other groups.

3.3 The correlation between GDF-15 and indicators of collagen metabolism

We found significant correlation between GDF-15 and ICTP ($r = 0.302$, $P < 0.001$) or PIIINP ($r = 0.206$, $P = 0.006$) (Figure 2); but the correlation between GDF-15 and PINP was not statistically significant.

Table 1. Clinical and Biochemical characteristics of the patients.

Parameters	Control group ($n = 35$)	AP group ($n = 50$)	OMI group ($n = 56$)	OMI-HF group ($n = 38$)	*P*-value
Clinical characteristics					
Sex, M/F	21/14	28/22	36/20	20/18	0.321
Age, yrs	60.8 ± 10.3	66.0 ± 8.1	61.7 ± 9.7	70.3 ± 11.0[a]	0.005
Smoking, %	-	20.0	76.8 [b]	73.7[b]	0.000
Hypertension, %	-	76.0	71.4	76.3	0.344
Hyperlipidemia, %	-	60.0	57.1	39.5	0.140
Diabetes mellitus, %	-	54.0	35.7	44.7	0.202
Biochemical characteristics					
White blood cell ($\times 10^9$/L)	6.27 ± 1.17	6.11 ± 1.10	6.86 ± 1.73	6.18 ± 0.99	0.055
cCr, mL/min	66.4 ± 14.7	82.1 ± 41.0	67.1 ± 21.2	52.9 ± 17.2[c]	0.001
UA, μmol/L	328.5 ± 61.6	330.3 ± 88.3	363.9 ± 60.1	411.2 ± 131.6[a]	0.001
TC, mmol/L	4.41 ± 0.94	4.33 ± 0.92	4.13 ± 1.28	3.93 ± 0.90	0.131
HbA1C, %	5.5 ± 0.2	6.6 ± 1.4	6.1 ± 0.4	7.6 ± 1.3[a]	0.000
*NT-proBNP, pg/mL	-	61.5 (24.1–191.8)	537.6 (262.7–1224.2)	1863.0 (912.2–2442.5)[a]	0.000

Data are presented as mean ± SD or percent unless other indicated. *Data are expressed as median (1/4 quartile ranges-3/4 quartile ranges) in abnormal distribution. [a]OMI-HF group was significantly higher than the other three in terms of age, uric acid, NT-proBNP, HbA1C; [b]OMI & OMI-HF groups had more smokers; and [c]OMI-HF group had lower levels in terms of creatinine clearance. There was no significant difference in other terms. AP: stable angina pectoris; cCr: creatinine clearance; HbAlC: Glycosylated hemoglobin; HF: heart failure; NT-proBNP: N-terminal B-type pronatriuretic peptide; OMI: old myocardial infarction; TC: total cholesterol; UA: uric acid.

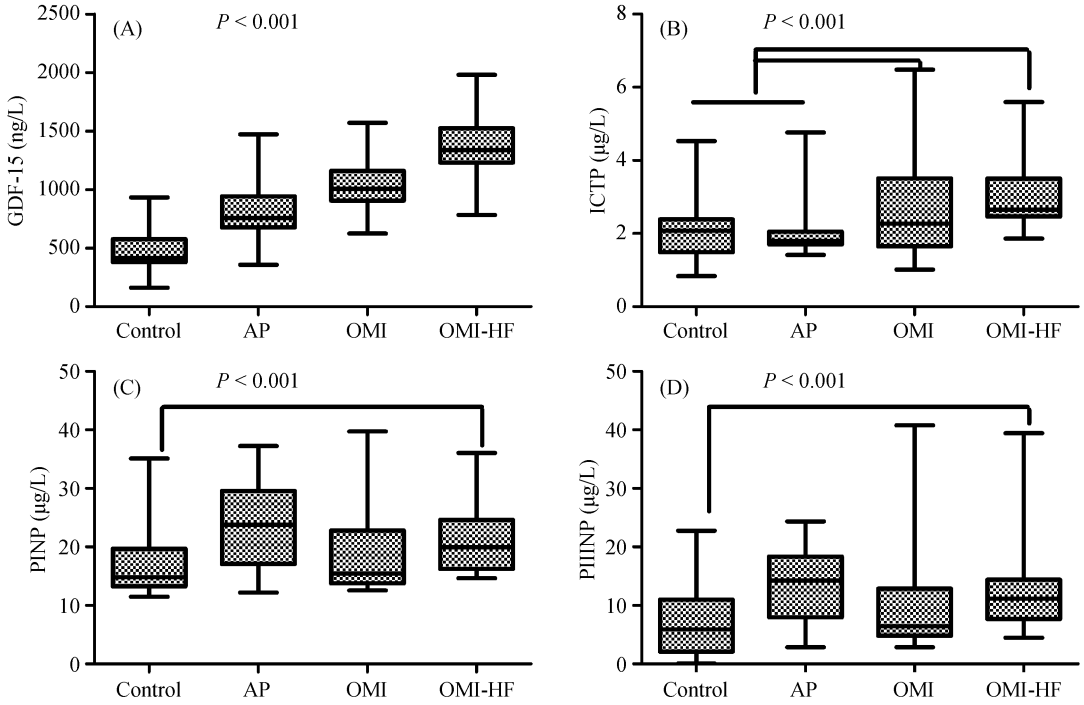

Figure 1. GDF-15 and levels of collagen metabolism indicators in clinical groups. AP: stable angina pectoris; GDF: Growth differentiation factor; ICTP: type I collagen carboxy-terminal peptide; OMI: old myocardial infarction; OMI-HF: old myocardial infarction with symptoms of past or present heart failure; PINP: precollagen I N-terminal peptide; PIIINP: precollagen III N-terminal peptide.

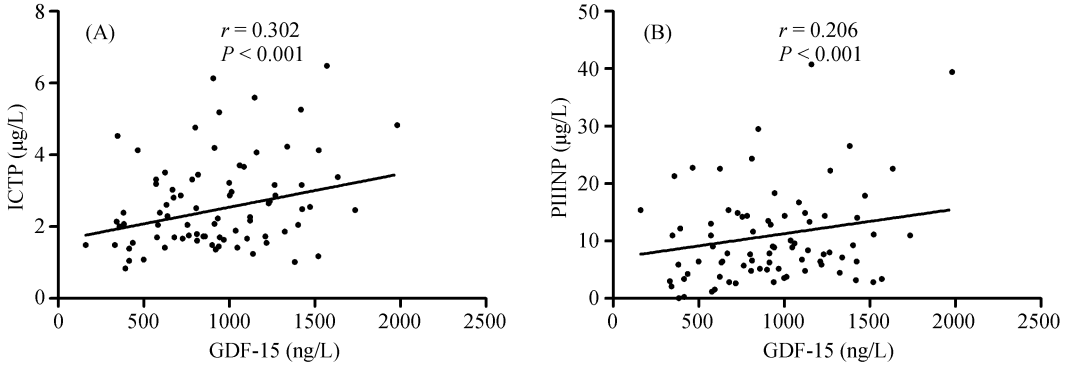

Figure 2. The correlation between GDF-15 and collagen metabolism indicators. Plasma GDF-15 level was positively correlated to ICTP (A) and PIIINP (B). GDF: growth differentiation factor; ICTP: type I collagen carboxy-terminal peptide; PIIINP: precollagen III N-terminal peptide.

3.4 The correlation between echocardiography functional indicators and GDF-15 & collagen metabolism indicators level

Among the echocardiography functional indicators, GDF-15 positively correlated with E/Em and left atrial pressure, but inversely correlated to LVEF and the average of peak systolic myocardial velocities (Sm). ICTP, PINP and PIIINP was inversely correlated to systolic function LVEF or Sm, but have no correlation with diastolic function E/Em and left atrial pressure (Table 2).

4 Discussion

In a cross-sectional study, we found a significant correlation between GDF-15 and HF stages.[2] Furthermore, the higher levels of indicators of collagen metabolism in old MI with HF patients and both ICTP and PIIINP were positively correlated to GDF-15. As a non-invasive method for *in vivo* evaluation of myocardial fibrosis, there is no prior study on the correlation between GDF-15 and ECM collagen metabolism in HF patients. Current data in our study suggests that GDF-15 maybe involved in the ECM collagen metabolic process, whereas the mechanism studies, such as more relative genes expression profile during the process, should be included in the future studies.

Table 2. The correlations between GDF-15, indicators of collagen metabolism and echocardiography functional indicators.

Parameters	GDF-15, ng/L	ICTP, μg/L	PIIINP, μg/L	PINP, μg/L
Systolic characteristics				
LVEF	–0.623**	–0.269**	–0.220**	-
Sm	–0.365**	-	–0.270**	–0.256**
Diastolic characteristics				
E/Em	0.349**	-	-	-
LAP	0.358**	-	-	-

E: flow velocities from early peak trans mitral; Em: myocardial peak early diastolic mitral annular velocities; GDF: Growth differentiation factor; ICTP: type I collagen carboxy-terminal peptide; LAP: left atrial pressure; LVEF: left ventricular ejection fraction; PINP: precollagen I N-terminal peptide; PIIINP: precollagen III N-terminal peptide; Sm: myocardial peak systolic mitral annular velocities, $^{**}P < 0.01$.

Highly expressed in myocardial tissue, collagen is the principal component in the cardiac collagen network.[10] Some fragments containing amino- or carboxy-terminals of collagen protein pre-peptides are released into blood during the process of biosynthesis and degradation.[11] Circulating levels of several components of collagen peptide fragments can be reliably determined: PINP is an amino-terminal fragment and reflects the synthesis rate of type I collagen; ICTP reflects the *in vivo* degradation rate of type I collagen; PIIINP, on the other hand, reflects the synthesis and degradation of type III collagen.[12] In animal experiments, these biomarkers of collagen metabolism are significantly correlated to the degree of myocardial fibrosis;[13] in humans, immunohistochemical evaluation of tissues obtained from heart transplantation or interventricular septum biopsy showed that circulatory biomarkers may be used as a non-invasive method for evaluating *in vivo* myocardial fibrosis.[14,15]

Collagen synthesis-degradation disequilibrium will result in changes of the ECM skeleton structure of HF patients.[16] In this study, we found that plasma levels of ICTP, PINP and PIIINP were all increased in the OMI-HF group compared to Control group. The results indicated that, in patients with old MI-HF, both synthesis and degradation rates of type I and type III collagen are elevated. The increased levels of plasma ICTP, PINP and PIIINP were significantly correlated with left ventricular systolic hypofunction. Graham's study on the progression from compensatory myocardial hypertrophy to symptomatic HF in rats discovered

that the myocardial collagen of rats in the stage of symptomatic HF were decreased and the collagen reduction was a crucial step in the progression to left ventricular dilatation and clinical HF.[17]

ECM is a dynamic microenvironment and the principal contributor to post-MI ventricular remodeling. In the following stage of HF progression, the plasma GDF-15 level also increased accordingly. The TGF-β family consisted of more than 30 structural-related proteins with multiple cellular functions which regulate wound repair and tissue fibrosis.[18] In the cardiovascular system, TGF-β plays an important role in ECM metabolism, and not only regulates the members in the ECM network, but also controls the expression of collagenase inhibitors (e.g., TIMPs) which inhibit the degradation of collagen in ECM.[19] GDF-15, as a member in the GDF family, was identified to have protective effects on anti-apoptosis and anti-hypertrophy in animal models; However, its role in myocardial fibrosis has rarely been mentioned. Bjornstad, *et al*.[20] reported that plasma GDF-15, matrix metalloproteinase (MMP)-3 and MMP-9 are all increased in two days after aortic valve replacement, indicating GDF-15 and MMPs may play a certain role in reversing ventricular remodeling. Interestingly, we found the level of GDF-15 is related to ICTP and PIIINP content. As we know, myocardial fibrosis as the principal determinant of diastolic function insufficiency and pumping function impairment, may lead to arrhythmia if the conducting system of the cardiac structure is affected, thus gradually resulting in HF and sudden death. In the current study, GDF-15 was correlated with diastolic function indicators such as E/Em, and LAP.

We conclude that GDF-15 might play a role in the metabolism of type I and type III collagen, particularly in collagen degradation. It also corresponds to its biological activities in preventing hypertrophy and apoptosis in animal experiments. However, collagen metabolism is a complicated dynamic process, and the interaction between GDF-15 and collagen deposition during HF progression is yet to be understood. Myocardial fibrosis is related to the impaired cardiac function and poor prognosis, while collagen deposition may affect the cardiac function through different pathways.

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