



Pericardial fluid levels of growth differentiation factor 15 in patients with or without coronary artery disease: a prospective study

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Background: Growth differentiation factor 15 (GDF15) has already been reported as a novel efficient biomarker in patients with coronary artery diseases (CAD). However, very little is demonstrated about the potential impact of pericardial fluid GDF-15 accumulation on CAD. The aim of this study was to evaluate pericardial fluid and plasma GDF15 levels in patients with ischemic heart disease.

Methods: In this study, 42 consecutive patients (21 patients with significant CAD; 21 patients without CAD) undergoing open heart surgery were recruited in this study. Pericardial fluid were obtained at the time of surgery, and GDF15 levels in the samples were measured by enzyme-linked immunosorbent assay. Plasma glucose, creatinine, CK-MB, cTnI and N-terminal pro-B-type natriuretic peptide (NT-proBNP) measurements were performed.

Results: The plasma GDF15 levels were markedly higher than the pericardial fluid levels both in the CAD group and non-CAD group ($1,174.0 \pm 148.7$ vs. 677.8 ± 77.2 pg/mL, $P < 0.01$; 925.8 ± 127.4 vs. 617.4 ± 76.2 pg/mL, $P < 0.01$). The levels of pericardial fluid GDF15, was not statistically different between the CAD and non-CAD groups ($P > 0.05$). An obvious correlation was observed between plasma and pericardial fluid GDF15 concentration both in the CAD group and non-CAD group ($R = 0.53$, $P < 0.01$; $R = 0.54$, $P < 0.01$). An obvious positive correlation was found between pericardial fluid GDF15 and plasma creatinine levels in CAD patients but not in non-CAD patients ($R = 0.65$, $P < 0.01$). In the CAD group, an obvious correlation was also observed between pericardial fluid GDF15 levels and NT-ProBNP ($R = 0.63$, $P < 0.01$), while no relationship was found in non-CAD group. There was a positive correlation between pericardial fluid GDF15 and LVEF in non-CAD group but not in CAD group patients ($R = -0.44$, $P < 0.05$).

Conclusions: Our study first revealed an association between pericardial fluid GDF15 and baseline characteristics. Pericardial fluid GDF15 levels are associated with cardiac and kidney function in patients with coronary artery disease and may be a valuable marker for assessing CAD severity and predicting its complications.

Keywords: Pericardial fluid; growth differentiation factor-15 (GDF15); coronary artery disease; heart failure

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Introduction

Growth-differentiation factor-15 (GDF-15) was reported to be a novel transforming growth factor- β superfamily member (1). Elevated plasma levels of GDF-15 is associated with elevated clinical non-cardiovascular and cardiovascular morbidity; it plays a vital role in progression of heart diseases such as atrial fibrillation, coronary artery diseases and heart failure (2). The GDF15 protein is upregulated as part of the circulating anti-inflammatory cytokines within the cardiomyocyte in response to ischemia and injury (3). It is weakly expressed by cardiomyocytes under normal environmental and physiological conditions (4). In response to ischemia and oxidative stress, there is a significant rise in the expression level of GDF-15 (5). Therefore, it seems that this biomarker is of great importance in Coronary artery disease, which is involved with these above mechanisms (6).

Coronary artery disease (CAD) is the major cause of mortality in the society with elevating incidence in recent years. The pathological processes include chronic inflammation, endothelial dysfunction, oxidative stress and vascular calcification which together contribute to plaque formation, ending up with a cardiovascular event (7). It is a combination of different syndromes including acute coronary syndrome (ACS) and stable angina, arrhythmia, and heart failure (8).

Based on the data and information of several clinical trials, GDF-15 could be considered as a reliable biomarker in the examination of cardiac disease (9-11). It has a strong prognostic significance in predicting heart failure, coronary artery disease and atrial fibrillation (12).

Pericardium is well known for its protective content and contains a mixture of fluid which provides typically a microenvironment with proangiogenic cytokines secreted by cardiomyocytes (13). Studies with pericardial fluid are scarce mainly due to the fact that collecting pericardial fluid samples is of great difficulty (14). Pericardial fluid can be harvested by heart surgery. It can be a window for evaluating the pathophysiological processes of the heart (15). Mounting evidence suggests that vasoactive and cardioactive agents exist at high levels in the human pericardial fluid (16-18). However, to date, no analyzed data exist regarding the pericardial fluid GDF-15 in clinical patients with heart disease. Our study was aimed to make an evaluation on the pericardial fluid GDF-15 levels and their clinical diagnostic validity in the patients with or without ischemic heart disease, and further make an assessment on the relationship between pericardial GDF-15 and baseline biochemical

variables and echocardiography parameters.

Methods

Patients population

A total of 42 patients participated in this observational, prospective clinical study. Patients undergoing open heart surgery in our Cardiovascular Surgery were consecutively registered. Patients were excluded as follows: primary and secondary cardiomyopathy, age <18 or >80 years, cardiac dilation and low LVEF (<45%), emergency operation, acute myocardial infarction within 30 days, malignant tumor, autoimmune disease or inflammatory disease.

Clinical data collection

Based on the clinical medical records, patients' clinical data was collected comprehensively, such as sex, age, cardiovascular risk factors, and the LVEF parameters of transthoracic echocardiography. LVEF is a parameter of the percentage of blood ejecting the heart each time it contracts and was defined by the American Heart Association as a measurement of heart failure. From short-axis imaging, M-mode left ventricular ejection fraction based on the cubed method was equal to (end-diastolic volume end-systolic volume)/end-systolic volume.

Blood and pericardial fluid Biochemical analyses

The blood samples of enrolled patients were taken as the baseline reference on the second day after admission to the hospital. Many measurements were performed, including blood glucose, creatinine, CK-MB, cTnI and NT-proBNP. Blood collected from patients was stored in an anticoagulant tube. During the open heart procedure, following incision of the pericardium, undiluted pericardial fluid was immediately obtained prior to heparinization. All suspected blood-polluted pericardial fluid samples were excluded. Blood and pericardial fluid samples were centrifuged at 3,000 r/min for 15min and the supernatant were stored at -80°C until analysis. Commercially available instruments and kits were used according to instructions

from the manufacturer. Biochemical measurements were performed using standard laboratory techniques. Plasma and pericardial fluid GDF15 concentrations were determined by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, USA). The color

Table 1 Demographic and perioperative characteristics of enrolled patients

Baseline parameter	CAD	Non-CAD	P value
Age, years	68.9±1.3	54.95±3.6	0.087
Gender, male	13 (61.9)	15 (71.4)	0.721
hypertension	11 (52.4)	9(42.9)	0.325
Diabetes mellitus	14 (28.6)	10 (47.6)	0.337
NYHA	2 (2–3)	2 (2–3)	0.582
Preoperative LVEF (%)	56.3±2.3	60.9±1.5	0.426
Plasma creatinine, mmol/L	86.4±6.6	83.4±4.9	0.083
Hemoglobin, g/L	128.1±2.7	133.0±3.8	0.142
NT-proBNP, ng/L	864.4±240.0	1,169.0±337.7	0.097
Troponin I, ng/mL	0.01 (0.01–0.45)	0.01 (0.01–0.49)	0.453
Creatine kinase MB, U/L	1.8±0.2	1.2±0.1	0.326

Data are presented as n (%), median (interquartile range) and mean ± SEM. NYHA, New York Heart Association grade for heart failure; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

intensity was measured with a multiwell spectrophotometer (BioTek, Winooski, VT, USA) at 450 nm.

Statistical analyses

Experimental data were analyzed by statistical software GraphPad Prism 7.0 (California, USA) and SPSS version 22.0 (Chicago, Illinois, USA). All of the continuous variables were shown as means ± Standard Error of Mean (SEM); Normality was tested using the Kolmogorov-Smirnov test. In all of the tests, a double-sided value of $P < 0.05$ was considered to be statistically significant. Spearman's correlation test was used to calculate the associations between pericardial GDF15 and other measured clinical laboratory parameters.

Results

Clinical characteristics of patients

Clinical and preoperative biochemical laboratory data of the 42 enrolled patients are listed in *Table 1*. The demographics and clinical presentations among patients in two groups are

summarized. There was no statistically significant difference in clinical baseline presentations between the CAD and non-CAD group patients.

Pericardial fluid and plasma GDF15 levels in patients with or without CAD

Mean pericardial fluid GDF-15 levels were 677.8 ± 77.2 and 617.4 ± 76.2 pg/mL in CAD and non-CAD group respectively. Mean plasma GDF-15 levels were 1174.0 ± 148.7 and 925.8 ± 127.4 pg/mL in CAD and non-CAD Group respectively. As illustrated in *Figure 1A*, the concentration of GDF15 in plasma was significantly higher than the pericardial fluid GDF15 levels both in the CAD and non-CAD group ($P < 0.01$). The pericardial fluid GDF15 levels was similar in both groups ($P > 0.05$). Spearman's correlation test was used to calculate the associations between pericardial GDF15 and other measured biochemical parameters. A positive correlation was found between pericardial fluid and plasma GDF15 concentrations in CAD group patients ($R = 0.53$, $P < 0.01$, *Figure 1B*). An obvious correlation was also observed between plasma and pericardial fluid GDF15 concentration in non-CAD patients ($R = 0.54$, $P < 0.01$, *Figure 1C*).

Associations between pericardial fluid GDF15 levels and baseline characteristics in CAD patients

Associations between laboratory parameters and pericardial fluid GDF15 concentrations in CAD patients was tested by Spearman's correlation test. A significant correlation was found between pericardial fluid GDF15 and circulating creatinine concentrations in CAD group patients ($R = 0.65$, $P < 0.01$, *Figure 2A*). Furthermore, a significant correlation was found between pericardial fluid and plasma NT-ProBNP levels in CAD patients ($R = 0.63$, $P < 0.01$, *Figure 2B*). However, no significant correlation was found between the pericardial fluid GDF15 and cTnI ($R = -0.26$, $P > 0.05$).

Relationship between pericardial fluid GDF15 levels and baseline characteristics in non-CAD patients

To document a relationship between pericardial fluid GDF15 concentrations and heart failure, we correlated LVEF with pericardial fluid GDF15 concentrations. In the CAD patients, correlation was not observed between pericardial fluid GDF15 levels and LVEF ($R = -0.22$, $P > 0.05$). It is interesting that correlation was not observed between

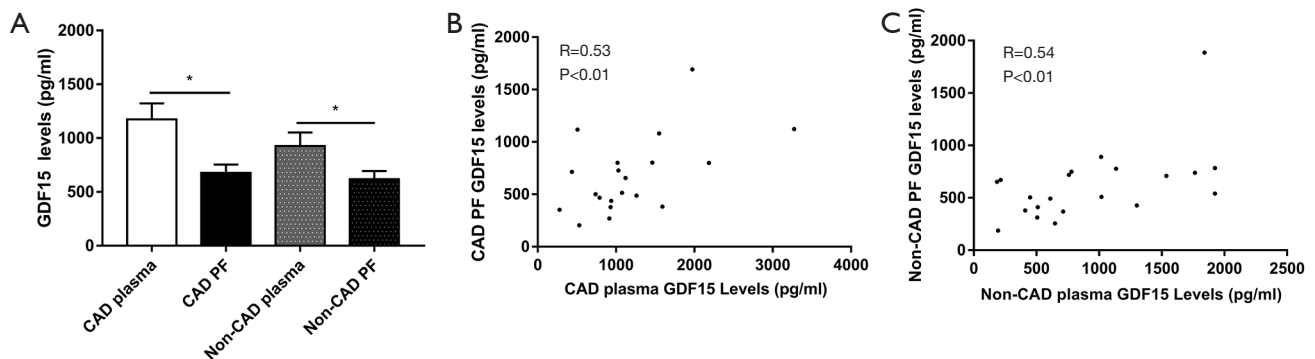


Figure 1 Pericardial fluid and plasma GDF15 levels in patients with or without coronary artery disease. The levels of GDF15 in plasma was significantly higher than the pericardial fluid both in the CAD and non-CAD group, *, $P < 0.01$ (A). An obvious correlation was observed between plasma and pericardial fluid GDF15 concentration both in the CAD group ($R = 0.53$, $P < 0.01$) (B). A positive correlation was observed between plasma and pericardial fluid GDF15 concentration both in the non-CAD group ($R = 0.54$, $P < 0.01$) (C).

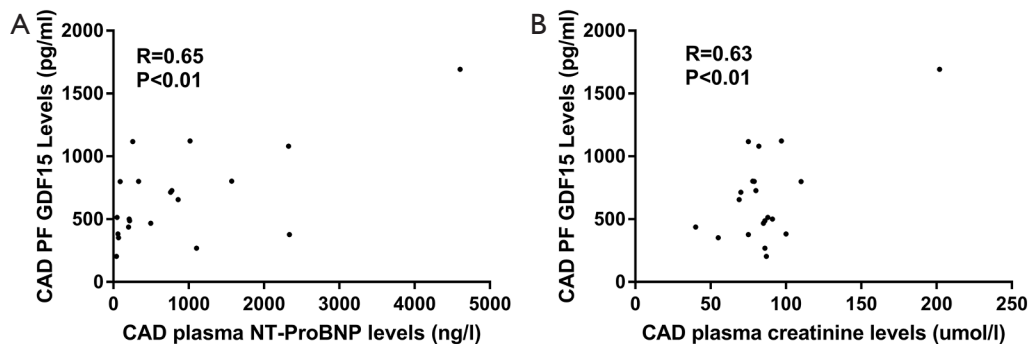


Figure 2 Associations between pericardial fluid GDF15 levels and baseline characteristics in CAD patients. There was a positive significant association between pericardial fluid GDF15 and plasma creatinine concentrations in CAD patients ($R = 0.65$, $P < 0.01$) (A). A significant correlation was found between pericardial fluid and plasma NT-ProBNP levels in patients with CAD ($R = 0.63$, $P < 0.01$) (B).

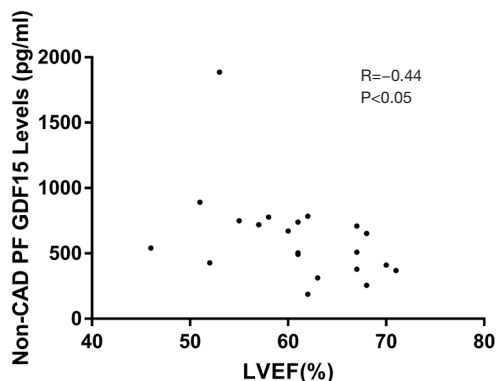


Figure 3 Relationship between pericardial fluid GDF15 levels and baseline characteristics in non-CAD patients. An obvious significant association was found between the pericardial fluid GDF15 levels and LVEF ($R = -0.44$; $P < 0.05$).

pericardial fluid GDF15 levels and plasma NT-ProBNP in non-CAD patients ($R = 0.36$, $R > 0.05$). Furthermore, an obvious significant association was observed between the pericardial fluid GDF15 levels and LVEF in non-CAD patients ($R = -0.44$; $P < 0.05$, *Figure 3*). The pericardial fluid GDF15 concentrations reflected the cardiac function as evaluated by LVEF in non-CAD patients.

Discussion

The heart tissue produces many physiologically active substances such as cardiac derived hormones, cytokines and growth factors (19). It is not completely understood that how the mechanisms work when it is involved with substances synthesis, secretion, and metabolism (20). Currently, it has not been emphasized that which is the

prominent pathway for the released substances from the heart (19,21). The traditional biochemical analysis of human PF showed that small molecules are similarly concentrated compared to plasma. However, some researchers revealing that PF is not only a simply ultrafiltrate of plasma and have potential diagnostic value for heart diseases (22).

This study demonstrated that the plasma GDF15 concentration was dramatically higher than the pericardial fluid in patients with or without coronary artery disease. Moreover, GDF levels in pericardial fluid were not significantly different between patients with ischemic and nonischemic heart disease. It revealed that we cannot distinguish patients with from those without CAD by pericardial fluid GDF15 only.

Biochemical markers are valuable and give prognostic information to cardiologists, including an established marker troponin and newer markers which are now finding their way into clinical decision-making (23). Some clinical research literatures also shown that plasma GDF15 has a significant association with myocardial injury (12,24,25). GDF15 has been well known to be elevated in patients with myocardial infarction and be a valuable biomarker of outcome in two clinical trials including patients with AMI (26,27). Our earlier study has reported that circulating GDF-15 can act as an effective biomarker for postoperative myocardial infarction in patients undergoing off pump CABG procedure (28).

To further demonstrate a correlation between pericardial fluid GDF15 levels and plasma biomarkers, in this study, we correlated baseline characteristics with pericardial fluid GDF15 levels. There was an obvious correlation between plasma and pericardial fluid GDF15 concentration both in the CAD and non-CAD group. This study is the first time to reveal pericardial fluid GDF15 in patients with heart disease. An obvious correlation was observed between pericardial fluid GDF15 levels and plasma NT-ProBNP in CAD patients. A positive correlation was also found between pericardial fluid GDF15 and plasma creatinine concentrations in CAD patients but not in non-CAD Patients. To our knowledge, these finding first reported GDF15 levels of pericardial fluid is associated with cardiac function and kidney function in patients with CAD.

It has been reported that the change of GDF15 levels could reveal both pathophysiological processes, as well cardiovascular inflammation (29). It is also demonstrated that higher plasma GDF15 concentrations in patients with chronic heart failure (30). GDF15 is induced in hypertrophic and dilated cardiomyopathy after volume

overload, ischemia, and heart failure (30).

In the present study, even no obvious correlation was observed between pericardial fluid GDF15 levels and plasma NT-ProBNP in non-CAD patients, there was a positive correlation between pericardial fluid GDF15 and LVEF. We propose that it is valuable to identify and measure pericardial fluid GDF15 levels combined with traditional markers as a novel method to improve the accuracy of the traditional marker approach in future.

A few limitations deserve mention in this study. First of all, only the pericardial fluid GDF15 concentrations and clinical parameters were analyzed in this study, so to get the experimental data in a deeper level is very necessary when exploring the biological changes of pericardial fluid. Secondly, a relatively small sample size may decrease and fade the significance of correlation in this study. Thus, it is essential to have a wider range of participants join the study to verify that pericardial fluid GDF15 can be used as a clinical indicator for cardiac dysfunction.

Conclusions

This is the first time to demonstrate that the levels of GDF15 in plasma was significantly higher than the pericardial fluid both in the CAD and non-CAD group. In some degree, pericardial fluid GDF15 cannot distinguish patients with from those without CAD. Pericardial fluid GDF15 levels is associated with cardiac and kidney function in patients with coronary artery disease and might serve as a clinically validated role for predicting the complication and severity of CAD.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was

approved by the independent Medical Ethics Committee of the Ruijin Hospital, Shanghai Jiaotong University School of Medicine (2016095).

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