Low plasma growth/differentiation factor 1 levels are associated with liver fibrosis in patients with stable angina

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Abstract

Background: Growth differentiation factor 1 (GDF1) is a member of the transforming growth factor-β (TGF-β) superfamily and a protective mediator against the development of post-infarction cardiac remodeling by negatively regulating MEK-ERK1/2 and Smad signaling pathways in the heart. The TGF-β/SMAD pathway has been shown to play a key role in the development of hepatic fibrosis. In addition, fatty liver disease has been associated with reduced MEK/ERK1/2 signaling. However, no previous study has investigated the association between GDF1 and liver fibrosis. Therefore, the aim of this study was to investigate the association between plasma GDF1 and liver fibrosis in patients with stable angina.

Methods: We included 327 consecutive patients with stable angina. ELISA was used to measure circulating levels of GDF1, and the fibrosis-4 index was used to assess liver fibrosis.

Results: The advanced liver fibrosis group had lower median plasma GDF1 levels than those with minimal liver fibrosis. There was a significant negative association between...
1 | INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, and is precipitated by cardiometabolic risk factors including chronic liver disease, diabetes, metabolic syndrome, and chronic renal disease. Chronic liver disease is a progressive deterioration of liver function, and the most common causes are hepatitis, alcohol abuse, and nonalcoholic fatty liver disease (NAFLD). NAFLD is common in patients with CVD, and both diseases share common risks factor such as high cholesterol intake, alcohol consumption, diabetes and other metabolic abnormalities. In addition, recent studies have reported associations between liver fibrosis, which is the irreversible progression of NAFLD, and coronary artery disease (CAD), cardiac arrhythmias, and impaired heart function. Furthermore, previous study also revealed that higher liver fibrosis scores are associated with increased risks of cardiovascular and all-cause mortality among patients with CAD. Liver fibrosis scores might play a potential role in CAD prognosis prediction. However, the underlying mechanisms and interactions remain unknown.

Growth differentiation factors (GDFs) are proteins which belong to the transforming growth factor-β (TGF-β) superfamily, and 15 GDFs have been identified to date (GDF1 to GDF15). The TGF-β superfamily comprised structurally related polypeptides which have been shown to regulate cell differentiation and growth in various adult and embryonic tissues. GDF1 has been shown to be specifically expressed in the nervous systems of adult mice and late-stage embryos. A previous study showed that GDF1 had favorable effects on vascular endothelial function, sodium excretion, and post-infarction cardiac remodeling in an animal study and also in patients with type 2 diabetes and normal individuals. The underlying mechanism is believed to be through the negative regulation of MEK-ERK1/2 and Smad signaling pathways. Interestingly, the TGF-β/SMAD pathway has also been found to play a key role in the development of hepatic fibrosis in subjects with fatty liver disease via suppression of the MEK/ERK1/2 signaling pathway. However, few studies have investigated the relationship between plasma GDF1 level and liver fibrosis in a cohort of Taiwanese patients with stable angina.

2 | MATERIALS AND METHODS

2.1 | Patient population

We enrolled a total of 327 patients with stable angina from July 2012 to December 2021 who visited the cardiovascular clinic at E-Da Hospital. The inclusion criteria were patients: (1) with stable angina pectoris, defined as effort-related chest pain without evidence of recent deterioration or rest pain in the previous 6 months, and diagnosed by the cardiologist in charge as previously described; and (2) who underwent a successful percutaneous coronary intervention, defined as <30% residual stenosis in a final angiogram as assessed using quantitative coronary angiography with no occlusion of the large branch (>1 mm) or dissection limiting flow, and Thrombolysis in Myocardial Infarction grade 3. The exclusion criteria were patients: (1) who used steroids; (2) had inflammatory diseases (such as infection/sepsis), malignancy, liver diseases, and collagen diseases; (3) with a history of psychosis; and (4) with heart valve disease, myocardial infarction, or had undergone heart surgery. In addition, we also excluded patients who were unable or unwilling to provide informed consent. All study patients lived in the same area during the study period, and they were all of Han Chinese ethnicity. Each patient provided written informed consent before being enrolled into the study. This cross-sectional study was approved by the Human Research Ethics Committee of E-Da Hospital.

2.2 | Baseline data collection

A detailed interview was conducted with each patient about their medical and personal history, as well as their demographic characteristics before the coronary angiography examination. We classified smoking status as follows: never smokers, former smokers (those who had stopped smoking for ≥1 year), or current smokers.
We grouped the current and former smokers in the analysis and compared them with the never smokers. A trained nurse performed all blood pressure readings with the patients seated using an automated blood pressure monitor (HEM-907; Omron) after 5 min of rest. Anthropometric parameters including body mass index (BMI) and waist circumference were also recorded.

**2.3 | Laboratory measurements**

Fasting (8 h) peripheral blood samples were obtained from the antecubital vein before the patients underwent coronary angiography. Levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, plasma triglycerides, serum glucose, serum albumin, serum creatinine, and complete blood cell count were measured in all patients using a parallel, multi-channel analyzer (Hitachi 7170A) as described previously.\(^{17,18}\) We used high-performance liquid chromatography (Tosoh Automated Glycohemoglobin Analyzer, HLC-723G8) to measure hemoglobin A1c (HbA1c). In addition, we measured levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following the methods of the Japan Society of Clinical Chemistry (“Hitachi” Discrete photometric chemistry analyzer for clinical use, LAbOSPECT 008AS), which is compatible to the methods by the International Federation of Clinical Chemistry. Serum creatinine level was measured using the Jaffe method, and estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation within 3–6 months of admission.\(^{19}\) The fibrosis-4 (FIB-4) index was calculated as: FIB-4 = age (years) x AST (IU/L)/platelet count (10\(^9\)/L) x √ALT (IU/L).\(^{20}\) The aspartate transaminase/platelet ratio index (APRI) was calculated as: APRI = AST/upper limit of normal of AST/platelet count x 100 (where the upper limit of normal of AST = 40 IU/L).

**2.4 | Measurements of plasma GDF1 and high-sensitive C-reactive protein (hs-CRP)**

After obtaining overnight fasting blood samples, the plasma was kept at –80°C until assay. We used a commercial ELISA kit to measure concentrations of plasma GDF1 (Cloud-Clone Corp.). The standard and dilution curves were parallel, and the inter-assay and intra-assay coefficients of variation for the assay were <12% and <10%, respectively (both n = 3). Plasma levels of hs-CRP were measured using an immunochemistry system (Beckman Coulter IMMAGE), which had a detection of 0.2 mg/L. All measurements were made in duplicate during a single experiment.

**2.5 | Definitions**

We used the World Health Organization criteria to define type 2 diabetes; as receiving medical therapy for diabetes and/or a history of type 2 diabetes.\(^{21}\) Patients with a systolic/diastolic blood pressure (SBP/DBP) of ≥140/90 mmHg, or a prescription for antihypertensive drugs were defined as having hypertension. We used the Adult Treatment Panel III criteria\(^{22}\) to define hyperlipidemia as: elevated total cholesterol (≥200 mg/dl), and/or elevated LDL-C (≥130 mg/dl), and/or low HDL-C (<35 mg/dl in men or <39 mg/dl in women), and/or elevated triglycerides (≥150 mg/dl), or being treated for a lipid disorder. Liver fibrosis was defined according to FIB-4 index categories as: minimal fibrosis (FIB-4 < 1.45), moderate fibrosis (FIB-4 1.45–3.25), and advanced fibrosis (FIB-4 > 3.25).\(^{20,23,24}\)

**2.6 | Statistical analysis**

Continuous data are presented as mean ± SD or median (interquartile range) as appropriate. Between-group differences were analyzed with one-way ANOVA for normally distributed variables followed by Tukey’s pairwise test. Categorical data are presented as frequency and percentage, and between-group comparisons were analyzed using the chi-square test. Because the distributions of serum AST, ALT, triglycerides, creatinine, plasma hs-CRP, and GDF1 values were skewed, we used logarithmically transformed values in the analysis. The association between GDF1 and liver fibrosis was evaluated using multivariate logistic regression models: (1) GDF1 and age; (2) GDF1, age, and sex; (3) GDF1, age, sex, and BMI; (4) GDF1, age, sex, BMI, and ALT; (5) GDF1, age, sex, BMI, ALT, hemoglobin, and albumin; and (6) GDF1, age, sex, BMI, ALT, hemoglobin, albumin, creatinine, and hs-CRP. We then classified the concentration of GDF1 into tertiles, and analyzed trends among the tertiles using general linear and logistic regression analyses. Using the highest tertile as the reference, we also estimated the odds ratio (OR) with 95% confidence interval (CI) of liver fibrosis in each tertile.

Associations between plasma GDF1 concentration and other variables were analyzed using simple and multiple linear regression analyses. In addition, the values of FIB-4 index, AST/ALT ratio and APRI in each GDF1 concentration tertile were analyzed for trends. All tests were 2-sided, and a p value <0.05 was considered to be statistically significant. All statistical analyses were performed using JMP version 7.0 for Windows (SAS Institute).

**3 | RESULTS**

**3.1 | Characteristics of the patients by liver fibrosis category**

Table 1 shows the biochemical and clinical characteristics of the 327 enrolled patients according to liver fibrosis category. The prevalence rates of minimal liver fibrosis (FIB-4 < 1.45), moderate liver fibrosis (FIB-4 1.45–3.25), and advanced liver fibrosis (FIB-4 > 3.25) were 24.5%, 43.1%, and 32.4%, respectively. The advanced liver fibrosis group were older, had higher levels of AST, blood urea nitrogen, and
Furthermore, the advanced liver fibrosis group had higher ALT, white hemoglobin than those with minimal and moderate liver fibrosis. Moreover, the advanced liver fibrosis group had a lower triglyceride level than the moderate liver fibrosis group. No significant differences were found in hypertension, male sex, hyperlipidemia, current smoking, diabetes mellitus, DBP, SBP, fasting glucose, HbA1c, total cholesterol, LDL-C, HDL-C, Gensini score or number of diseased coronary arteries among the three groups.

### 3.2 Association between plasma GDF1 and liver fibrosis

We found a significant association between a lower plasma GDF1 concentration and liver fibrosis, even after controlling for anthropometric

| Variable                          | All          | Minimal (FIB-4: <1.45) | Moderate (FIB-4: 1.45–3.25) | Advanced (FIB-4: >3.25) | p Value  |
|-----------------------------------|--------------|------------------------|-----------------------------|-------------------------|----------|
| N                                 | 327          | 80                     | 141                         | 106                     |          |
| Age (years)                       | 69.2 ± 13.5  | 59.0 ± 13.2            | 68.6 ± 10.8                 | 77.8 ± 11.3             | <0.0001  |
| Sex, male (%)                     | 241 (73.7)   | 63 (78.8)              | 102 (72.3)                  | 76 (71.7)               | 0.495    |
| BMI (kg/m²)                       | 25.2 ± 4.2   | 27.2 ± 4.1             | 25.6 ± 3.9                  | 23.1 ± 3.7              | <0.0001  |
| Waist circumference (cm)          | 90.9 ± 10.3  | 92.9 ± 10.2            | 92.0 ± 9.2                  | 87.9 ± 11.3             | 0.011    |
| Hypertension (n, %)               | 231 (70.6)   | 51 (63.8)              | 104 (73.8)                  | 76 (71.7)               | 0.280    |
| Hyperlipidemia (n, %)             | 192 (58.7)   | 46 (57.5)              | 84 (59.6)                   | 62 (58.5)               | 0.954    |
| Diabetes mellitus (n, %)          | 139 (42.5)   | 32 (40.0)              | 55 (39.0)                   | 52 (49.1)               | 0.250    |
| Current smoking (n, %)            | 110 (33.6)   | 34 (42.5)              | 42 (29.8)                   | 34 (32.1)               | 0.145    |
| Alcohol drinking (n, %)           | 87 (26.6)    | 30 (37.5)              | 36 (25.5)                   | 21 (19.8)               | 0.024    |
| Systolic BP (mmHg)                | 132 ± 22     | 133 ± 22               | 133 ± 22                    | 129 ± 22                | 0.378    |
| Diastolic BP (mmHg)               | 76 ± 14      | 77 ± 13                | 77 ± 14                     | 74 ± 14                 | 0.170    |
| Fasting glucose (mmol/L)          | 8.6 ± 4.6    | 8.5 ± 4.1              | 8.2 ± 4.7                   | 9.1 ± 4.8               | 0.399    |
| HbA1c (%)                         | 6.9 ± 1.6    | 7.1 ± 1.5              | 6.9 ± 1.4                   | 7.0 ± 1.9               | 0.736    |
| AST (μkat/L)                      | 0.6 (0.4–1.0)| 0.4 (0.3–0.5)          | 0.5 (0.4–0.7)               | 1.2 (0.6–2.3)           | <0.0001  |
| ALT (μkat/L)                      | 0.47 (0.30–0.73) | 0.45 (0.30–0.68) | 0.48 (0.29–0.98)           | 0.51 (0.33–0.79)        | 0.045    |
| Total cholesterol (mmol/L)        | 4.6 ± 1.2    | 4.4 ± 1.1              | 4.7 ± 1.1                   | 4.4 ± 1.2               | 0.076    |
| Triglycerides (mmol/L)            | 1.3 (0.9–1.9)| 1.3 (1.0–1.9)          | 1.4 (1.0–1.9)               | 1.1 (0.8–1.5)           | 0.016    |
| HDL-cholesterol (mmol/L)          | 1.04 ± 0.33  | 0.98 ± 0.30            | 1.06 ± 0.33                 | 1.06 ± 0.35             | 0.241    |
| LDL-cholesterol (mmol/L)          | 2.6 ± 0.9    | 2.6 ± 1.0              | 2.8 ± 0.8                   | 2.6 ± 1.0               | 0.180    |
| BUN (mmol/L)                      | 9.5 ± 6.4    | 8.8 ± 5.5              | 8.2 ± 5.0                   | 12.1 ± 8.1              | <0.0001  |
| Creatinine (μmol/L)               | 114.9 (97.2–150.3) | 106.1 (88.4–132.6) | 114.9 (97.2–141.4)          | 132.6 (106.1–247.5)     | 0.016    |
| eGFR (ml/min/1.73m²)              | 54.1 ± 26.6  | 63.6 ± 26.7            | 54.2 ± 22.1                 | 41.4 ± 23.3             | <0.0001  |
| Albumin (g/L)                     | 38 ± 5.0     | 39 ± 5.0               | 39 ± 5.0                    | 36 ± 4.0                | 0.001    |
| Platelets (10³/µl)                | 208.3 ± 67.1 | 268.3 ± 80.6           | 204.8 ± 52.0                | 174.7 ± 57.3            | <0.0001  |
| Hematocrit (%)                    | 38.5 ± 6.4   | 39.2 ± 6.7             | 39.1 ± 5.8                  | 36.5 ± 6.9              | 0.005    |
| Hemoglobin (g/L)                  | 12.8 ± 2.3   | 13.1 ± 2.4             | 13.0 ± 2.0                  | 12.1 ± 2.4              | 0.006    |
| WBC count (x10³/L)                | 8.613 ± 3.916| 7.796 ± 3.043          | 8.944 ± 3.662               | 9.215 ± 4.803           | 0.013    |
| Hs-CRP (mg/L)                     | 3.4 (1.0–10.0)| 2.4 (0.7–6.4)         | 3.2 (1.2–10.0)             | 5.0 (1.2–17.2)          | 0.041    |
| GDF1 (ng/ml)                      | 114.7 (60.0–174.0) | 138.8 (90.9–202.2) | 114.7 (62.4–173.6)          | 91.8 (45.2–155.0)       | 0.001    |
| No. of diseased coronary arteries | 1.8 ± 1.1    | 1.6 ± 1.2              | 1.8 ± 1.1                   | 2.0 ± 1.1               | 0.098    |
| Gensini score a                   | 27.5 (10.0–68.5)| 38.0 (12.0–75.5) | 24.0 (9.0–53.0)            | 31.5 (13.0–91.5)        | 0.162    |

Note: Data are mean ± SD or frequency (percentage).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; FIB, fibrosis; GDF1, growth/differentiation factor 1; HDL, high-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; WBC, white blood cell; WHR, waist-to-hip ratio.

aSignificant difference was tested using log-transformed data.
factors, ALT, hemoglobin, albumin, creatinine, and hs-CRP (Table 2). Furthermore, a lower concentration of GDF1 showed a significant linear trend and was independently associated with liver fibrosis, especially when analyzing the concentration both as a continuous variable and by tertile (Tables 2 and 3). In the multiple logistic regression analysis, fully adjusted ORs for liver fibrosis in the second and third tertiles were 2.02 (95% CI, 0.70–6.30) and 5.04 (95% CI, 1.35–25.60), respectively.

### 3.3 Associations between plasma GDF1 level and clinical laboratory data

Simple linear regression analysis showed that plasma GDF1 was negatively associated with the FIB-4 index, blood urea nitrogen, and creatinine, and positively associated with platelet count and eGFR. In multiple linear regression analysis, plasma GDF1 level was positively associated with platelet count, and negatively associated with the FIB-4 index, blood urea nitrogen, and creatinine (Table 4). In addition, in analysis of the subjects by tertile of GDF1 concentration, the FIB-4 index, APRI, and AST/ALT ratio were significantly associated with GDF1 concentration (p for trend < 0.05, Figure 1).

### 4 DISCUSSION

According to a previous clinical study, fatty liver disease is common in patients with CAD, and it can progress to liver fibrosis resulting in higher rates of all-cause and cardiovascular mortality. Similarly, in the current study, we found that among the 327 stable angina patients, more than 75% had moderate to advanced liver fibrosis (Table 1). In the past decade, several studies have evaluated the relationship between fatty liver disease and CAD. Although some chronic liver diseases such as NAFLD share similar risk factors with CAD (such as hyperglycemia, insulin resistance, hypertension, inflammation, dyslipidemia, hyperuricemia, hypoadiponectinemia, renal failure, and obesity), the underlying molecular relationship between chronic liver disease and CAD is unknown. Clinically, a meta-analysis found that NAFLD was associated with the severity of atherosclerosis, including coronary calcification levels, carotid intima-media thickness, arterial stiffness and endothelial dysfunction. Furthermore, the review by Meex et al. in 2017 also showed that many hepatokines such as fetuin A, fetuin B, retinol-binding protein 4, and selenoprotein P are involved in both fatty liver disease and insulin resistance, which in turn influences the process of atherosclerosis. However, no previous report has described the underlying molecular pathogenesis between chronic liver disease and CAD. To the best of our knowledge, this is the first study to show that GDF1, which is involved in both post-infarction cardiac remodeling and the development of fatty liver disease, was associated with liver fibrosis in patients with stable angina.

The cytokine system is very complex. Maintaining a balanced healthy environment for all human cells is difficult, and a factor which is beneficial for certain circumstances may be harmful for another. GDF1 is a member of the TGF-β superfamily and is known to be a protective mediator against the development of post-infarction cardiac remodeling via negative regulation of the Smad signaling and MEK-ERK1/2 pathways in the heart. A

| Model adjusted for | Liver fibrosis |
|--------------------|---------------|
|                    | OR   | 95% CI | p Value |
| Plasma log: GDF1   |      |       |         |
| Age                | 0.28 | 0.12–0.68 | 0.005 |
| Age, sex           | 0.29 | 0.12–0.70 | 0.006 |
| Age, sex, BMI      | 0.33 | 0.14–0.78 | 0.012 |
| Age, sex, BMI, ALT | 0.33 | 1.14–0.79 | 0.013 |
| Age, sex, BMI, ALT, hemoglobin, albumin | 0.25 | 0.08–0.78 | 0.016 |
| Age, sex, BMI, ALT, hemoglobin, albumin, creatinine, hs-CRP | 0.08 | 0.01–0.46 | 0.005 |

**Note:** Results of multivariate logistic regression analysis are presented as the odds ratio (OR) of having a liver fibrosis status and decreased plasma GDF1 level.

**Abbreviations:** ALT, alanine aminotransferase; BMI, body mass index; GDF1, growth/differentiation factor 1; Hs-CRP, high-sensitivity C-reactive protein.

| Factor                              | Tertiles of GDF1                  |
|-------------------------------------|-----------------------------------|
|                                     | T3 (95% CI) | T2 (95% CI) | T1 (95% CI) | p Value |
| All subjects                        |            |            |            |         |
| No. of cases/reference              | 92/17      | 79/29      | 76/34      | 0.024 |
| Cut off GDF1 concentration (ng/mL) | <75        | 75–150     | >150       |         |
| Univariate                          | 2.42 (1.27–4.76) | 1.22 (0.68–2.20) | 1.00 |         |
| Multivariate*                       | 5.04 (1.35–25.60) | 2.02 (0.70–6.30) | 1.00 |         |

**Note:** Values shown are cut-off values of plasma GDF1 levels of all subjects, and odds ratios (ORs) with 95% confidence intervals (CIs).

**Abbreviation:** GDF1, growth/differentiation factor 1.

*Adjusted for age, sex, body mass index, alanine aminotransferase, hemoglobin, albumin, creatinine, and high-sensitivity C-reactive protein.
suppressed MEK/ERK1/2 signaling pathway has been associated with fatty liver disease, liver fibrosis, and poor hepatoma differentiation in recent studies.\(^\text{12,13}\) In this study, a lower plasma GDF1 concentration was significantly independently associated with and showed a significant linear trend with liver fibrosis (Tables 2 and 3). It is reasonable that a decrease in GDF1 could enhance the progress of liver fibrosis. In addition, the Gensini score and number of diseased coronary arteries, which was used to evaluate CAD severity, did not show a significant difference among tertiles of GDF1 (data not shown). The role of GDF1 post infarction is known, however it remains unclear in CAD.

In analysis of the associations between common risk factors for liver fibrosis, we found no significant differences in hypertension, male sex, hyperlipidemia, current smoking, diabetes mellitus, DBP, SBP, LDL-C, HDL-C, total cholesterol, HbA1c or fasting glucose among the liver fibrosis severity groups (Table 1). This suggests that the pathogenesis of liver fibrosis in patients with stable angina may have a different mechanism to that of liver fibrosis, even though they share similar risk factors.

In this study, we found that plasma GDF1 level was independently associated and showed a significant linear trend with liver fibrosis after controlling for all anthropometric variables, liver enzymes, albumin, renal function and inflammation markers (Tables 2 and 3). In linear regression analysis, we further showed that plasma GDF1 level was positively associated with renal function and liver function and condition (such as albumin and platelet levels), but negatively associated with the FIB-4 index (Table 4). More importantly, the common CAD risk factors such as BMI, blood pressure, Hba1c, LDL, and hs-CRP were not associated with GDF1. These findings imply that

### Table 4: Linear regression analysis of variables associated with plasma log GDF1 levels in the study subjects.

| Variable              | Simple β | Simple p Value | Multiple β | Multiple p Value |
|-----------------------|----------|----------------|------------|------------------|
| Age                   | -0.076   | 0.173          |            | -                |
| Sex                   | -0.030   | 0.593          |            | -                |
| BMI                   | 0.073    | 0.190          | 0.049      | 0.425            |
| Waist circumference   | 0.006    | 0.926          | 0.004      | 0.957            |
| Systolic BP           | 0.007    | 0.909          | 0.004      | 0.949            |
| Diastolic BP          | 0.085    | 0.140          | 0.077      | 0.181            |
| Fibrosis-4            | -0.135   | 0.019          | -0.126     | 0.045            |
| Fasting glucose       | -0.060   | 0.291          | -0.060     | 0.292            |
| HbA1c                 | 0.002    | 0.969          | -0.008     | 0.896            |
| AST                   | -0.077   | 0.181          | -0.072     | 0.214            |
| ALT                   | 0.028    | 0.616          | 0.030      | 0.597            |
| Total cholesterol     | 0.023    | 0.684          | 0.016      | 0.786            |
| Triglycerides         | 0.063    | 0.255          | 0.049      | 0.403            |
| HDL-cholesterol       | -0.002   | 0.965          | -0.002     | 0.967            |
| LDL-cholesterol       | 0.001    | 0.981          | -0.002     | 0.968            |
| Blood urea nitrogen   | -0.145   | 0.011          | -0.125     | 0.032            |
| Creatinine            | -0.146   | 0.008          | -0.138     | 0.013            |
| Estimated GFR         | 0.110    | 0.048          | 0.087      | 0.164            |
| Albumin               | 0.041    | 0.519          | 0.015      | 0.824            |
| Platelets             | 0.177    | 0.001          | 0.163      | 0.004            |
| Hematocrit            | 0.001    | 0.986          | -0.017     | 0.778            |
| Hemoglobin            | 0.008    | 0.885          | -0.009     | 0.884            |
| White blood cell count| -0.049   | 0.376          | -0.046     | 0.408            |
| Hs-CRP                | -0.029   | 0.652          | -0.014     | 0.833            |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GDF1, growth/differentiation factor 1; GFR, glomerular filtration rate; HDL, high-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.

\(^\text{a}\)Regression coefficient adjusted for age and sex.
the role of GDF1 in liver fibrosis in patients with stable angina may be different from the traditional shared risk factors between CAD and NAFLD. Further studies are warranted to elucidate this issue.

After classifying the subjects into tertiles according to plasma GDF1 concentration, we found that GDF1 was associated with the FIB-4 index and APRI, which is used to predict fibrosis and cirrhosis in patients with chronic hepatitis (Figure 1).23,32 The average AST/ALT ratio in our patients was 1.78, neither <1 or >2 (Figure 1). This revealed that a high FIB-4 index in our angina patients was not strongly related to the causes of alcoholic fatty liver disease or NAFLD.33–35 With regard to the general demographics, we found no significant differences in age (69.3 ± 13.4 vs. 69.9 ± 13.9 vs. 68.6 ± 13.3 years, p = 0.769) or alcohol consumption (22.9% vs. 22.2% vs. 34.6%, p = 0.068) among the tertiles of GDF1 (data not shown).

There are several limitations to this study. First, we lacked liver echo evaluation reports and liver biopsy proof. This is because it is not reasonable to ask all stable angina patients to undergo such examinations when there is no strong evidence of liver disease. Second, the role of GDF1 in coronary arteriosclerosis remains unknown. Third, our results showed only that there was an association between plasma GDF1 levels and liver fibrosis. Further investigations are needed to investigate whether the mechanism is through the MEK/ERK1/2 signaling pathway or other underlying pathways. Fourth, if the study population had different diseases (e.g., acute coronary syndrome and myocardial infarction), the diverse condition and disease severity of the study population may have impacted the results. To avoid selection bias, we chose individuals with stable angina for this study, thus the results of the present study might not be generalizable to other populations. Finally, further investigations are also needed to investigate whether other hepatokines are also involved and interact with GDF1 in the liver fibrosis process in patients with stable angina.

5 | CONCLUSIONS

Our results indicated that in patients with stable angina, low plasma GDF1 was associated with liver fibrosis. Further studies are warranted to investigate the association between plasma GDF1 and the pathogenesis of liver fibrosis.

AUTHOR CONTRIBUTIONS

All authors contributed to this study. W.-C.H., W.-H.T., and C.-C.H. conceived and designed the study. W.-C.H., W.-H.T., Y.-J.L., and C.-C.H. provided the methodology. F.-M.C. performed the formal analysis, and project administration. T.-H.Y., C.-C.W., and C.-C.H. validated the data. T.-H.Y., C.-C.W., W.-C.H., and C.-P.W. performed the investigation, resources, and data curation. T.-H.Y., W.-C.H., C.-C.W., C.-P.W., Y.-C.L., and C.-T.W. prepared the manuscript. W.-H.T., T.-H.Y., W.-C.H., C.-C.W., C.-P.W., Y.-C.L., and C.-T.W. reviewed and edited the manuscript. W.-C.H., W.-H.T., Y.-J.L., and C.-C.H. performed the visualization. W.-C.H. and C.-C.H. performed the supervision and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors have declared that no competing interest exists.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

INFORMED CONSENT

Each patient provided written informed consent before being enrolled into the study.

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