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The Role of Growth Differentiation Factor-15 in the Diagnosis of Patients with Chronic Angina Undergoing Coronary Angiography

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ABSTRACT

Coronary artery disease (CAD) is considered the most prevalent leading cause of myocardial ischemia and mortality worldwide and can lead to angina pectoris and myocardial infarction (MI). Growth Differentiation Factor-15 (GDF-15) is usually measured together with other biomarkers to predict all-cause mortality. During stressful conditions associated with tissue injury and inflammation like myocardial ischemia, it is released into circulation and detected at high concentration in foam cells of the atherosclerotic plaque. Its circulating levels are associated with chronic diseases rather than acute diseases mainly cardiovascular diseases (CVDs) like CAD and chronic heart failure (CHF). The aim of the current study was to detect the role of GDF-15 as a new biomarker for the diagnosis of stable angina patients with normal left ventricular ejection fraction who were presented as having stable chest pain, so that to avoid endangering them to the invasive coronary angiography. Fasting venous blood samples were taken from 90 participants who were presented as having chest pain. They were all subjected to echocardiography, electrocardiography and coronary angiography. The left ventricular ejection fraction was calculated using the biplane M mode method. GDF-15 serum level was measured by using Human GDF-15 Sandwich ELISA Kit following the manufacturer's instructions.The current study detected a high significant difference in the serum levels of GDF-15 and serum creatinine between the patients and the control group (P≤0.01). On the other hand, there were significant differences in the serum levels of triglycerides and VLDL-C between both study groups (P≤0.05). ROC curve analysis showed that GDF-15 had AUC= 1.00, the best cut off= 254.16 with sensitivity and specificity of 100%. So, it can be concluded that GDF-15 could be used as a biomarker for the diagnosis of stable angina patients with obstructive atherosclerotic plaque and normal LV ejection fraction.

Keywords: Angiography, Diagnosis, GDF-15, Patients, Stable angina.

INTRODUCTION

CAD is considered the most prevalent leading cause of myocardial ischemia and mortality worldwide and can lead to angina pectoris and myocardial infarction (MI) (Doenst *et al*., 2019). It is usually caused either by vasospasm in the coronary arteries or by the presence of atherosclerotic plaques or blood clot within these arteries that may lead to coronary stenosis or occlusion which in turn limits the blood supply to a specific region of the myocardium leading to ischemia (Criteria, 2010). CAD can progress from asymptomatic CAD, reversible ischemia (stable angina pectoris), irreversible ischemia (unstable angina), necrosis (acute myocardial infarction AMI) and finally cardiac dysfunction (Lippi *et al*., 2013). Atherosclerosis represents the major pathophysiological cause of CAD and ischemic heart disease (Bauersachs *et al*., 2019). There are many risk factors for having CAD like: age, gender, family history, ethnicity, hypertension, dyslipidemia, smoking and obesity (Brown *et al*., 2023; Duggan *et al*., 2022; GHARIB *et al*., 2022; Levin *et al*., 2021). Stable angina can be defined as a symptom of CAD which refers to chest pain that may radiate to neck, jaw or arm and described as

being squeezing and relieved by rest or nitroglycerine (Ferraro *et al*., 2020; Gillen & Goyal, 2023; Tu *et al*., 2021). It occurs as a result of an imbalance between myocardial need and oxygen supply (Tamargo & Lopez-Sendon, 2022). The diagnosis of stable angina depends on the physical examination of the patients and their history regarding the severity, type and duration of the pain, since those patients will usually experience a squeezing chest pain occurs on exertion and relieved at rest. The medical history of the patient, assessing the blood pressure, measuring the body weight, cigarette smoking, alcohol consumption and exercise tolerability are very important points in the diagnosis (Gillen & Goyal, 2023). There are many techniques used for the diagnosis of stable angina some of which are non-invasive like: Pre-Test Probability (PTP), Coronary Computed Tomography Angiography (CCTA), Stress echocardiography, Stress electrocardiogram (ECG) and Single-photon emission computed tomography (Bertolone *et al*., 2022; Knuuti *et al*., 2018); and some others are invasive like: Invasive Coronary Angiography (ICA) (Bertolone *et al*., 2022). Growth Differentiation Factor-15 (GDF-15) is usually measured together with other biomarkers to predict all-cause mortality like: high-sensitivity C-reactive protein (hs-CRP), hs-troponin T and N-Terminal B-Type natriuretic peptide (NT-proBNP). During stressful conditions associated with tissue injury and inflammation like myocardial ischemia, GDF-15 is released into circulation and detected at high concentration in foam cells of the atherosclerotic plaque, while in the absence of stress its circulating levels cannot be detected (Zhang *et al*., 2023). It is a member of a family called transforming growth factor beta (TGF-β) and is mainly expressed by placenta and prostate ("GDF-15 in Plasma and Circulating Mononuclear Cells and NT-proBNP for Diagnosis of Chronic Heart Failure and Predicting Cardiovascular Disease Events," 2019; May *et al*., n.d.).GDF-15 prevents cardiac hypertrophy, dilation and cell death, therefore it has a cardio-protective role via its anti-inflammatory, anti-oxidant and anti-apoptotic actions. It is released under stressful conditions (May *et al*., n.d.; Rochette *et al*., 2021; Zhu *et al*., 2019). Its circulating levels are associated with chronic diseases rather than acute diseases mainly CVDs like CAD and CHF (Dalos *et al*., 2019). Its levels are thought to be predictive of mortality, ischemic events including MI in CAD patients (Li *et al*., 2021).

MATERIALS AND METHODS

Design of the study and patient selection

The study design was a case-control study. It was ethically approved by the local ethical committee of the College of Pharmacy/Mustansiriyah University. It was performed on 90 male and female participants who were presented to the outpatient clinic as having chest pain. All participants were asked to fill signed informed consents. Fasting venous blood samples were collected from the Iraqi center for heart diseases at Al-Shaheed Ghazi Al-Hareery/ Medical City teaching hospital. All were examined by echocardiography by the cardiologist and then subjected to coronary angiography which was also performed by the cardiologist and his team to check for the severity of coronary arteries occlusion and ischemia based on American Heart Association (Gulati *et al*., 2021) (stenosis < 50 was considered low risk, stenosis 50-69% was considered intermediate and stenosis > 70% was considered critical), as well as the number of the diseased vessels: single vessel, two vessels or three vessels. Based on the results of the angiography, the subjects were divided into two groups: angina (atherosclerosis) patients (n=60) and control (n=30). The subjects who showed no evidence of CAD or ischemia on coronary angiography, no medical history including hypertension, dyslipidemia, or DM were enrolled in the control group.

Inclusion criteria

Male and female patients who were presented with chest pain with or without ECG changes reflecting ischemia, age of 40-76 years old, left ventricular ejection fraction LVEF ≥ 56%, no or controlled hypertension and diabetes mellitus.

Exclusion criteria

Patients with myocardial infarction, renal failure or chronic kidney disease, valvular heart disease, congenital heart disease, stroke, chronic heart failure, atrial fibrillation, malignancy and immune diseases.

Methods

Fasting venous blood samples (6 ml) were drawn from each participant. Then, the samples were centrifuged at 3000 rpm for 10 min. to obtain the serum which was placed in eppendorf tubes and frozen at -40 c^0 until measurements were applied. Echocardiography and ECG were applied to all.

Echocardiography.

It was performed by the cardiologist at his clinic to detect any regional wall motion abnormality and any ischemic changes. The left ventricular ejection fraction was calculated using the biplane M mode method (it is an operator-dependent method and performed by a sub-specialty doctor by taking the ratio between end diastolic dimension to end systolic dimension, results of ≥ 56 were considered as normal).

X-ray conventional coronary angiography

It was performed under local anesthesia using a catheter with a contrast dye inserted into the radial or the femoral artery. The number of the diseased vessels and the degree of stenosis were determined. The participants were asked to be fasting for at least 8 hours before the procedure. Clopidogrel 600 mg and aspirin 300 mg tablets, were prescribed to be taken prior the procedure as loading doses. Heparin was injected during the session. The most potential challenges had been faced during the procedure were unstable left main stem (LMS) artery, perforation and dissection which required an urgent surgical intervention.

GDF-15 estimation

The serum level was measured by using Human GDF-15 Sandwich ELISA Kit/ Cloud-Clone Corp./USA following the manufacturer's instructions.

Other measured biomarkers

Serum triglycerides, total cholesterol, HDL-C, urea, creatinine and fasting blood glucose were measured using Triglycerides SL, Cholesterol SL, HDL cholesterol, Urea UV SL, Creatinine PAP SL and Glucose PAP SL kits, respectively, of Selectra pro M biochemistry analyzer/ ELITech/ France. On the other hand, Friedewald equation was used to estimate the serum levels of VLDL-C and LDL-C.

The most important limitations of the current study were the small sample size, significant differences in gender distribution between the patients and the control and the study was performed in a single center.

The statistical analysis

IBM SPSS Statistics 26 program was used to detect the effect of different factors on study parameters. One-way ANOVA and T-test was used to significantly compare between means. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability). GraphPad Prism 9 program was used to draw the figures in this study (Forthofer *et al*., 2007; Glover, n.d.).

Ethical approval

The study was ethically approved by the local ethical committee of the College of Pharmacy/Mustansiriyah University (ref.number: 93 at 21/1/2024). All participants were asked to fill signed informed consents based on Helsinki statement by The World Medical Association (WMA).

RESULTS AND DISCUSSION

Demographic distribution and the clinical characteristics of the study groups:

Both of the study groups were presented by their gender, age, BMI, LVEF, smoking status and family history, besides, medical and medication history of the patients also was taken regarding hypertension, diabetes mellitus, dyslipidemia, taking statins and beta-blockers as in (Table I).

Regarding the age distribution, family history, BMI, LVEF and smoking status, there were no significant differences found between the study groups (P>0.05). This result may not agree with other studies which suggested that the risk of having CAD, atherosclerosis and angina is increased with aging as it is considered an independent risk factor for cardiovascular diseases together with environmental and genetic factors (Nanayakkara *et al*., 2018; Piccirillo *et al*., 2019). where there are changes which occur in the coronary circulation like loss of elasticity, increased intimal thickness, collagen deposition, platelets aggregations and other changes (Ali Sheikh, 2020; Shi *et al*., 2021). In addition, regarding BMI, the result was agreed with other study which also found no effect of BMI on stable angina (Soliman *et al*., 2023). On the other hand, there was no significant effect detected of family history and smoking as risk factors for having CAD and angina, there was a study which support this result (Kang *et al*., 2023) and other studies which did not support the result (Ashour, 2017; Frančula-Zaninović & Nola, 2018; Salehi *et al*., 2021). While, regarding gender distribution, there was a high significant difference between both groups (P≤0.01).It was found that males constituted the higher percentage of the participated patients (78%) whereas females constituted only 22% of them, which indicated that males were more likely to have CAD and stable angina than females. This result was supported by many studies (Costa *et al*., 2020). There was a high significant difference between patients who had dyslipidemia (52%) and those who did not have (48%), (P≤0.01).

Table I. Demographic distribution data and the clinical characteristics of the study groups

Characteristics		Patients (n=60)	Control (n=30)	*P*- value
Age (years) Mean ± SD		59.35 ± 9.32	58.53 ± 9.40	0.6 NS
BMI (kg/m^2) Mean ± SD		28.35 ± 4.11	28.67 ± 3.97	0.7 NS
Gender:	Male n (%)	47 (78%)	12 (40%)	0.001**
	Female n (%)	13 (22%)	18 (60%)	
Total		60 (100%)	30 (100%)	
Family history:	yes	25 (42%)	12 (40%)	0.8 NS
	No	35 (58%)	18 (60%)	
Total		60 (100%)	30 (100%)	
Smoking:	Yes	9 (15%)	6 (20%)	0.5 NS
	No	51 (85%)	24 (80%)	
Total		60 (100%)	30 (100%)	
Dyslipidemia:	Yes	31 (52%)	–	–
	No	29 (48%)		
P-value		0.001**		
Hypertension:	Yes	39 (65%)	–	–
	No	21 (35%)		
P-value		0.001**		
DM:	Yes	28 (46.7%)	–	–
	No	32(53.3%)		
P-value		0.001**		
LVEF (%) Mean ± SD		63.42 ± 7.24	64.07 ± 6.51	0.6 NS
Statins:	Yes	30 (50%)		
	No	30 (50%)	–	–
P-value		1 NS		
Beta blockers:	Yes	20 (33%)	–	–
	No	40 (67%)		
P-value		0.001**		

The data were expressed as Mean ± SD, Chi-square test was used to significantly compare between percentages (since it is descriptive); * Significant difference (*P*≤0.05); ** Highly Significant difference (*P*≤0.01); NS: non significant difference; BMI: Body Mass Index; n: number; % percentage; SD: standard deviation; LVEF: left ventricular ejection fraction.

Besides, the current study also detected a high significant difference between the patients who had hypertension (65%) and those who did not have hypertension (35%) with (*P*≤0.01). Also, It was detected that (46.7%) of the patients had DM while (53.3%) did not have DM with a high significant difference (*P*≤0.01). As risk factors for having CAD and stable angina, this result might indicate that patients with hypertension, dyslipidemia, and DM were more prone to develop stable angina and CAD than those patients who did not have these risk factors. This was clarified also by other studies (Abdulaali *et al*., 2018; Fadhil *et al*., 2022; Mohammed, 2017; Shatari *et al*., 2021; Wang *et al*., 2022) . In addition the present study detected no significant difference between patients who were on statins (50%) and those who were not (50%) (*P*>0.05), while there was a high significant difference between patients who were on β-blockers (33%) and those who were not (67%) (*P*≤0.01).

Comparison in the serum levels of the studied biomarkers between the study groups:

(Table II) explains the differences in the serum levels of the studied biomarkers between the patients and the control. The current study detected a high significant difference in the serum levels of GDF-15 (Figure 1) and serum creatinine between the patients and the control group (*P*≤0.01). On the other hand, there were significant differences in the serum levels of triglycerides and VLDL-C between both study groups (*P*≤0.05). This result agreed with other studies (Mei *et al*., 2022). However, there were no significant differences detected in the serum levels of total cholesterol, HDL-C, LDL-C, urea and fasting blood glucose (*P*>0.05).

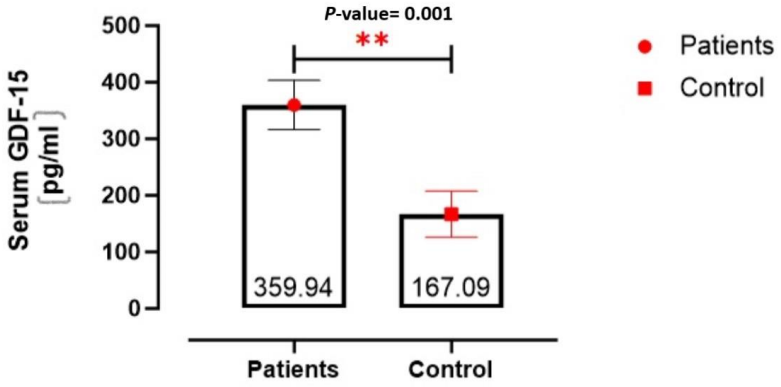

Figure 1. The difference in the serum level of GDF-15 between the patients and the control groups.

Table II. Comparison in the serum levels of the studied biomarkers between the study groups

Biomarker	Mean±SD		*P*-value
	Patients (n=60)	Control (n=30)	
GDF-15 (pg/ml)	359.94±43.35	167.09±40.57	0.001**
Total cholesterol (mg/dl)	148.73±48.06	143.96±32.27	0.6
TG (mg/dl)	185.50±100.88	143.46±76.73	0.04*
HDL-C(mg/dl)	36.70±13.15	40.47±11.18	0.1
LDL-C(mg/dl)	75.01±40.85	74.83±33.40	0.9
VLDL-C(mg/dl)	37.10±20.18	28.87±15.14	0.05*
S.cr. (mg/dl)	0.89±0.20	0.76±0.19	0.004**
B.U. (mg/dl)	33.61±7.27	32.11±7.42	0.3
FBG(mg/dl)	132.78±77.10	105.36±25.72	0.06

The data were expressed as Mean ± SD, T-test was used to significantly compare between means (since it is between two groups); * Significant difference (*P*≤0.05); ** Highly Significant difference (*P*≤0.01); GDF-15: growth differentiation factor-15; S.cr.: serum creatinine; B.U.: blood urea; FBG: fasting blood glucose.

Receiver Operating Characteristic (ROC)

In purpose to assess the sensitivity and the specificity of the new biomarker GDF-15 in the diagnosis of stable angina patients with normal LVEF, ROC analysis was applied. Based on the results of ROC data analysis, the current study detected that GDF-15 had AUC= 1.00, the best cut off= 254.16 with sensitivity and specificity of 100% as shown in (Figure 2). There was another study which detected that GDF-15 had a prognostic value in patients with CAD where ROC showed that GDF-15 showed 80% sensitivity and 91.7% specificity (Wang *et al*., 2016). Accordingly, based on the results of the current study, GDF-15 could be used independently in the diagnosis of stable angina patients with normal LVEF and thus reducing the need for exposing all patients who are suffering from stable chest pain to the risks of coronary angiography. There are many biomarkers which are used routinely in the diagnosis of cardiovascular diseases in multi-biomarker approach. Some of them are cardiac specific like troponin and some others are non-cardiac specific like C-reactive protein (CRP) which are mostly used to diagnose acute coronary syndrome like MI and unstable angina. However, since the current study aimed at diagnosing chronic angina patients which were presented with stable chest pain, so both of those biomarkers were not considered.

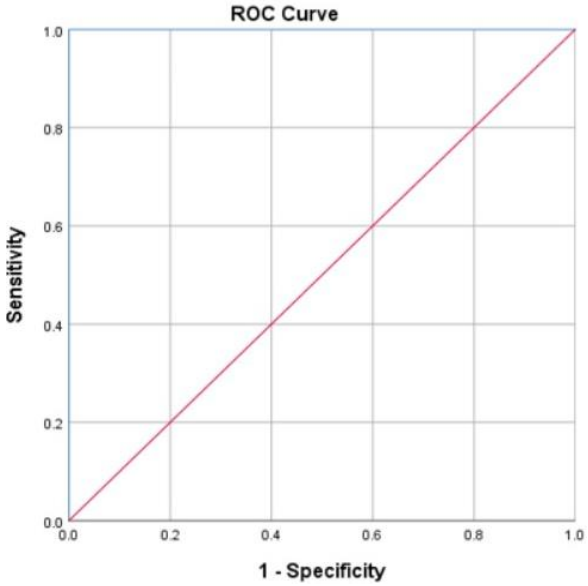

Figure 2. Receiver Operating Characteristic curve of GDF-15. AUC= 1.00; the best cut off= 254.16; sensitivity=100%; specificity= 100%.

Table III. The effect of the number of diseased coronary vessels on the serum levels of the studied biomarkers: 1-Specificity

Biomarker	Mean±SD			*P*-value
	Patients SVD (n=12)	Patients 2VD (n=23)	Patients 3VD (n=25)	
GDF-15 (pg/ml)	356.36±40.31	361.53±42.29	360.18±47.20	0.9
T.Cholesterol (mg/dl)	155.08±49.16	144.00±52.35	150.04±44.89	0.8
HDL-C (mg/dl)	40.75±12.33	34.65±11.78	36.64±14.70	0.4
TG (mg/dl)	190.42±78.94	188.96±104.01	179.96±110.40	0.9
LDL-C (mg/dl)	76.51±44.70	71.63±48.76	77.40±31.46	0.8
VLDL-C (mg/dl)	38.09±15.82	37.80±20.79	35.98±22.08	0.9
B.urea (mg/dl)	35.00±6.95	33.48±8.20	33.06±6.69	0.7
S.cr. (mg/dl)	0.90±0.17	0.87±0.22	0.91±0.21	0.7
FBG (mg/dl)	107.25±62.18	121.04±53.18	155.82±96.11	0.1

The data were expressed as Mean ± SD; One-way ANOVA was used to significantly compare between means (since it is between more than two groups); * Significant difference ($P \leq 0.05$);** Highly Significant difference ($P \leq 0.01$); SVD: single vessel disease; 2VD: two-vessels disease; 3VD: three-vessels disease.

The effect of the number of diseased coronary vessels on the serum levels of the studied biomarkers:

The effect of the number of diseased coronary vessels on the serum levels of the studied biomarkers was summarized in (Table III). The current study detected no significant effect of the number of the occluded coronary arteries (single-vessel, two-vessel and three-vessel disease) on the serum levels of GDF-15, total cholesterol, TG, HDL-C, LDL-C, VLDL-C, FBG, creatinine and urea as elucidated in (Table III), where ($P>0.05$). Although Framingham risk score (FRS) is usually used to assess the severity of CAD (Farhood *et al*., 2023), and Gensini score also used to assess the degree of occlusion in different coronary arteries where a study found that GDF-15 serum level was significantly increased with increased degree of occlusion (Wang *et al*., 2016), the current study tried to explore if there is any effect of the number of occluded coronary arteries on the serum levels of GDF-15. However, there were no other studies found to explore if there is a relationship between the number of the occluded coronary vessels and GDF-15 serum level.

CONCLUSION

It was concluded that GDF-15 could be used as a useful biomarker for the diagnosis of stable angina patients with obstructive atherosclerotic plaque and normal LV ejection fraction with high sensitivity and specificity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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