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Serum Growth Differentiation Factor 15 as a Biomarker for Chronic Heart Failure in Coronary Artery Disease Patients

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ABSTRACT

Background: GDF-15, a member of the superfamily of transforming GF beta, regulates pathways of inflammation and apoptosis in both short-term and long-term tissue injury. Among novel biomarkers is GDF-15, which is used to diagnose chronic heart failure conditions. LVEF is associated with increased end diastolic diameter, increase in LV mass index, and increased GDF-15. **Objective:** For assessing the validity of the GDF-15 test to predict CHF onset in people with coronary atherosclerosis. **Subjects and methods:** Our research was done on sixty-nine subjects, who were categorized as the following: 23 patients with CAD, 23 patients who developed CHF on top of CAD according to the revised Framingham criteria, and 23 subjects who represented the control group. CAD was evidenced by history of MI or PCI or CABG or positive treadmill or imaging stress test or coronary angiography (CA) revealing ≥50% stenosis in ≥1 coronary vessels. **Results:** Although there was no age difference between the CAD and CHF groups, there were high statistical significance difference regarding age among studied groups. However, no statistical significance difference was found regarding gender in the study groups. Smoking-related differences between the two groups and the control group were statistically significant, but not those between the CAD and CHF groups. Although there was a very statistically significant discrepancy between both the CAD and CHF groups and the control group, there was no a statistically significant in comparing hypertension between the CAD and CHF groups. There was not a significant difference in terms of DM between the CAD and CHF groups, however there was a statistically significant difference between the two groups and the control group. **Conclusion:** Our findings suggest that GDF-15 might be a valuable biomarker for predicting HF onset in CAD patients. GDF-15 levels were highly significantly different among CAD patients when compared to persons in good condition.

Keywords: CAD, CHF, GDF-15.

INTRODUCTION

Stable coronary artery disease (CAD), a major public health concern with significant morbidity and mortality worldwide [1], is caused by ACS and chronic inflammatory atherosclerosis. According to studies, inflammatory reactions may have a major impact on the development of CAD[2].

CHF, which is caused by coronary artery atherosclerosis, is the most common kind of HF. CHF has become a pandemic in recent years [3]. Circulating biomarkers are increasingly being used to improve patient management and understand the pathogenesis of HF [4]. Given that HF affects several organ systems, biomarkers for cardiac and extracardiac disorders may offer additional insight above cardiac indicators such cardiac troponins or natriuretic peptides [5].

Hs-CTnT increases among CHF diseased people have been connected to the gradually increasing frequency of cardiovascular events, despite the fact that conventional cardiac troponin T tests are routinely utilised as a positive or negative categorical variable. Hs-CTnT at baseline and during follow-up is a powerful predictor of cardiac events in people with heart failure [6].

A possible cardiovascular biomarker, GDF-15, which integrates information from cardiac and extracardiac disease pathways linked to the incidence, progression, and prognosis of HF [7], is developing. GDF-15, a member of the superfamily of transforming growth factors, is linked to pathways of inflammation and apoptosis [8].

Measuring blood levels of GDF-15, a consequence of functioning macrophages, may aid in the early detection of atherosclerosis. Numerous investigations have demonstrated that macrophages are crucial in the onset of arterial thrombosis, a life-threatening consequence of atherosclerosis [9].

Increased expression levels of GDF-15 have been linked to worsened conditions for persons with heart disease, particularly ACS patients, independent of troponin or BNP levels. Reports indicating GDF-15 has a protective impact against heart disease have been attributed to the antiapoptotic, anti-inflammatory, or anti-hypertrophic activities reported in animal models [10]. Interest in GDF-15 as possible heart failure indicators is now rising[11].

The purpose of this study is to evaluate the reliability of the GDF-15 test to forecast the development of CHF in CAD patients.

PATIENTS AND METHODS

Forty-six individuals with CAD who were hospitalized in Cardiology Departments were included, at Zagazig University Hospitals, as well as 23 control individuals, and they were divided into three groups:

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(1) **First group: Coronary artery disease (CAD) group:**
Included 23 patients, who had CAD, as indicated by at least one of the following: treadmill electrocardiogram or stress nuclear perfusion imaging evidence of exercise-induced ischemia, a history of MI, angiographic evidence of less than fifty percent stenosis in one coronary vessel, and a history of coronary revascularization (percutaneous coronary intervention or coronary artery bypass graft), nonetheless, not meeting the updated Framingham criteria for CHF [12].

(2) **Second group: (CAD-CHF) group: Included also 23 patients, they had CAD and CHF.**

(3) **Third group (Control group): Also included 23 subjects, who didn't have CHF** (Evidenced by the revised Framingham criteria) **nor significant CAD** (Evidenced by angiographic stenosis from 0-50% or negative treadmill electrocardiograms for exercise-induced ischemia or stress nuclear perfusion imaging).

Inclusion criteria: Patients suffering from CAD with and without chronic heart failure.

Exclusion criteria:

1. Sudden cardiac failure two weeks prior to the blood sample.
2. Primary and secondary cardiomyopathies (other than ischemic).
3. A congenital cardiac condition and combination.
4. A stroke during the previous year.
5. Serum creatinine >2 mg/dl.
6. Liver cirrhosis or hepatocellular carcinoma.
7. Involvement of asthma, pulmonary emphysema, and chronic bronchitis.
8. Inclusion of peripheral arterial disease, an autoimmune or inflammatory condition, an ongoing infection, thalassemia, or cancer.

Study type: Case control study**.**

All patients underwent the following procedures:

1- **History taking:** including gender, age, smoking, high blood pressure, DM and previous MI, PCI, or CABG, or positive treadmill or imaging stress test or coronary angiography (CA) revealing ≥50% stenosis in ≥1 coronary vessels.

2- **Clinical examination:** Applying the revised Framingham criteria as evidence for CHF.

Table (1): The Framingham criteria for diagnosing HF [12]

Revised Framingham Criteria		
Major Criteria	**Minor criteria**	**Major or minor criteria**
Night-time paroxysmal dyspnea or orthopnea	Swelling of ankle	> loss of BW of four and half kg in five days as a result of treatment
Neck-vein enlargement	Evening cough	
Rales	Dyspnea with effort	
Cardiomegaly	Hepatomegaly	
Pulmonary edema acute	Lung effusion	
S3 gallop	Vital capacity decreased by a third from its peak.	
>16 cm H_2O of elevated vein pressure	Increased heart rate (more-than 120 beats /min)	
Hepatojugular reflux		

According to the Framingham criteria, at least two major criteria must be present at the same time, or one major criterion must be present together with two minor criteria. Minor criteria (including nephrotic syndrome, chronic lung disease, cirrhosis, ascites, and pulmonary hypertension) are only acceptable if they cannot be attributable to another medical condition [12].

3- **Resting ECG:** To detect ischemic changes.

4- **Conventional transthoracic echo-Doppler study:**
Using a 2.5 MHz transducer on an HP Sonos 5500 set (USA), a transthoracic echo examination was carried out. When the patient was supine or in the left lateral position, images were taken utilizing the left parasternal long axis, apical 4 and apical 2 chamber views. The American Society of Echocardiography's [13] recommendations were followed, and numerous parameters were recorded and calculated, the ejection fraction was calculated from apical 4- and 2-chamber pictures using a modified Simpson's technique [14] to assess the systolic function in every single patient.

5- Blood sample for GDF-15 and (hs-CTnT):
Five millilitres of blood were drawn from each subject. Whole blood was collected using conventional sample tubes; the blood was left undisturbed at room temperature for the blood to clot. This typically takes between 10 and 20 minutes. The clog was eliminated by centrifuging for twenty minutes between 2000 and 3000 rpm. Centrifugation was repeated if precipitation started to fall while the sample was being retained. Collection and storage of serum at -80 degrees Celsius was done until it was needed.

Assay:

1- The **hs-CTnT** was measured on full automated Cobas e411.

2- GDF-15:

The test kit was supplied from Bioneovan co., Ltd.

The technique is enzyme linked immunosorbent assay (ELISA).

There were ten conventional wells set up on a Microelisa stripplate. Fifty µl of the standard dilution buffer and hundred µl of the standard solution were added to and thoroughly mixed in the wells one and two. The solutions from wells one and two were added in equal amounts to wells three and four, totaling hundred µl for each well. Fifty µl of standard dil. buffer were then added and well mixed. The solution was released in fifty µl from wells three and four. The solutions from wells three and four were added in quantities of fifty µl each to wells five and six respectively. Fifty µl of standard dilution buffer were then added and well mixed. The solutions from wells five and six were added to wells seven and eight respectively in a volume of fifty µl. After that, fifty µl of standard dilution buffer were added and well mixed. 50 µl of the solutions from wells 7 and 8 were added to wells 9 and 10, respectively. Fifty µl of standard dilution buffer were added and well mixed after that. The solutions from wells seven and eight were added in quantities of 50 µl each to wells nine and ten, respectively. 50 µl of standard dil. buffer were added and well mixed after that. The solution was pumped out of wells nine and ten in an amount of 50 µl. After dilution, each well had a volume of 50 µl and the corresponding conc. of 900 pg/ml, 600 pg/ml, 300 pg/ml, 150 pg/ml, and 75 pg/ml.

Assay range: 16 pg/ml-1000 pg/ml [8].

Ethical approval:

This study was approved by the Zagazig Medical Ethics Committee of the Zagazig Faculty of Medicine. All participants gave written consent after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

Using SPSS V. 16, statistical data from the current study were presented and examined. The quantitative data were presented as mean and standard deviation (SD) and were compared by one-way ANOVA test, followed by post-hoc test if P was significant. Qualitative data were presented as frequency and percentage and were compared by the chi-square test (X^2). The serum Hs-CTnT and GDF-15 cutoff points were determined using the receiver operating characteristic (ROC) curve. P value less than 0.05 was considered significant.

RESULTS

The age difference between the control group and both sick groups was statistically very significant. There were no statistically significant differences between the study groups regarding gender. Pertaining to smoking, although there was no difference between the CAD and CHF groups, there was a difference of statistical significance between the studied groups. Regarding HTN, there was highly significant statistical rise in blood pressure between both groups of patients and control group. But no significant difference between CAD and CHF groups. As regards DM, it was present in CHF patient group more than in CAD patient group and it was absent in control group (Table 2).

Table (2): The demographic data of the study groups

Demographic data	CAD group Mean±SD (Range)	CHF group Mean±SD (Range)	Control group Mean±SD (Range)	P-value
Age (yrs)	55.7±7 (42-66)	58.4±8.6 (36-75)	30±4 (25-37)	<0.001**
Male gender	74%	70%	74%	0.743
Smoking	52%	44%	17%	0.02*
HTN	57%	57%	0%	<0.001**
DM	57%	74%	0%	<0.001**

*: Significant, **: Highly significant

One way-ANOVA and post-hoc test showed that, there was highly statistically significant rise in Hs-CTnT and GDF15 among patients' group in relation to control group, with higher level in both marker among those with CHF as detected in table (3).

Table (3): The Hs-CTnT and GDF15 levels in all studied groups

Variable	CAD group Mean±SD	CHF group Mean±SD	Control group Mean±SD	P-value
Hs-CTnT (pg/ml)	**837±24.4**	**1745±43.1**	**3.8±0.2**	**<0.001****
GDF15 (pg/ml)	**895±28.6**	**1309±32.3**	**529±7**	**<0.001****

*: Significant

The test showed highly significant positive correlation between Hs-CTnT and GDF15 among CAD patients' group (Fig. 1).

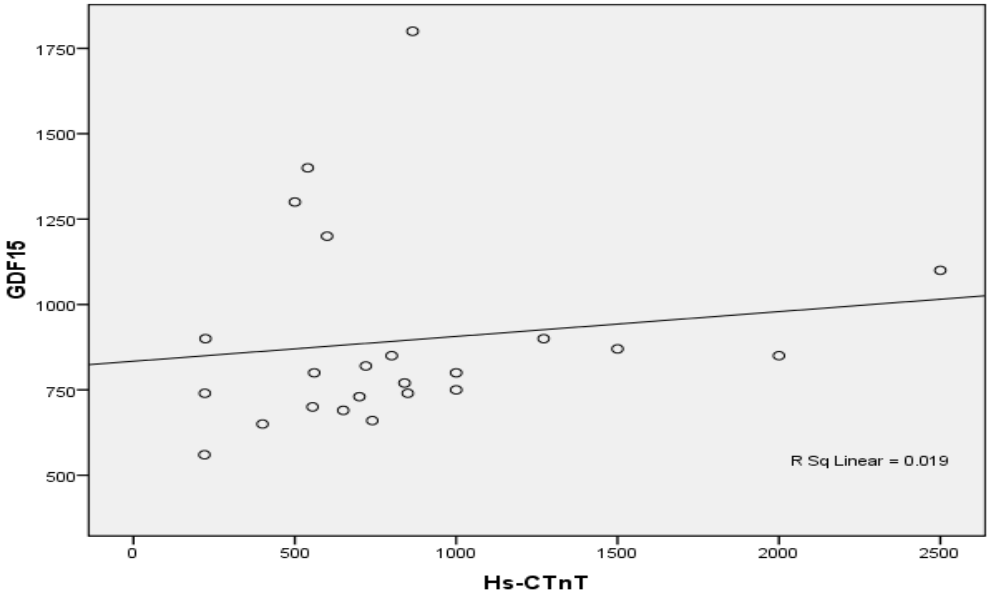

Fig (1): Shows correlation between serum level of Hs-CTnT and GDF15 among CAD patients' group

As shown in figure (2), the test showed highly significant positive correlation between Hs-CTnT and GDF15 among CHF patients' group **(r: 0.8, P: >0.001).**

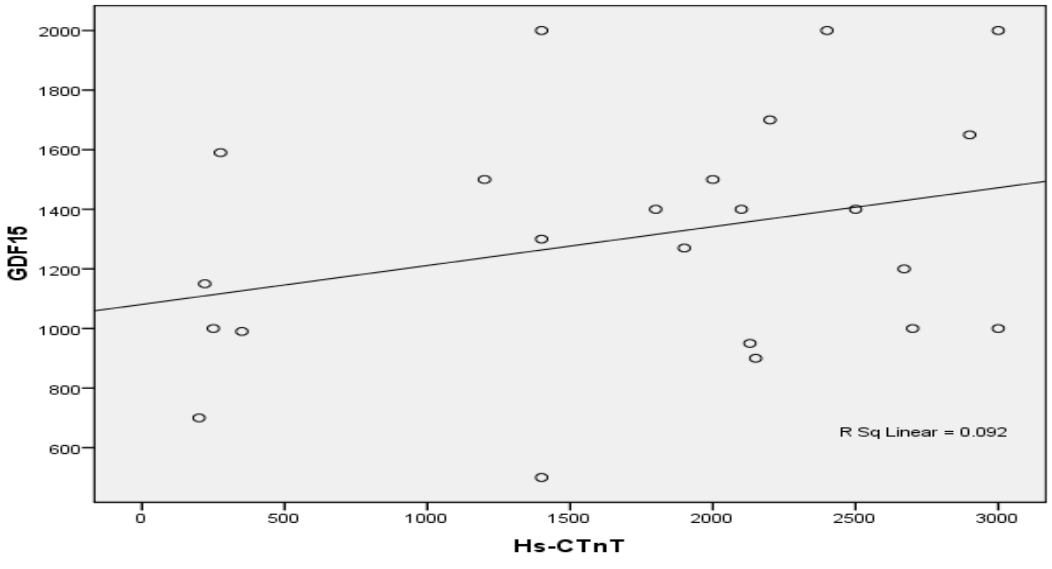

Figure (2): Shows correlation between serum level of Hs-CTnT and GDF15 among CHF patients' group

With a sensitivity of 78.3 percent, 82.6 percent as specificity, 82 percent as positive predictive value, eighty-five percent as a negative predictive value, and an accuracy of eighty-five percent ROC analysis revealed that AUC for the prediction of CHF in CAD patients was 0.755 at the ideal Hs-CTnT cutoff value of 1100 ng/ml (Figure 3). At the optimal GDF15 cutoff value of 885 pg/ml, ROC analysis revealed that AUC for predicting CHF in CAD patients was 0.820, with a sensitivity of 91.3 percent, 69.6 percent specificity, positive predictive value of ninety-three percent, negative predictive value of eighty percent, and accuracy of ninety-two percent (Figure 4).

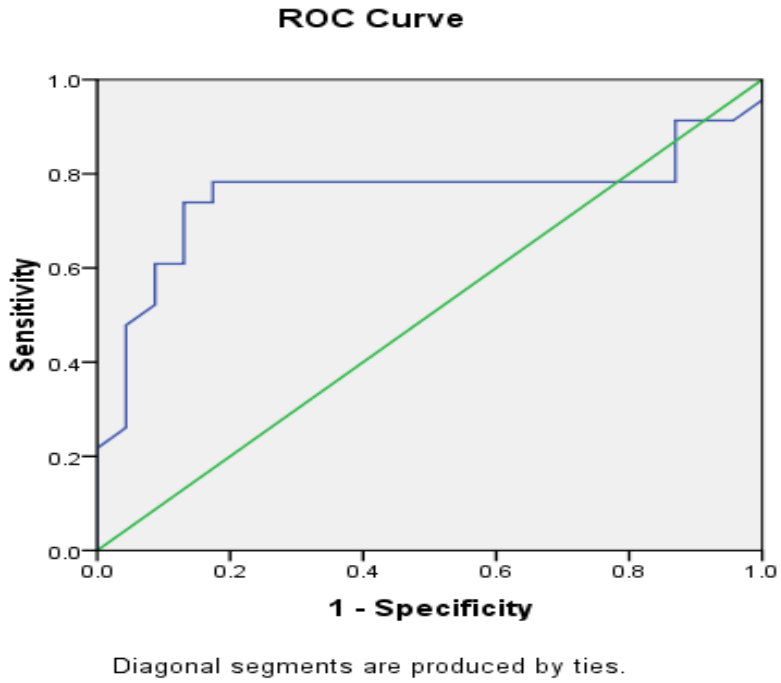

Fig (3): ROC curve for Hs-CTnT.

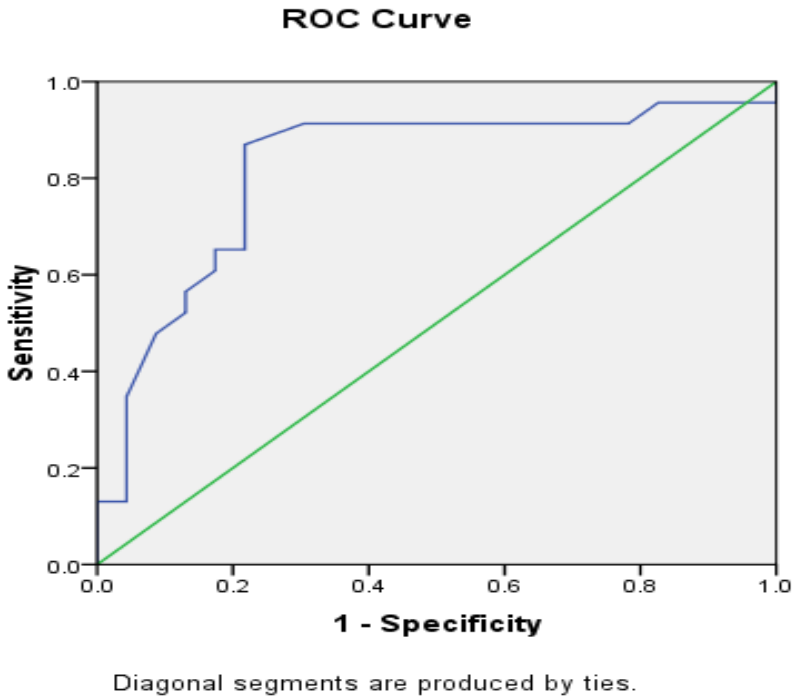

Fig (4): ROC curve for GDF-15

DISCUSSION

Cardiovascular disease is more widespread than ever as a result of dietary and lifestyle changes. Coronary artery atherosclerosis is the most frequent cause of death from cardiovascular diseases. CHF, which is caused by coronary artery atherosclerosis, is the most common kind of HF. Therefore, it is essential to identify acute coronary syndrome (ACS) early and begin treatment right once [15]. Growth differentiation factor-15, a member of the transforming growth factor beta superfamily, controls the inflammatory and apoptotic pathways in both short-term and long-term tissue damage [16].

The current study's objective was to assess the accuracy of the GDF15 test in predicting the onset of CHF in CAD patients. The results of the present investigation showed that, despite the absence of a statistical significance disparity between the CAD and CHF groups, there was an extremely significant difference in age between studied groups. This result disagrees with that of **Zhu and Sun** [17] who reported that, there were no discernible variations in lipids, blood pressure, or age amongst CAD patients, CHF, and control group.

As regards gender, in the groups under study, there was no statistically significant difference. As regards smoking, there was significant statistical difference between both groups of patients and control groups. However, between the CHF and CAD groups, there was no statistical difference. As regards hypertension, there was highly significant statistical rise in blood pressure between both groups of patients and control group, but there was no significant difference between CAD, CHF groups. The results disagree with that of **Wang *et al.*** [2] who reported that, there was similar value in age between CAD and control group, and agree with present study as regard gender. While CAD patients had greater rates of hypertension than healthy controls (P 0.01),

As regards presence of diabetics among studied groups, the present study revealed that, the CHF patients had higher percent (74%) followed by CAD (57%) and no one of control persons had diabetes. This outcome was consistent with that of **Wang *et al.*** [2] who reported that, the CAD participants exhibited higher frequencies of diabetes than control.

The current study's findings showed that on comparing to the control group, the serum GDF-15 level was statistically significantly higher in the CAD group than in the CHF group (P 0.001), and both groups showed extremely substantial increases. these findings concurred with those of other earlier researches. According to **Bonaca *et al.*** [18], CHF patients have greater concentrations of GDF-15. HF caused the median plasma concentration of GDF-15 to increase by 4.4 times, according to **Mueller *et al.*** [19], who also found that all of the ill groups they looked at had greater levels of GDF-15 than healthy controls (P <0.001 for each).

GDF-15 was found to be a helpful biomarker for heart failure with a normal EF in a subsequent investigation. Regardless of the existence of CAD or other risk factors, the risk is higher in people with mild or moderate to severe left ventricular diastolic dysfunction [20].

In terms of Hs-CTnT levels, the current research showed an increase in Hs-CTnT among the patient group in comparison to the control group. Patients with CHF attained the highest level (P < 0.001).

According to **Mueller *et al.*** [19] and **Gaggin and Januzzi** [21], patients with HF and sepsis had considerably greater cardiac troponin concentrations. These findings were also in agreement with each other. This investigation found a highly significant positive correlation between Hs-CTnT and GDF15. This supports the results of **Eggers *et al.*** [22], who found a strong correlation between GDF-15 and cTnI >0.01 g/L and cTnT level at presentation 0.1 mg/L.

The current investigation discovered a statistically significant difference in Hs-CTnT and GDF-15 levels between the three groups. These findings indicate that CHF patients had considerably higher Hs-CTnT and GDF-15 levels than CAD patients, implying that GDF-15 can be employed as myocardial damage biomarker. This is in agreement with **Wang *et al.*** [2] who studied 179 patients. They reported that GDF15 level was highest in old myocardial infarction patients who got heart failure (OMI-HF) and lowest among stable angina patients and with intermediate level in old myocardial infarction patients without heart failure.

Also, our results are in agreement with **Xanthakis *et al.*** [23]**,** who discovered that individuals with advanced coronary artery disease, substantially decreased systolic function, a history of myocardial infarction, or high blood pressure have greater GDF-15 levels. After an incident of ACS, people who have high GDF-15 levels are more likely to experience unfavourable left ventricular remodeling and HF. In ACS patients who participated in the PROVE-IT research, pre-discharge levels of GDF-15 were associated with higher risks of death, recurrent MI, and HF [18]. Beyond hs-CRP and hs-TnT, GDF-15 is a predictive marker that is highly related with poor outcomes in individuals with stable CAD and ACS [24].

A ROC curve of GDF-15 was constructed to discriminate between individuals with or without CHF and coronary atherosclerosis. AUC for this result was 0.820. At the threshold value of 885pg/ml, the diagnostic sensitivity and specificity were ninety-one and 69.6%, respectively, while accuracy was ninety-two percent. The PPV and NPV were ninety-three and eighty percent respectively, of the positive and negative predictive values.

This result was in conflict to many research. **Zhu and Sun** [17] reported that in the ROC curve, which was constructed to distinguish between CAS with or without CHF, AUC was 0.804 and cutoff value was1086.38 pg/ml, the sensitivity and specificity were 72.4, 93.6 respectively.

According to **Wang *et al.*** [2]**,** the blood level of GDF-15 had an AUC of 0.96 with a sensitivity of 80% and ninety-one percent specificity for predicting CAD. Their research showed that a high level of GDF-15 was positively associated with CAD and might be used to establish a diagnosis.

The findings of the present investigation are also at odds with those presented by **Farhan *et al.*** [25]. The receiver operating curve study of GDF-15 for prediction of CV mortality showed an AUC of 0.852 and an estimated cutoff of 2094.6 pg/ml with a sensitivity of seventy-six percent and specificity of 80%. The level of GDF-15 was found to be 1212.8 pg/ml. Thus, they came to the conclusion through ROC analysis that GDF-15 was an accurate predictor of cardiovascular mortality.

The potential biomarker role of GDF-15 in HF was examined in a cohort of 455 individuals with chronic HF with a median LVEF of thirty-two percent. When they were diagnosed, the majority of the patients exhibited NYHA classes 2 and 3 symptoms. GDF-15 levels in seventy-five percent of these individuals were more than 1200 ng/L. Higher GDF-15 levels were present in patients with ischemia or non-ischemic HF, and these levels significantly linked with the illness's severity [26].

LIMITATIONS

Our main limitations were the small sample size and just one-center study. Longer clinical investigations are necessary to fully comprehend the benefits of GDF-15 in predicting the prognosis of people with ACS.

CONCLUSION

Our findings suggest that GDF-15 might be a valuable biomarker for predicting the onset of HF in CAD patients. GDF-15 levels were highly significant in CAD diseased than in persons in good condition. Additionally, in CAD patients with CHF, it increased more quickly and had a positive correlation with the Hs-CTnT level.

RECOMMENDATIONS

We recommend using GDF-15 as a novel biomarker to identify heart failure, diagnose coronary artery disease, and predict HF onset in those who already have the condition.

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Competing interests: Nil.

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