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Relation of Growth Differentiation Factor- 15(GDF-15) to Major Cardiovascular Complications in Type Two Diabetes in Zagazig University Hospitals

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ABSTRACT

Background: Diabetes mellitus (DM), the coming worldwide epidemic, markedly increases mortality by its cardiovascular complications. Growth Differentiation Factor (GDF)-15 is a promising tool for the detection of DM and its related cardiometabolic complications.**Aim:** Assessment of GDF-15 levels in type 2 DM with and without cardiovascular complications in comparison to control and to detect its relation to diabetes clinical and glycemic parameters to find out its diagnostic and prognostic value in these conditions.**Patients and Methods:** an observational descriptive case-control study included 96 subjects,32 of them were healthy volunteers and the remaining 64subjects have type 2 diabetes(32 patients of the last group without CV complications and the other 32 patients had cardiovascular complications). All subjects were subjected to full history, clinical examination,and routine laboratory investigations, in addition to measurement of GDF-15 level by ELISA.**Results:** GDF-15 in the present study was significantly increased in diabetics compared to controlsand in complicated than the non-complicated group with a cutoff value of 170.13 for detection of diabetes and cutoff value of 327 for detection of CV complications with sensitivity and specificity above 65%. GDF-15 had apositive correlation with each of FBG, HbA1c, HOMA IR, TG, ESR and serum cholesterol also it was found that BMI, HbA1c, HOMA IR and TG are significant independent predictors to GDF-15 elevation in diabetic patients.**Conclusion:** GDF-15 elevation could be used as single or part of multi marker approach for diagnosis and prognosis for type 2 DM and its cardiovascular complications.

Key Words: Cardiovascular Complications, Diabetes Mellitus, Growth Differentiation Factor-15.

Introduction:

The prevalence of diabetes mellitus (DM) is reaching epidemic proportions in adults due to an increase in life expectancy, sedentary lifestyle, and obesity. Adults with diabetes and obesity are more prone to cardiovascular complications according to World health statistics2014. Prevalence, incidence, and mortality of cardiovascular diseases (CVD) are 2-8-fold higher in persons having diabetes than those without diabetes (1).According to International Diabetes Federation (IDF) 2018, the prevalence of diabetes in Egypt was around 15.1% among adults (2). The significant impact of diabetes on CVD events and outcomes relates to the interplay of preexisted traditional CVD risk factors with the negative effect of glucose metabolism impairment, lipotoxicity, adipose tissue dysfunction, excessive oxidative stress (3). All these factors can induce endothelial dysfunction, leading to cardiac remodeling and hypertrophy, worse vascular integrity, and cardiac function (4).

Growth differentiation factor-15 (GDF-15) (previously known as macrophage inhibitory cytokine-1) is a part of the transforming growth factor-beta (TGF-b) superfamily (5). It is a stress-responsive cytokine whose expression increases with age, smoking, environmental factors, protein glycation, hormonal changes, obesity, insulin resistance, diabetes, oxidative stress, inflammation, and tissue injury through either p53 or early growth response protein-1 (EGR-1) transcription factors (6). It is highly produced in cardiomyocytes, adipocytes, macrophages, endothelial cells, and vascular smooth muscle cells which is associated with cardiometabolic risk, progression, and prognosis of the disease condition (7).The GDF-15 levels were elevated more in Type II DM than the non-diabetic population in most of studies (8**).**In contrast some studies reported that GDF-15 was nonsignificantly elevated in diabetes (9).

There are insufficient data concerning the pathophysiological role of GDF-15 in diabetes, CAD, hypertension, and diabetes associated with cardiovascular diseases. It was stated that there was an inverse correlation between insulin sensitivity and GDF-15 values (5). A higher level of GDF-15 was associated with augmented cardiovascular and non-cardiovascular mortality (10),so these studies

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displayed that the deficiency of GDF-15 is beneficial against vascular damage and inflammation. On the other hand, other studies stated that GDF-15 plays a protective role in the heart, adipose tissue, and endothelial cells (6) (18). Before accepting GDF-15 as a clinically beneficial biomarker, the following inquiries need to be answered; whether its measurement can support therapeutic management, can it be used for routine clinical practice or clinical measurement, whether its level can give any diagnostic and prognostic information, whether it can be used to make a clinical decision for any particular diseases, and can GDF-15 be used as a single marker or as a part of multi-marker approach along with other individual markers. As a result of these controversies, The present study was designed firstly to measure GDF-15 concentration in diabetic patients in comparison to control subjects and cardiovascular complicated diabetic patients in comparison to diabetic patients without cardiovascular complications, secondly to find out any relation between serum level of GDF-15 and each of glycemic parameters and major cardiovascular complications in diabetes mellitus and finally to find out if GDF-15 can be used as a diagnostic and prognostic biomarker for DM and its major cardiovascular complications.

Materials and Methods

2.1. Study design and settings

Observational descriptive case-control and analytic study including patients from the medical ICU and outpatient diabetic clinic of the internal medicine department of Zagazig University in the period between April 2017 to March 2018 were assessed in the study.

2.2. patient selection

In this study, 96 subjects were recruited. 32 of them were healthy volunteer subjects as control (group1)and 64 type 2 diabetic patients (group 2)which wasdiagnosed according to ADA 2014and were subdivided into 32 patients without cardiovascular complications (group 2A) the other 32 patients had diabetic cardiovascular complications (group 2B),eight patients of themwith Ischemic heart diseases, eight patients with heart failure, eight patients with ischemic stroke and eight patients with hypertension after exclusion of patients with pregnancy, malignancy, smoking , immunodeficiency diseases, septicemia, an Acquired immunodeficiency syndrome (AIDS), Evident acute infection, Connective tissue diseases, and Patients receiving drugs that may modify the immune response like corticosteroids. Both groups were matched in age, sex, and BMI. 48 patients were female and 48 were male. All of them were overweight. The mean age of included diabetic patients was 61.2±6.3 years and for control was 61.7±6.2 years.

2.3. Ethical clearance

All procedures performed in the study involving human participants were approved bytheinstitutional review board (IRB number 3406) of Zagazig University.

2.4. Patient assessment

Full medical history, clinical examination, and routine laboratory investigations were performed for each subject including Fasting blood glucose after 8h of fasting,2hPP blood glucose, Hemoglobin A1C.Fasting insulin,HOMA-IR,Complete blood picture, Liver function tests: serum bilirubin (total and direct), serum albumin, serum alanine transferase, and aspartate transferase measured by kinetic method, Renal function tests: serum creatinine, serum urea, Bleeding profile: INR, Prothrombin time (PT) and Partial Thromboplastin Time (PTT), Fasting lipid profile (Total cholesterol, Serum triglycerides, LDL and HDL), CRP, ESR, ECG, Abdominal ultrasoundand Brain CT scan for stroke patients. Echocardiography for CV complicated group ,in addition to specific laboratory investigation including measurement of serum GDF-15 level by ELIZA (Shino-Test Corporation,Kanagawa ,Japan).

2.5. Serum GDF-15 level

Buffer is heated to room temperature and merged gently till completely dissolve,then watered down into deionized water to prepare 500 ml of Wash Buffer. Dilute to the working concentration using Biotin-antibody Diluent (1:100), respectively. HRP-avidinCentrifuge the vial before opening.100 µl of Standard, Incubated for 2 hours at 37°C. 100 µl of Biotin-antibody working solution is added to each well incubated for 1 hour at 37°C., repeat the process three times for a total of three washes. 100 µl of

ISSN:0975-3583,0976-2833 VOL12,ISSUE07,2021

HRP-avidin working solution is added to each well incubated for 1 hour at 37°c Repeat the aspiration and wash five times. Add 90 µl of TMB Substrate to each well. Incubate for 10-30 minutes at 37°C.Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. The serum is removed and assayed immediately or aliquot and stored samples at -20°C.

2.5.*Statistical analysis*

All data were collected, tabulated, and statistically analyzed using SPSS 26.0 for windows (SPSS Inc., Chicago, IL, USA).

Quantitative data were expressed as the mean ± SD & (range), and qualitative data were expressed as absolute frequencies (number)& relative frequencies (percentage).

Independent samples Student's t-test was used to compare two groups of normally distributed variables while Mann Whitney test was used to compare two groups of non-normally distributed variables and the ANOVA test for comparison of more than 2groups.

Percentage of categorical variables were compared using the Chi-square test or Fisher's exact test when appropriate.

Spearman's rank correlation coefficient was calculated to assess the relationship between various study variables, (+) sign indicates a direct correlation and (-) sign indicates an inverse correlation, also values near to 1indicate strong correlation, and values near 0indicate weak correlation.

Multivariate Logistic Regression was done to detect the predictors of GDF-15.

Sensitivity, specificity, predictive value for positive (PVP), predictive value for negative (PVN), and accuracy were calculated at 95% CI to measure the validity of GDF-15 as a screening test for diabetic complications

All tests were two-sided. p-value < 0.05 was considered statistically significant (S), p-value ≥ 0.05 was considered statistically insignificant (NS).

Results:

There was no statistically significant difference between the studied groups regarding age, BMI and sex ensuring matching of the studied groups. There was highly statistically significant increase in FBS, 2hPPS, HbA1c , serum cholesterol ,TG and HOMA IR in diabetic patients compared to control group and there was highly statistically significant increase in FBS, PPS, HbA1c , serum cholesterol , TG and HOMA IR in complicated diabetic patients compared to non- complicated diabetic group**(Table 1)**. There was highly statistically significant increase in GDF-15 concentration in diabetic patients(group 2) compared to control group(group 1) (p-value =0.001 and GDF-15 cutoff value 170.13),There was a highly statistically significant increase in GDF-15 concentration in CVcomplicated diabetic patients (group 2B)compared to the non-complicated diabetic group(group 2A) (p=<0.001 and cutoff value 327) **(Table 2)**.There was a statistically significant positive correlation between GDF-15 and Fasting blood glucose, HbA1c, HOMA IR, TG, ESR, and serum Cholesterol **(Table 3)**.

The study shows that BMI, HbA1c, HOMA IR, and TG are significant independent predictors of elevated GDF-15 levels in DM as shown in table (4).For the performance of GDF-15 as a biomarker of diabetes among studied subjects,the ROC curve was performed. It was found that the best cutoff value of GDF-15 in the diagnosis of diabetes is ≥ 170.3 with an area under the curve (AUC) of 0.714, sensitivity of 70.3% and specificity of 66%, the positive predictive value of 80.4 %, negative predictive value 52.5 % and accuracy 68.8 %**(Figure 1)**. On the other hand, for the performance of GDF-15 as a biomarker for major cardiovascular complications in the diabetic group ROC curve was performed. It was found that the best cutoff value of GDF-15 in the prediction of major cardiovascular complications in diabetes is ≥ 327 with an area under the curve (AUC) of 0.901, a sensitivity 78.1% and specificity of 81.2%, positive predictive value 80.6 %, negative predictive value 78.8 % and accuracy 79.7% **(Figure 2)**

Table (1): Comparison of Demographic and metabolic parameters between control, non-complicated and CV complicated diabetic groups of the study:

ISSN:0975-3583,0976-2833 VOL12,ISSUE07,2021

Variable	Control Group (group 1) (n=32)	Non complicated DM Group (group2A)(n=32)	Complicated DM group (group 2B)(n=32)	Tests	
	Mean ±SD	Mean ±SD	Mean ±SD	*test*	*P* value
Age (years)	61.7±6.2	61.03±6.1	61.4±6.5	0.1	0.9
BMI (Kg/m^2)	33.3±2.2	33.8±2.4	33.5±2.9	F 0.4	0.7
Fasting blood glucose(mg/ dl)	91.3 ± 11.3	172.1±274.2 a	262.1±88.8 ab	F 50.4	<0.001 HS
2h PPS(mg/ dl)	122.7±10.9	270.2±67.8 a	326.3±75.7 ab	KW 8.9	0.01 S
HbA1c:(gm%)	5.2±0.34	9.2±1.7 a	9.9±1.4 ab	F 118.3	<0.001 HS
HOMA IR	1.7±0.5	2.5±0.6 a	3.5±1.1 ab	KW 11.1	0.004 S
HDL(mg/ dl)	34.9 ± 6.5	34.96±6.8	37.09±6.76	F 1.1	0.3
LDL(mg/ dl)	136.6 ± 42.5	146.06±36.9	148.03±32.1	KW 3.3	0.2
TG(mg/ dl)	138.3 ± 50.6	154.4±49.7 a	180.2±49.7 ab	KW 5.9	0.043
Cholesterol(mg/ d)	205.1 ± 50.8	214.8±37.4 a	261.9±68.1 ab	F 10.3	<0.001 HS
Variable	**No (%)**	**No (%)**	**No (%)**	**test**	**P value**
Sex					
• Female	16 (50)	16 (50)	16 (50)	NA	NA
• Male	16 (50)	16 (50)	16 (50)		

Independent t-Test (t), Kruskal Wallis test (**KW), a**=significant difference between group 1and 2A, **b**=significant difference between group 2A and 2B, Hb A1C: GlycatedHeamoglubin, HDL: High Density Lipoprotein**,** LDH: Low Density Lipoprotein**,** TG: Triglyceride

Table (2): Comparison of GDF-15 concentration in control,non-complicated and complicated diabetic patients

Variable	Control Group (group 1) (n=32)	non complicated DM Group (n=32)	Complicated DM group (n=32)	tests	
				test	*P* value
S.GDF-15(ng/dl) Mean ± SD	184± 145.1	211.9±197.8 a	622.3±287.2 ab	KW 9.6	0.008 S

Kruskal Wallis test (KW), a=significant difference between group 1and 2A ,b=significant difference between group 2A and 2B

ISSN:0975-3583,0976-2833 VOL12,ISSUE07,2021

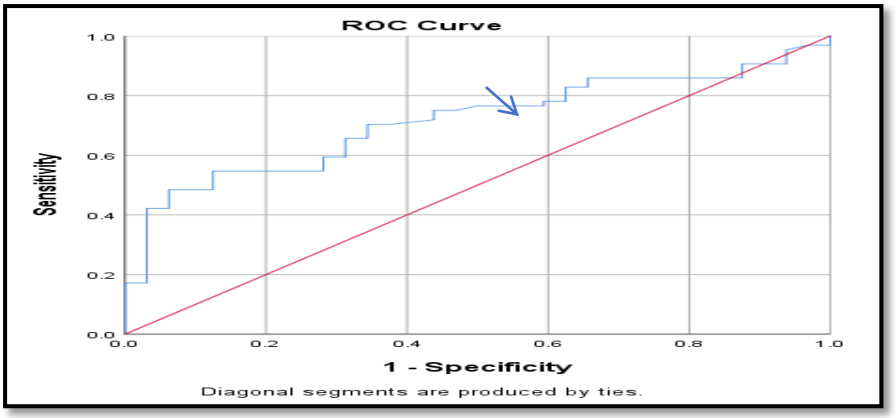
Table (3): Correlation between GDF-15 and different variables in diabetic and control subjects:

Variables	Diabetic groups		Control Group	
	r	p	r	p
Age (years)	0.008	0.948	-0.163	0.374
BMI (Kg/m^2)	-0.104	0.414	-0.207	0.255
Fasting blood glucose(mg/ dl)	0.281	0.024*	-0.020	0.914
Hb A1c(gm%)	0.182	0.04*	-0.055	0.764
2h PPS(mg/ dl)	0.171	0.177	0.06	0.745
HOMA IR	0.291	0.02*	0.95	0.604
HDL(mg/ dl)	0.123	0.333	0.104	0.572
LDL(mg/ dl)	-0.117	0.356	0.007	0.969
TG(mg/ dl)	0.324	0.009**	-0.027	0.884
Cholesterol(mg/d)	0.292	0.018*	-0.161	0.378
ESR (mm/hr)	0.382	0.002**	0.000	0.999
CRP (mg/l)	-0.173	0.173	0.204	0.264

Table (4): Multivariate logistic regression for prediction of GDF-15 elevation in diabetic patients:

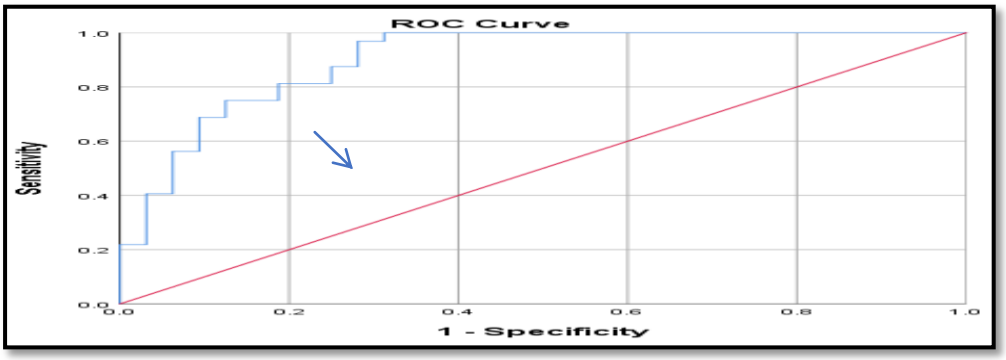
Parameters	B	S.E.	Wald	Sig.	OR	95% C.I. for EXP(B)	
						Lower	Upper
Age (years)	-.044	.055	.647	.421	0.957	.859	1.066
BMI (Kg/m^2)	.330	.145	5.171	.023*	1.391	1.047	1.849
Fasting blood glucose(mg/ dl)	-.009	.008	1.281	.258	0.991	.975	1.007
HbA1c(gm%)	.008	.009	.854	.355	1.008	.991	1.026
2h PPS(mg/ dl)	.242	.233	1.076	.039*	1.237	.807	2.010
HOMA IR	.278	.368	.572	.032*	1.321	.042	2.716
HDL(mg/ dl)	-.008	.053	.025	.874	.992	.893	1.101
LDL(mg/ dl)	.021	.011	3.751	.053	1.021	1.000	1.043
TG(mg/ dl)	-.016	.008	4.098	.043*	.984	.969	.999
Cholesterol(mg/d)	-.008	.007	1.580	.209	.992	.979	1.005
ESR (mm/hr)	.017	.024	.503	.478	1.017	.970	1.066
CRP (mg/l)	.065	.040	2.683	.101	1.067	.987	1.154

Figure (1):Roc curve showing validity of GDF-15 in predicting diabetic from non-diabetic patients



ISSN:0975-3583,0976-2833 VOL12,ISSUE07,2021

Figure (2):Roc curve showing validity of GDF 15 in complicated diabetic patients



Discussion

Type 2 diabetes mellitus (T2DM) remains a significant health problem and one of the most widespread metabolic diseases worldwide, which displays relentless growth in the general population (11). Recent observation, epidemiological and clinical investigations have shown strong evidence regarding sufficient link between T2DM with cardiovascular (CV) mortality and morbidity (1).The different roles of GDF-15 were demonstrated in many studies; however, the exact mechanism and downstream signal are not known. The GDF-15 levels were elevated more in Type II DM than the non-diabetic population in most of studies (8).In contrast some studies reported that GDF-15 was nonsignificantly elevated in diabetes(9).It is clear that GDF-15 offers beneficial effects to manage metabolism and insulin sensitivity through its binding and activation of GFRAL (GD Family Receptor Alpha Like). However, the data about the mechanism in which GDF-15 acts in other environments remains controversial. While it appears that GDF-15 acts as a compensatory agent in diabetes and CVD, its role as a potential driver of these pathologies cannot be ruled outs due to its ability to cause anorexia and cachexia, or weakness(5) .And other studies stated that increased GDF-15 plays a protective role in the heart, adipose tissue, and endothelial cells (7) through alteration in the hypertrophic responseor antagonism of hypertrophy and reductions in cell death.Therefor the present study was designed firstly to measure GDF-15 concentration in diabetic patients in comparison to control subjects and in cardiovascular complicated diabetic patients in comparison to diabetic patients without cardiovascular complications , secondly to find out any relation between serum level of GDF-15 and each of glycemic parameters and individual major cardiovascular complications in diabetes mellitus and finally to find out if GDF-15 can be used as diagnostic and prognostic biomarker for DM and its major cardiovascular complications .

The current study shows that there was a highly statistically significant increase in serum GDF-15 concentration in diabetic patients(group2)compared to the control group (group 1)(GDF-15 cutoff value 170.13) for detection of DM and a highly statistically significant increase in GDF-15 concentration in CVcomplicated diabetic patients (group2B) compared to non-complicated diabetic group (group2A) with GDF-15 cutoff value 327 for detection of CV complications .There was a highly statistically significant increase in FBS, PPS, HbA1c, serum cholesterol, TG, and HOMA IR in CV complicated diabetic patients compared to the non-complicated diabetic group.

Our study showed an elevated level of GDF-15 in the diabetic group compared to the control group,this was in agreement with Berezin, et al (8),and this was attributed to the impairment of glucose metabolism, dyslipidemia, adipose tissue dysfunction, and excessive oxidative stress are factors that induce endothelial dysfunction in diabetic subjects (3).these factors worsen vascular integrity, cardiac function and lead to the release of GDF-15 from adipocytes and cardiovascular cells (4).In contrast to Au-yeung et al (9)who suggested that GDF-15 is unrelated to type 2 diabetes and glycaemic traits which was attributed to the well control of glycemic status of the patients involved in their study.

The current study showed an elevated level of GDF-15 in the cardiovascular complicated diabetic group compared to the non-complicated diabetic group,this was in agreement with Adela, et al (7)who reported an increased level of GDF-15 in diabetic patients with cardiovascular complications compared to the non-complicated diabetic group which was attributed to antiapoptotic effect against ischemia-

ISSN:0975-3583,0976-2833 VOL12,ISSUE07,2021

reperfusion (I/R) and reduced the size of myocardial infarction (MI). GDF-15 has been described as a promising cardioprotective agent (12). Moreover, GDF-15 might induce angiogenesis, which plays an essential role in the recovery of damaged myocardium (13).In contrast to Tuegel et al (14*)* who suggested that GDF-15 was chronically elevated in individuals with CVD, specifically in individuals with a history of myocardial infarction or heart failure.

The present study showed an increased level of GDF-15 in patients with ischemic heart diseases in agreement with Zhang et al (15).This was inconsistent to Au-yeung et al (9)who suggested a potential inverse association of GDF-15 with CAD occurred for several reasons,GDF-15 could simply be an early marker of underlying disease, a reflection of confusing by ill-health.

This study results in heart failure patients are also consistent with George et al (16),In contrast to Pareek et al(17*)*who demonstrated that, GDF-15 showed stronger association with composite outcomes including all-cause mortality, but weaker association with incident heart failure or CVD and did not offer a significantly greater improvement in discriminative ability than that provided by other biomarkers.

Our study results concerning GDF-15 levels in diabetic patients with an ischemic stroke showed elevation in the GDF-15 level compared to uncomplicated diabetic group which are in agreement with Dong et al (18)In contrast to Bao etal (19*)* who mentioned that the associations of GDF-15 with ischemic stroke was attenuated and non-significant as the associations were likely to be confounded by preexisting CV dysfunction or by the competing risk of death.

The present study results in diabetic patients with hypertension showed elevation in the GDF-15 levels compared to control groups which agree with the study of sokemen et al (20).In contrast Xu et al (21) who suggested that GDF-15 has biphasic effects on cellular survival by cardiac remodeling, ischemia/reperfusion injury and atherosclerotic plaque formation.

There was statistically significant positive correlation between GDF 15 and each of Fasting blood glucose, HbA1c, HOMA IR, TG, ESR and serum Cholesterol

Multivariate linearregression analysis was performed in the present study for analyzing the factors that were significantly correlated with GDF-15 elevation in the diabetic group. It was found that BMI, HbA1c, HOMA IR, and TG are significant independent predictors ofGDF-15 elevations in DM Similarresults were found in Adela,et al (7) also Hong, et al (22)whoshowed that GDF-15 had a positive correlation with insulin resistance independent of age and BMI, and the serum level of GDF-15 had a positive correlation with impaired fasting glucose and type 2 diabetes and insisted that GDF-15 may be a novel biomarker for detecting impaired fasting glucose.

For the performance of GDF-15 as a biomarker of diabetes among studied subjects,the ROC curve was performed. It was found that the best cutoff value of GDF-15 in the diagnosis of diabetes is $\geq$ 170.3 with an area under the curve (AUC) of 0.714, a sensitivity of 70.3% and specificity of 66%,the positive predictive value of 80.4 %, negative predictive value 52.5 % and accuracy 68.8 %,similar results were shown by Adela,et al (7).On the other hand, for the performance of GDF-15 as a biomarker for major cardiovascular complications in the diabetic group ROC curve was performed. It was found that the best cutoff value of GDF-15 in the prediction of major cardiovascular complications in diabetes is $\geq$ 327 with an area under the curve (AUC) of 0.901, a sensitivity of 78.1%, and specificity of 81.2%, positive predictive value 80.6 %, negative predictive value 78.8 % and accuracy 79.7% in agreement with Eddy,et al (5)

The current study showed some limitationsincluding the monocentric work that limits the generalization of the results and the data should be interpreted with caution. In addition, there was no follow-up of the complicated groups to assess GDF-15 levels in the prediction of mortality. Also, the effect of the medical interventions on GDF-15 concentrations was not considered in the current study.

With a better understanding of the upstream disease pathways reflected by GDF-15, new treatment targets may emerge. Increasing GDF-15 concentrations over time are indicative of a worse prognosis in the community and patients with CAD or HF. Further evaluations of the impact of environmental influences, lifestyle changes, and (medical) treatments on GDF-15 concentrations over time are eagerly awaited. Eventually, interventions that affect GDF-15 may be associated with better health and

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improved outcomes. Prospective randomized studies of such interventions stratified for and monitored by GDF-15 concentrations, therefore, appear to be an exciting opportunity.

Conclusion

The current study revealed thatGDF-15 may be useful as single or part of multi marker approach for diagnosis and prognosis for type 2 DM and its cardiovascular complications. Further understanding regarding the signaling pathways of GDF-15 may help to discover novel therapies against diabetes and cardiovascular complications. Patients with increased GDF-15 concentrations may potentially benefit from anti-inflammatory, antioxidant, or antiaging therapies.

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Author's contribution:

Dr.AymanMagdEldin Mohammad Sadek, Usama Ahmed Khalil and AbdallahAbdelazizAbdallah : design of the work. Ghada Samir, andEssam Adel Abdelrahman: data collection, data analysis, interpretation, and drafting the article. FathyaAbdelghaffarMetwally: statistical analysis. Mohamed SamyFawzi: laboratory investigations, drafting the article. All authors were involved in the critical revision of the article, and final approval of the version to be published.

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Compliance with ethical standards:

Conflict of interest

The author(s) declare that they have no conflict of interest.

Statement of human and animal rights

The work described has been carried out under The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Informed consent

Written informed consent was obtained from all individual participants included in the study. For patients with altered sensorium, informed consent was obtained from their first-degree relatives.

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