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Relation between Common Carotid Intima-Media Thickness and Molecular Markers in Patients with Newly Diagnosed versus Established Diabetes Mellitus: A Retrospective Study

Osama I. Ramadan[1]*, Lashin S. Ali[2,3], Randa M. Abobaker[4], Hoda M. Flifel[4], Ahmed S. Abdelmonsef[5], Osama M. Abdelhay[6,7], Ahmed E. Moustafa[8], Sameer Alqassimi[9], Eman S. Albeltagy[10,11], Husseini F. EL boraey[12]

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a prevalent chronic condition associated with early vascular complications. Identifying relevant biomarkers early in the disease course may facilitate better risk stratification and management. This study aimed to assess serum levels of Growth Factor Receptor-Bound Protein-2 (GRB2), advanced glycation end-products (AGEs), and carotid intima-media thickness (CIMT) in patients with early versus established T2DM.

Methods: This retrospective study included 140 patients with T2DM (70 newly diagnosed and untreated, 70 with established disease, and 70 healthy controls). GRB2, AGEs, and CIMT were measured and correlated with demographic and biochemical data. The Statistical Package for Social Science for Windows, version 20 (IBM, Chicago, USA), was used to statistically analyze the collected data.

Results: GRB2, AGEs, and CIMT were significantly higher in both early and established T2DM compared to controls (P<0.001), and significantly higher in established compared to early T2DM (P<0.001). Mean GRB2 levels were 3.87±0.37 ng/dL (early), 4.33±0.57 ng/dL (established), and 3.01±0.46 ng/dL (controls). CIMT was 0.66±0.04 mm (early), 0.73±0.06 mm (established), and 0.60±0.05 mm (controls). GRB2 positively correlated with CIMT, AGEs, and glycemic indicators. Carboxymethyl-lysine (CML) ≤ 4.9 ng/mL yielded 100% sensitivity and specificity; methylglyoxal ≤ 3.5 ng/mL showed 88.57% sensitivity; CIMT ≤ 0.71 mm showed 85.71% sensitivity and 60.0% specificity; GRB2 ≤ 3.99 ng/dL had 71.43% sensitivity.

Conclusion: GRB2, AGEs, and CIMT levels increase with the progression of T2DM. These markers may aid in distinguishing early from established T2DM; however, further longitudinal studies are warranted to validate their prognostic and clinical relevance.

Keywords: Diabetes Mellitus, Oxidative Stress, Advanced Glycation End Products, Growth Factor Receptor-Bound Protein-2, Carotid Intima Media Thickness

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Corresponding author: *Dr Osama I. Ramadan, dr.osama784@yahoo.com*

1. Oral Surgery and Diagnostic Sciences Department, Faculty of Dentistry, Applied Science Private University, Amman, Jordan
2. Department of Basic Medical Science, Faculty of Dentistry, Al-Ahliyya Amman University, Amman, Jordan
3. Department of Medical Physiology, Faculty of Medicine, Mansoura University, Egypt
4. Maternal and child health nursing, North Private College of Nursing, KSA
5. Department of Anatomy and Physiology, College of Medicine, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, Saudi Arabia
6. Department of Medicine & Surgery, Vision Medical College, Jeddah, KSA
7. Department of Physiology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
8. Medical Biochemistry Department, Damietta Faculty of Medicine, Al-Azhar University, Egypt
9. Department of Internal Medicine, Faculty of Medicine, Jazan University, KSA
10. Internal Medicine Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt
11. Internal Medicine Department, General Medicine Practice Program, Batterjee Medical College, Aseer, 62451, Saudi Arabia
12. Diagnostic Radiology Department, Damietta faculty of medicine, Al-Azhar University, Egypt

↑What is "already known" in this topic:
Type 2 diabetes mellitus (T2DM) is a prevalent chronic condition associated with early vascular complications. The development of atherosclerotic lesions or overt clinical manifestations, endothelial dysfunction is considered a reliable indicator of subclinical cardiovascular diseases associated with diabetes mellitus; however, findings are inconsistent. Few comparative studies have compared the levels of GRB2, AGEs, and CIMT in patients with newly diagnosed and established D.M.

→What this article adds:
This study aimed to assess serum levels of Growth Factor Receptor-Bound Protein-2 (GRB2), advanced glycation end-products (AGEs), and carotid intima-media thickness (CIMT) in patients with early versus established T2DM. Our findings highlight that GRB2, AGEs, and CIMT levels increase with the progression of T2DM. These markers may aid in distinguishing early from established T2D.

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Introduction

Type-II diabetes mellitus (DM) is an important global health problem with higher morbidity (e.g., metabolic, cardiovascular, neurological, renal, and hepatic) and mortality. Thus, it imposes a significant burden on the patient, his/her family, and the overall all society with increasing healthcare cost (1, 2).

Peripheral artery disease, stroke, cardiovascular changes, coronary heart disease, diabetic kidney disease, retinopathy, and peripheral neuropathy are examples of macrovascular illnesses that have historically been associated with difficulties in individuals with diabetes mellitus (3-5).

The cardiovascular changes in diabetes are largely due to accelerated coronary atherosclerosis. This usually starts early, before the appearance of the clinically significant events, when compared to people without diabetes (6, 7).

Coronary atherosclerosis, associated with diabetes mellitus, is attributed to different metabolic, hormonal, obesity, and hemodynamic factors, all of which lead to endothelial dysfunction. However, there was no clear glycemic threshold at which the cardiovascular complications started to develop. But, it gradually increased with the elevation of blood glucose levels (8).

As it appears before the development of atherosclerotic lesions or overt clinical manifestations, endothelial dysfunction is considered a reliable indicator of subclinical cardiovascular diseases associated with diabetes mellitus (9, 10). Thus, building strategies to prevent or ameliorate endothelial dysfunction may minimize the risk of developing atherosclerotic cardiovascular disorders (CVD) (11).

In clinical settings, the flow-mediated vasodilatation of the brachial artery a widely used diagnostic aid of the endothelial dysfunction. However, it more advanced stages of the disease, the changes (increase) in the common carotid intima media thickness (CIMT) can be used as a surrogate indicator of elevated CVD risk (12).

Advanced glycation end products (AGEs) are a complex group of oxidants formed by slow non-enzymatic glycation between reducing sugars (e.g., glucose or fructose) and proteins or lipids. These AGEs play a crucial role in the pathogenesis of vascular diseases associated with DM (13).

Normally, a small amount of AGEs is produced as a normal consequence of metabolism. However, the production of AGEs increased with age, especially in the presence of chronic diseases (14).

The production of AGEs in DM is enhanced by the chronic hyperglycemia, with a reduction of their clearance at the same time. The increased accumulation of AGEs triggers oxidative stress and inflammation. In addition, apoptosis is increased (15).

AGEs can also decrease the production of nitric oxide (NO) and endothelial NO synthase activity with increased concentrations of glucose (16). It is well-known that AGEs are increased in human atherosclerotic tissues. Thus, AGEs may play a role in the vascular endothelial dysfunction in DM (17). However, studies comparing levels of AGEs in newly diagnosed and well-established type 2 diabetes mellitus (DM) are scarce.

Growth factor receptor-bound protein 2 (GRB2) is a bridging protein, normally expressed in many cells. It regulates many signaling pathways essential for the preservation of numerous physiological functions (e.g., cellular proliferation, growth, and differentiation). The abnormal activation or expression of GRB2 is correlated with the increase invasion of cells in cancers and the development of DM and other chronic diseases (18, 19).

In previous studies, GRB2 levels are associated with dyslipidemia, atherosclerosis, and inflammatory infiltration (20, 21). Thus, destruction of GRB2 could have anti-atherosclerotic effects. These findings suggest that GRB2 may be a novel biomarker of metabolic and vascular dysfunction in T2DM. However, clinical data on GRB2 in different stages of diabetes remain limited

To our knowledge, few comparative studies have compared the levels of GRB2, AGEs, and CIMT in patients with newly diagnosed and established T2DM (22, 23). To better understand their potential role in early vascular risk stratification, this investigation is a valuable contribution to identifying early markers of diabetic vascular pathology.

The current work was designed to estimate the different biomarkers (Growth Factor Receptor-Bound Protein-2, Common Carotid Intima Media Thickness, and advanced glycation end products) in patients with early versus established diabetes mellitus.

Methods

Patients

This was a retrospective study, collecting data from medical records of patients attending our university hospital, at both the radiology department and the internal medicine department, Damietta faculty of medicine, Al-Azhar University. In addition, data from healthy subjects were included as a control group. Patients were divided into three groups: control, early T2DM, and established T2DM. Early T2DM was defined as newly diagnosed type 2 diabetes with a disease duration of <1 year, based on American Diabetes Association criteria, and not yet receiving long-term antidiabetic therapy. Established T2DM was defined as a confirmed diagnosis for ≥5 years with patients on stable treatment regimens, including oral hypoglycemic agents and/or insulin.

Before the start of the study, informed consent was obtained and the study protocol was submitted for evaluation and approved by the local research and ethics committee.

The final assessment was carried out for 70 patients with early diagnosis of diabetes mellitus and 70 patients with established diagnosis of diabetes mellitus, besides the control group (70 subjects). Early diabetes mellitus was defined by the first diagnosis of diabetes mellitus at the time of inclusion in the study with no previous treatment for the disease. However, the established diabetes mellitus was defined if patients received previous treatment (in the form of diet or medications).

The exclusion criteria were as follows: Other types of diabetes than T2DM; severe acute or chronic complications of DM, other chronic medical diseases (e.g., liver and renal

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failure, cancers, hypertension, coronary heart disease, or cerebral infarction). In addition, patients with other stressful conditions (e.g., sepsis, surgery, or trauma) were excluded.

The collected data included anthropometric and biochemical variables. The anthropometric measurements included patient weight, height, body mass index (BMI), and waist circumference. The body mass index was calculated from the equation (BMI= Weight (kg)/squared height (m)). Waist circumference was measured midway between the last rib and the iliac crest. Blood pressure was measured by a sphygmomanometer.

Blood samples for biochemical analyses were collected early in the morning (8-10 AM) after an overnight fast. The antecubital vein was the site of collection. Samples were collected and divided into two samples, the first added to an EDTA-containing tube and the other to another tube with no anticoagulation. An automatic analyzer was used to measure serum glucose, glycated hemoglobin, and lipid profile on a spectrophotometric basis. However, plasma insulin was measured by chemiluminescent microparticle immunoassay. Insulin resistance was defined by the homeostasis model assessment of insulin resistance index (HOMA-IR). It was calculated from the equation (HOMA-IR = fasting insulin (mU/L) x fasting glucose (mg/dl)/405.

Ultrasound Measurement of CIMT

Carotid arteries were examined by a B-mode and color mode, high-resolution, Doppler ultrasound according to the guidelines of the American Society of Echocardiography Carotid Intima-Media Thickness Task Force (24)**.** A semi-automatic software was used to register measurements, "QLAB Advance Ultrasound Quantification Software, v5.0, Phillips, Eindhoven, The Netherlands". Measurements were performed on both right and left sides in triplets, and the mean value was recorded for statistical analysis.

Determination of Serum Levels of AGEs

The OxiSelectTM Methylglyoxal Competitive ELISA Kit and OxiSelectTM N-epsilon-(Carboxymethyl) Lysine Competitive ELISA Kit, manufactured by Cell Biolabs, Inc. in San Diego, CA, USA, were used to quantify the levels of methylglyoxal (MG) and N-carboxymethyl lysine (CML) in the serum. The measurements were taken in compliance with the manufacturer's specifications.

Determination of serum GRB2 concentration

The serum was obtained and kept at -80 ˚C till the time of analysis. Then, GRB2 concentrations were determined by the available Human GRB2 ELISA kit as directed by the manufacturer (JL Biologicals). An enzyme analyzer was used to measure the absorbance at 450 nm. A standard curve was built using the standard concentration (X) and the standard optical density (OD) at 450 nm (Y), and the standard curve was fitted using a logistic equation, and the sample concentration was measured.

Statistical analysis of data

The collected data were submitted to statistical analysis using the Statistical Package for Social Science for Windows, version 20 (IBM, Chicago, USA), running on an IBM-compatible personal computer, with Windows 10 (as operating system). The arithmetic means and standard deviations were used to express the quantitative data, while relative frequency and percentages were used to represent the qualitative data. Groups were compared by One-Way Analysis of Variance (ANOVA) test with post-hoc least significant differences to compare between two groups (for quantitative data). On the other side, the chi-square test was used to investigate the association between categorical variables. Then, the correlation between the studied parameters was achieved by the calculation of Pearson's correlation coefficient. The ability of the studied parameters to differentiate between early and well-established diabetes mellitus was tested by the receiver operating characteristic (ROC) curve, and the area under the curve (AUC) was an indicator of the test's ability. Values >0.7 were considered a reflection of the accepted power of the test. P value < 0.05 was considered significant for interpretation of the results.

Results

Demographic and clinical data

The results showed that the studied groups were comparable in terms of age, gender, and blood pressure, with no statistically significant differences. However, weight, BMI, and waist circumference were significantly higher in both the early and established diabetes mellitus (DM) groups compared to the control group, although the differences between the early and established DM groups were not statistically significant. Conversely, height was significantly lower in the diabetic groups than in the control group, with no significant difference between early and established DM groups (Table 1).

Biochemical tests

Fasting glucose, fasting insulin, and HOMA-IR levels were significantly elevated in both diabetic groups compared to controls, with further significant increases observed in the established DM group compared to the early DM group. Similar trends were noted in glycated hemoglobin and lipid profile components (total cholesterol, LDL, and triglycerides). In contrast, HDL cholesterol levels were significantly reduced in the diabetic groups compared to the control group, and further reduced in the established group relative to the early group (Table 2).

Ultrasound Measurement of CIMT and Serum Levels of AGEs

The Baseline ultrasonographic assessments of carotid atherosclerosis, including carotid intima-media thickness (CIMT) and plaque formation, were performed in 210 subjects with a median age of 51.2 years (Figure 1).

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Table 1. Comparison between study groups regarding patient demographics

Variable		Early DM	Established DM	Control	Test	*P*
Age (years)		50.50±4.94	51.81±4.92	50.80±5.40	1.28	0.281
Sex	Male	41(58.6%)	45(64.3%)	46(65.7%)	۰,۸٦	0.65
	Female	29(41.4%)	25(35.7%)	24(34.3%)		
Weight (kg)		84.48±5.59#	85.47±6.90#	76.04±3.26	٦٣,۰۱	<0.001*
Height (m)		1.7076±0.03906	1.7016±0.03025	1.7237±0.02509	۸,۹۷	<0.001*
BMI (kg/m^2)		28.98±1.77#	29.55±2.70#	25.58±0.69	۸۸,۲۲	<0.001*
Waist circumference (cm)		114.95±6.61#	116.02±8.30#	102.91±2.51	93.67	<0.001*
Blood pressure	Systolic	126.85±8.26	127.78±9.15	125.92±7.67	0.858	0.425
(mmHg)	Diastolic	81.00±6.68	81.28±7.30	79.57±5.75	1.35	0.260

* Indicates significant variance between groups; # Significant Increase when compared to control group; $ indicates significant increase when compared to early diabetes group.

Table 2. Comparison between study groups regarding patient biochemical tests

Variable	Early DM	Established DM	Control	Test	*P*
Fasting glucose (mg/dl)	156.70±5.78#	157.41±6.34#	110.31±5.47	1476.0	<0.001*
Fating insulin (mIU/L)	15.60±3.09#	17.49±3.69#$	2.90±0.53	591.95	<0.001*
HOMA IR	6.03±1.21#	6.80±1.47#$	0.79±0.14	611.57	<0.001*
HgA1c%	6.84±0.54#	7.73±0.62#$	4.83±0.37	570.33	<0.001*
Total cholesterol (mg/dl)	215.80±15.27#	219.38±14.87#	189.61±7.36	108.96	<0.001*
HDL (mg/dl)	30.28±2.78@	27.08±4.02@&	55.01±6.09	803.56	<0.001*
LDL (mg/dl)	109.62±8.09#	129.14±12.60#$	77.69±9.85	440.62	<0.001*
TG (mg/dl)	170.27±6.75#	176.75±7.78#$	132.00±6.75	808.77	<0.001*

* Indicates significant variance between groups; # Significant Increase when compared to control group; $ indicates significant increase when compared to early diabetes group; @ indicates significant decrease when compared to control group; & indicates significant decrease when compared to early diabetes group.

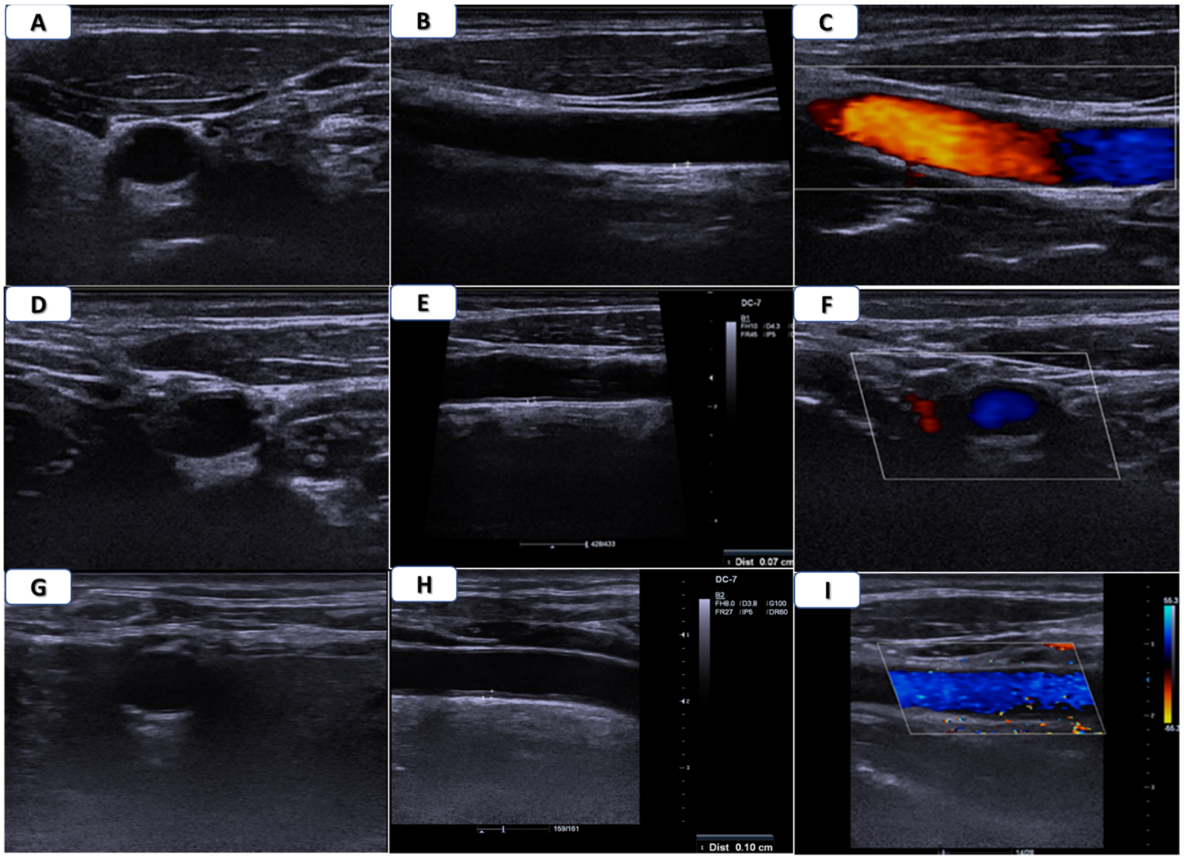

Figure 1. Ultrasonographic measures of carotid intima-media thickness in both B-mode and colored mode (A, B, C) in the healthy group; (D, E, F) in the early DM group; (G, H, I) in the established DM group.

Significant differences were observed among study groups in GRB2 levels, CIMT, and serum levels of advanced glycation end products (AGEs). GRB2, CIMT, methylglyoxal, and carboxymethyl-lysine levels were all significantly higher in early and established DM groups compared to controls. Moreover, these parameters were significantly higher in the established DM group than in the early group (Table 3).

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Table 3. Comparison between study groups regarding GRB2, CIMT, and AGEs

Variable	Early DM	Established DM	Control	Test	*P*
GRB2 (ng/ml)	3.87±0.37#	4.33±0.57#,$	3.01±0.46	140.62	<0.001*
CIMT (mm)	0.66±0.04#	0.73±0.06#$	0.60±0.05	107.81	<0.001*
Methylgyxal (µg/ml)	3.10±0.33#	3.62±0.43#$	2.64±0.40	109.99	<0.001*
Carboxymethyl-Lysine (µg/ml)	3.98±0.37#	6.85±0.61#$	3.27±0.36	1170.01	<0.001*

* Indicates significant variance between groups; # Significant Increase when compared to control group; $ significant increase when compared to early DM group.

Table 4. Correlation between GRB2, CIMT, Methylgyxal, Carboxymethyl-Lysine, and other variables

Variable	GRB2		CIMT		Methylglyxal		Carboxymethyl-Lysine	
	r	*p*	r	*p*	r	*p*	r	*p*
CIMT	0.622	<0.001*						
Methylglyxal	0.568	<0.001*	0.555	<0.001*				
Carboxymethyl-Lysine	0.644	<0.001*	0.656	<0.001*	0.681	<0.001*		
Age	0.102	0.140	0.030	0.663	0.022	0.748	0.093	0.181
BMI	0.480	<0.001*	0.554	<0.001*	0.525	<0.001*	0.472	<0.001*
Waist circumference	0.475	<0.001*	0.538	<0.001*	0.521	<0.001*	0.449	<0.001*
Systolic BP	-0.026	0.706	0.040	0.568	0.075	0.280	0.075	0.277
Diastolic BP	0.047	0.500	0.038	0.582	0.092	0.184	0.098	0.158
Fasting glucose	0.709	<0.001*	0.599	<0.001*	0.593	<0.001*	0.625	<0.001*
Fasting insulin	0.687	<0.001*	0.629	<0.001*	0.598	<0.001*	0.644	<0.001*
HOMA_IR	0.697	<0.001*	0.634	<0.001*	0.604	<0.001*	0.650	<0.001*
HA1C	0.696	<0.001*	0.660	<0.001*	0.652	<0.001*	0.748	<0.001*
Total cholesterol	0.579	<0.001*	0.443	<0.001*	0.478	<0.001*	0.510	<0.001*
LDL	0.722	<0.001*	0.832	<0.001*	0.645	<0.001*	0.769	<0.001*
HDL	-0.700	<0.001*	-0.690	<0.001*	-0.615	<0.001*	-0.658	<0.001*
TG	0.696	<0.001*	0.728	<0.001*	0.626	<0.001*	0.676	<0.001*

* Indicates significant variance between groups; # Significant Increase when compared to control group; $ significant increase when compared to early DM group.

Table 5. The ability of CIMT, GRB2, Methylglyxal, and Carboxymethyl-Lysine in differentiation between early and established diabetes mellitus

Variable	AUC	Cutoff	Sensitivity	Specificity
CIMT	0.800	≤0.71	85.71	60.00
GRB2	0.761	≤3.99	71.43	71.43
Methylglyxal	0.816	≤3.5	88.57	58.57
Carboxymethyl-Lysine	1.000	≤4.9	100.00	100.00

Correlation between CIMT, Serum Levels of AGEs, and other variables

GRB2 levels were significantly and positively correlated with CIMT, methylglyoxal, and carboxymethyl-lysine. Additionally, GRB2 showed significant positive correlations with BMI, waist circumference, fasting glucose, fasting insulin, HOMA-IR, HbA1c, total cholesterol, LDL, and triglycerides. A significant negative correlation was observed with HDL. Similar patterns of correlation were recorded for CIMT, methylglyoxal, and carboxymethyl-lysine (Table 4).

Diagnostic Performance in Differentiating Early and Established DM

Among the studied biomarkers, carboxymethyl-lysine showed the highest diagnostic accuracy for distinguishing between early and established DM, with an AUC of 1.000, yielding 100% sensitivity and specificity at a cutoff ≤4.9 µg/ml. Methylglyoxal showed good discriminatory ability (AUC = 0.816, sensitivity = 88.57%), followed by CIMT (AUC = 0.800, sensitivity = 85.71%, specificity = 60.00%). GRB2 was the least sensitive marker, with a sensitivity of 71.43% and specificity of 71.43% at a cutoff ≤3.99 ng/ml (Table 5, Figures 2, 3).

Discussion

Diabetes is a non-communicable disease with numerous consequences that require care (25). This study aimed to explore novel biomarkers that might distinguish between early and established type 2 DM (T2DM), and reflect the disease's cardiovascular burden. Specifically, we assessed growth factor receptor-bound protein 2 (GRB2), carotid intima-media thickness (CIMT), and advanced glycation end products (AGEs), in addition to standard biochemical markers.

Our results showed that GRB2, CIMT, and AGEs were significantly elevated in both early and established T2DM compared to healthy controls, with the highest values observed in the established group. These findings suggest that these markers may reflect progressive vascular and metabolic alterations that begin early in the disease course.

Our findings are consistent with a prior study that investigated blood levels of GRB2 in T2DM patients and discovered that: T2DM patients' serum GRB2 levels were noticeably greater than those of healthy controls. CIMT, fasting plasma glucose (FPG), HbA1c, and HOMA-IR also showed positive correlations with GRB2 levels (23). Given its function in lipid metabolism and inflammatory responses, these results imply that GRB2 may contribute to the pathophysiology of atherosclerosis in type 2 diabetes.

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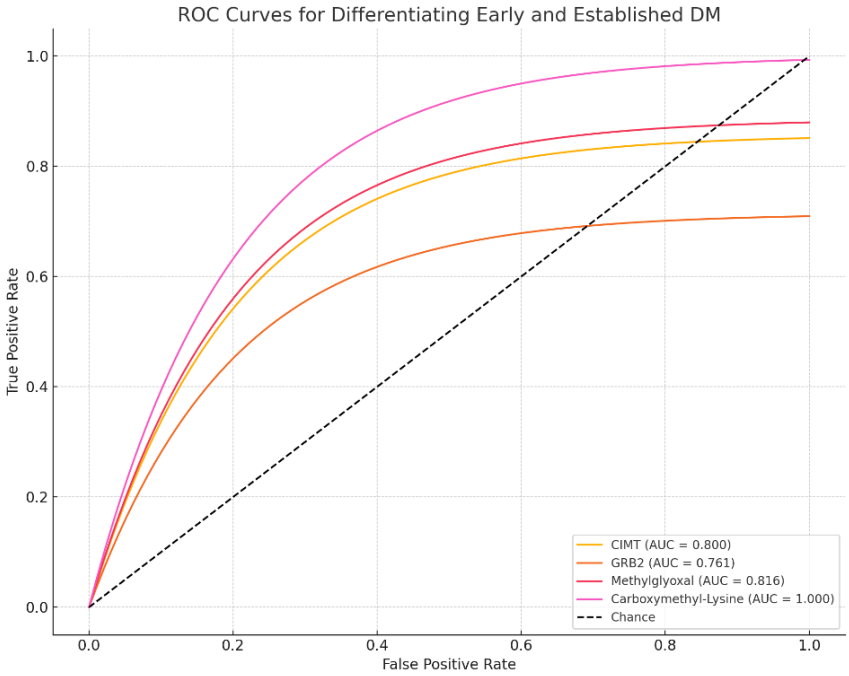

Figure 2. The ROC curve plot illustrating the diagnostic performance of CIMT, GRB2, Methylglyoxal, and Carboxymethyl-Lysine in differentiating between early and established diabetes mellitus. Each curve reflects the sensitivity.

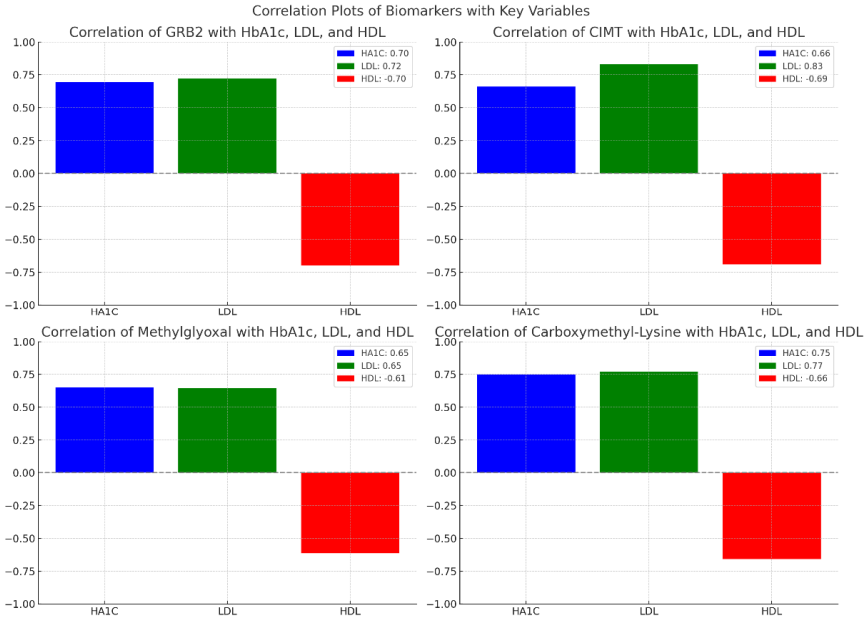

Figure 3. The correlation plots showing how GRB2, CIMT, Methylglyoxal, and Carboxymethyl-Lysine relate to key variables: HbA1c, LDL, and HDL. These visualizations highlight strong positive correlations with HbA1c and LDL and strong negative correlations with HDL across all biomarkers.

GRB2 levels are linked and fluctuating with inflammatory response and glucolipid metabolism. However, its values in type-2 DM were not documented. Values of GRB2 in the current work were significantly higher in early and established DM than healthy controls, and values of GRB2 positively correlated with CIMT. The pathogenesis of atherosclerosis associated with DM is attributed to an inflammatory response and lipid deposition. Different metabolic pathways share in this pathogenesis (e.g., mitogen-activated protein kinase (MAPK), among others). MAPK reg-

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ulates the proliferation and migration of vascular endothelial cells and smooth muscle cells, besides its role in the pathogenesis of atherosclerosis (26). GRB2 is a key element in the MAPK signaling pathway, as reported in human and experimental studies (27, 28).

GRB2 may also have a significant role in the development and progression of DM by binding to phosphorylated insulin receptor substrate one and subsequently regulating insulin levels. In addition, GRB2 expression was significantly upregulated in type-2 DM in experimental models (29).

The correlation between GRB2, insulin levels, fasting glucose, glycated hemoglobin, and others indicates that GRB2 had a direct role in the pathogenesis of diabetes mellitus, as there was a significant increase in GRB2 in early and established DM than in control subjects. As we strictly included subjects with hyperglycemia without significant complications, the higher levels of GRB2 should play a significant role in glucose metabolism. However, results must be treated cautiously due to the small sample size and the retrospective nature of the study. Future studies are recommended to explore the role of GRB2 in the pathogenesis of DM.

The ultrasound assessment of CIMT is a non-invasive, readily available, and rapid assessment of the possible risk of cardiovascular diseases associated with different diseases (e.g., DM). The current work confirms the changes of CIMT in early and well-established type-2 DM.

In the current work, the CIMT ≤0.71 mm was the best cutoff value to differentiate early from well-established diabetes mellitus with increased risk of cardiovascular diseases. However, previous literature provided controversial values to assess the cardiovascular risk. The value of > 1 mm had the highest specificity, but with low sensitivity. Other thresholds were >0.8 mm and>0.90 mm. However, one study indicated that the optimal values of CIMT >0.7 mm for females and 0.8 mm for males are the optimal classification threshold to indicate higher risk (30). In addition, the current results are in line with previous studies, which indicated that there was a greater CIMT in patients with prediabetes when compared to healthy controls (31, 32). Furthermore, Faeh et al. (33) reported a significant association between CIMT and diabetes mellitus. Other large studies and meta-analyses showed that CIMT is a good predictor of major cardiovascular events in patients with type-2 DM (30, 34, 35).

Regarding glycation end products, the results of the current work are in line with previous studies confirming the increased levels of advanced glycation end products (AGEs) in type-2 DM than healthy controls (36, 37). In addition, de la Cruz-Ares et al. (38) reported that the AGEs are significantly increased in established DM than in early diagnosed DM. There is experimental and clinical evidence that increased formation of AGEs leads to endothelial dysfunction in patients with T2DM. The increased values of AGEs are associated with vasoconstriction (through increased levels of endothelin-1) and reduce the production of nitric oxide (NO) levels (reduce vasodilatation) and stimulate the modification of extracellular matrix with subsequent progression of atherosclerosis (39).

Also, AGEs could lead to vascular inflammation and thus contribute to the vascular complications of DM (40). The accumulation of AGEs in the vessel's walls may be responsible for the deposition of rigid fibrin and collagen fibers, which contribute to the increased CIMT in DM (39).

Hence, the correlation between GRB2, AGEs, and CIMT observed in this study suggests that both GRB2 and AGEs may play a contributory role in the development and progression of type 2 diabetes mellitus (T2DM) and its associated vascular complications. The observed elevation of these biomarkers in early and established T2DM, along with their significant association with CIMT, underscores their potential as surrogate indicators of vascular damage and metabolic dysregulation.

Limitations of the study

This study has several limitations. First, the relatively small sample size and retrospective design limit the generalizability of the findings. Second, participants were not matched for body mass index (BMI), which may have influenced metabolic and inflammatory biomarkers such as GRB2 and AGEs, given the known associations between adiposity and systemic inflammation. Future studies should control for BMI to delineate its confounding effects more precisely. Also, Future well-designed prospective studies are essential to confirm the prognostic value of these biomarkers and to assess whether targeting GRB2 or AGEs can alter the course of vascular complications in diabetes.

Conclusion

A possible mechanistic connection between early vascular remodeling in type 2 diabetes and biochemical alterations brought on by hyperglycemia is supported by the correlations between GRB2, AGEs, and CIMT that have been found. These results point to the potential use of AGEs and GRB2 as biomarkers for vascular risk and disease progression. Furthermore, these markers' capacity to distinguish between early-stage and established diabetes raises the possibility of their use in disease staging and customized patient monitoring.

Authors' Contributions

All authors conceived the study, contributed to the manuscript, analyzed the data, wrote the first draft and revised the manuscript based on feedback from all reviewrs, supervised the project and provided resources.

Ethical Considerations

The study was authorized by the Damietta Faculty of Medicine, Al-Azhar University's Institutional Review Board (DFM-IRB 00012367-25-02-028). Every patient who participated in the study gave their informed consent.

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None.

Conflict of Interests

The authors declare that they have no competing interests.

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